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Automatic landmarking of cephalograms using active appearance models

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SUMMARY There have been many attempts to further improve and automate cephalometric analysis in order to increase accuracy, reduce errors due to subjectivity, and to provide more efficient use of clinicians' time. The aim of this research was to evaluate an automated system for landmarking of cephalograms based on the use of an active appearance model (AAM) that contains a statistical model of shape and grey-level appearance of an object of interest and represents both shape and texture variations of the region covered by the model.

Multi-resolution implementation was used, in which the AAM iterate to convergence at each level before projecting the current solution to the next level of the model. The AAM system was trained using 60 randomly selected, hand-annotated digital cephalograms of subjects between 7.2 and 25.6 years of age, and tested with a leave-five-out method that enabled testing not only of the accuracy of the AAM system but also the accuracy of each AAM. Differences between methods were examined using the non-parametric Wilcoxon signed rank test.

An average accuracy of 1.68 mm was obtained, with 61 per cent of landmarks detected within 2 mm and 95 per cent of landmarks detected within 5 mm precision. A noticeable increase in overall precision and detection of low-contrast cephalometric landmarks was achieved compared with other automated systems. These results suggest that the AAM approach can adequately represent the average shape and texture variations of craniofacial structures on digital radiographs. As such it can successfully be implemented for automatic localization of cephalometric landmarks.

Introduction

It was not until introduction of the cephalostat (Broadbent, 1931; Hofrath, 1931) that cephalometric analysis revolutionized diagnostics and treatment planning in orthodontics. For the first time, it was possible to analyze not only the dentoalveolar but also the underlying skeletal characteristics of the viscero- and neurocranium. Since then, it has become a standardized diagnostic method in everyday orthodontic practice and research.

Two approaches may be used for tracing lateral cephalograms: a manual approach and a computer-aided approach. The manual approach is the oldest and still most widely used and is carried out by placing a sheet of acetate paper over the cephalometric radiograph and manually tracing skeletal and soft tissue features, identifying landmarks, and measuring distances and angles between landmark locations. Computer-aided cephalometric analysis uses manually identified landmarks, based either on transferring landmarks from cephalometric radiographs with a digitizing pad connected to a computer, or direct landmark identification with a mouse cursor on the computer monitor (Baumrind and Miller, 1980; Richardson, 1981; Turner and Weerakone, 2001). The computer software then completes the cephalometric analysis by automatically measuring distances and angles.

It is widely acknowledged that both approaches are time-consuming and prone to errors, both systematic and random, due to problems and inconsistencies in features and landmark identification, drawing lines between landmarks and measuring with a ruler and protractor (Baumrind and Frantz, 1971; Kamoen *et al.*, 2001). The latter is successfully eliminated in computer-aided cephalometric analysis, but the greatest error still lies in landmark identification (Houston *et al.*, 1986; Nimkarn and Miles, 1995; Kamoen *et al.*, 2001). Variability in landmark identification has been determined to be five times greater than measurement variability, with both methods (Miller *et al.*, 1971; Savage *et al.*, 1987). In addition, the process is open to considerable subjectivity, since landmarks currently are defined using subjective criteria rather than strict mathematical specifications.

It is generally accepted that accuracy in landmark identification ideally should be less than 0.5 mm. However, measurements with errors within 2 mm are considered acceptable, and are often used as a reference to evaluate the recognition success rate, but the former level of precision is considered desirable (Forsyth and Davis, 1996). Research studies on the accuracy of landmark identification have also shown that manual landmark identification errors vary significantly depending on the landmark, observer, and quality of the radiograph (Cohen and Linney, 1986; Parthasarathy *et al.*, 1989). In some studies, the mean estimating error of expert landmarking identification has been reported to be 1.26 mm (Parthasarathy *et al.*, 1989).

There have been many attempts to further improve and automate cephalometric analysis. Computer systems, which automatically identify relevant skeletal and soft tissue structures and landmarks have the potential to increase accuracy, reduce errors due to subjectivity, provide more efficient use of clinician time, and improve the ability to correctly diagnose orthodontic cases.

The first attempt at automated landmarking of cephalograms was made by Cohen *et al.* (1984). Since then, automatic cephalometric analysis has been the subject of a number of studies, and the automatic identification of landmarks has been attempted by more than 20 independent researchers using different approaches and computer systems, with varying degrees of success. All these methods can be divided into three categories. The first, pure edge tracking, follows a strategy similar to that employed by clinicians and uses a combination of image processing techniques to detect and extract the important edges, subsequently used to locate landmarks on line crossings (Cohen *et al.*, 1984; Cohen and Linney, 1986; Lévy-Mandel *et al.*, 1986; Parthasarathy *et al.*, 1989; Davis and Taylor, 1991; Davis, 1994; Liu *et al.*, 2000). The second category, knowledge-based template matching methods, implements a grey-level model around each landmark to reduce the search area (Cardillo and Sid-Ahmed, 1994; Ren *et al.*, 1998; Rudolph *et al.*, 1998; Desvignes *et al.*, 2000; Hutton *et al.*, 2000; Grau *et al.*, 2001; Romaniuk *et al.*, 2002). The third category employs neural networks and fuzzy inference systems to locate the landmarks (Uchino and Yamakawa, 1995; Sanei *et al.*, 1999; Innes *et al.*, 2002; Ciesielski *et al.*, 2003; El-Feghi *et al.*, 2004).

The pure edge-based approach identifies pixels near the object boundaries. Boundaries are detected as areas with a high gradient value. Landmarks are then found in relation to these boundaries. Attempts to use this approach for automatic landmark identification have both experimental design flaws and very limited results. In most cases, the method was tested on the same set of radiographs used to develop the algorithm (Lévy-Mandel *et al.*, 1986; Parthasarathy *et al.*, 1989; Davis and Taylor, 1991; Liu *et al.*, 2000). Some of the studies used a very small number of radiographs (Lévy-Mandel *et al.*, 1986; Parthasarathy *et al.*, 1989). It was also observed that tested methods only worked on high quality images (Parthasarathy *et al.*, 1989). These heuristic methods are based on *ad hoc* rules for finding each specific landmark. The main problem is that the rules become increasingly difficult as more complex landmarks, structures, and variations in image quality and contrast are introduced. This may explain the limited accuracy of these algorithms and their inability to produce a potentially clinically applicable approach.

The knowledge-based template matching approach, also known as the learning approach, uses mathematical models to narrow down the search area for each landmark, subsequently applying various pattern-matching algorithms to pinpoint the exact location of the landmark (Cardillo and Sid-Ahmed, 1994; Uchino and Yamakawa, 1995; Ren *et al.*, 1998; Rudolph *et al.*, 1998; Desvignes *et al.*, 2000; Hutton *et al.*, 2000; Grau *et al.*, 2001; Romaniuk *et al.*, 2002). These methods have proved more accurate, especially in detecting complex landmarks with less contrast characteristics. They also produce consistently better results with radiographic images of varying quality. Nevertheless, overall accuracy is still far beyond applicability in everyday clinical practice and research.

The newer generation of knowledge-based systems make use of an additional statistical model that takes into account the variation of characteristics in the images. The first attempt was undertaken by Hutton *et al.* (2000), who applied active shape models (ASMs) to detect cephalometric landmarks. Those authors concluded that even though ASMs were not sufficiently accurate for clinical application, they should provide a model for future studies and a framework for further improvements.

Active appearance models (AAMs), recently proposed by Cootes *et al.* (2001), Cootes and Taylor (2001), and Stegmann (2004), modelling both shape and texture variability seen in a training set, should make the search more precise and robust.

The aim of this study was to evaluate the accuracy of a computerized automatic landmark identification system, based on the AAM approach.

Materials and methods

Experimental design

Sixty cephalograms were randomly selected from the records of patients who had attended for orthodontic assessment and treatment at the Orthodontic Department of the Clinic of Dentistry, Medical Faculty, University of Novi Sad, Serbia. The subjects were aged between 7.2 and 25.6 years (mean age 14.7 years; Table 1).

All the radiographs were taken on Soredex Cranex Tome Ceph digital X-ray machine (Soredex, Tuusula, Finland) using a phosphorus IP-plate (24 × 30 cm). The image plate was processed by a PCT-Digora medical image laser scanner (Soredex). This yielded images that were 2400 × 3000 pixels, giving a pixel size of 0.1 mm, with 256 grey levels in Bitmap format. According to visual assessment, the radiographs varied in quality from average

Table 1 Characteristics of image sample.

Characteristics	Number
Males	37
Females	23
Skeletal Class I	24
Skeletal Class II	27
Skeletal Class III	9
Normal face height	27
Short face height	16
Long face height	17

to high, and were overall considered of good rather than exceptional quality, and as such represented typical lateral cephalograms taken on a modern radiographic machine. By applying this non-selective method of sample collecting, it was hoped to obtain a wide range of variations of both morphological characteristics of skeletal and soft tissue structures and quality of the radiographs.

In order to compare the proposed system for automatic landmarking with previous studies, the images were reduced to 945 × 1181 pixels by pixel averaging. The pixel size in the resultant image was increased from 0.1 to 0.22 mm. The loss in accuracy due to this resolution reduction was considered negligible. The real impact of the full resolution AAMs on performance of the proposed method will be analyzed in future studies.

For building training sets and testing the accuracy of the algorithm, a modified drop-one-out scheme was used (Rudolph *et al.*, 1998; Hutton *et al.*, 2000). Instead of removing just one radiograph from the initial set of cephalograms, five radiographs that were later used for performance testing were removed. Thus, not only accuracy of the algorithm could be tested but also the accuracy of each AAM.

Building statistical models of shape and texture

The first step in building statistical models of appearance is data acquisition. The training set consists of annotated images, where key landmark points are marked on each example object. Suitable normalization is then undertaken after which the data are ready for analysis and can be described in terms of statistical models. The process is divided into three steps: capture, normalization, and analysis (Stegmann, 2000).

In total, 17 standard cephalometric landmarks and 114 pseudo-landmarks were used to define statistical models of shape and texture (Figure 1). For this purpose, the open C++ source code set of the AAM tools were partially modified and used in this study. Each of the 131 landmarks was manually identified by one observer on five occasions. The 'gold standard' (the closest assessment of a landmark position that can be achieved with existing technology and science) was defined as the mean of the five recordings. This gold standard was also used to assess and compare landmarking errors in both the automatic and manual approaches.

Given such a set, a statistical model of shape and texture variation can be generated by applying principal component analysis to the set of vectors describing the shapes and textures in the training set (Cootes *et al.*, 2001; Cootes and Taylor, 2001; Vucinic, 2006). The shape of an object can be represented as a vector x and the texture (or grey levels) as vector g. The appearance model has parameters c controlling the shape and texture according to:

$$x = \bar{x} + Q_s \cdot c,$$
$$g = \bar{g} + Q_g \cdot c,$$

where $\bar{x}$ is the mean shape, $\bar{g}$ the mean texture, and Q_s and Q_g the matrices describing the modes of variation derived from the training set.

Subsequently, a full synthetic image of modelled objects can be synthesized for a given c by generating a texture image from the vector g and warping it using the control points described by x (Figure 2).

AAM matching

To identify the landmarks on a new cephalogram, an initial template was placed over the image. The method described by Cootes *et al.* (2001) and Cootes and Taylor (2001) was used. AAM treat interpretation as an optimization problem in which it seeks to minimize the difference between the new image and the one synthesized by the appearance model. A multi-resolution implementation was used, in which AAMs iterate to convergence at each level before projecting the current solution to the next level of the model (Figure 3). This is more efficient and can converge to the correct solution

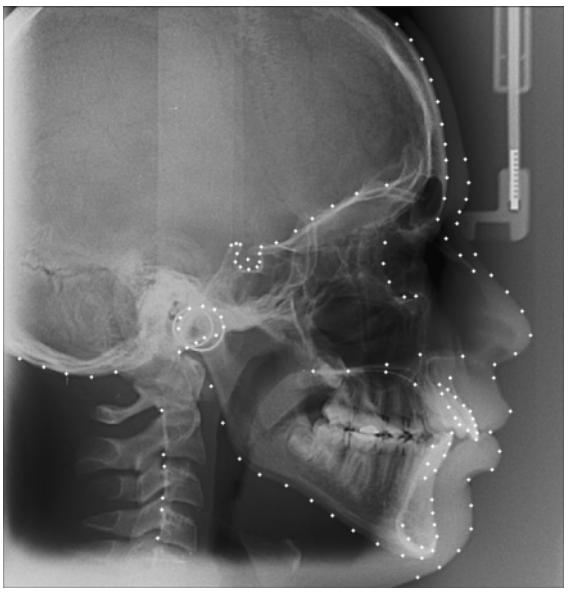

Figure 1 Example of cephalometric image annotated with 131 landmark points.

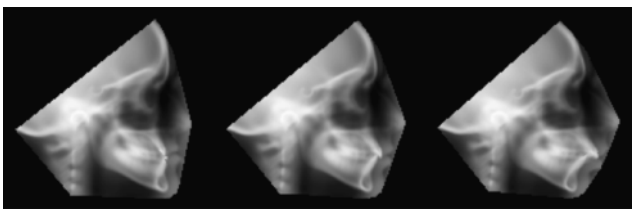

Figure 2 First mode of variation of an appearance model, describing some possible variations in both the shape and the texture component of the synthesized image, seen across 60 training images (Left, –28D; centre, mean; right, +28D).

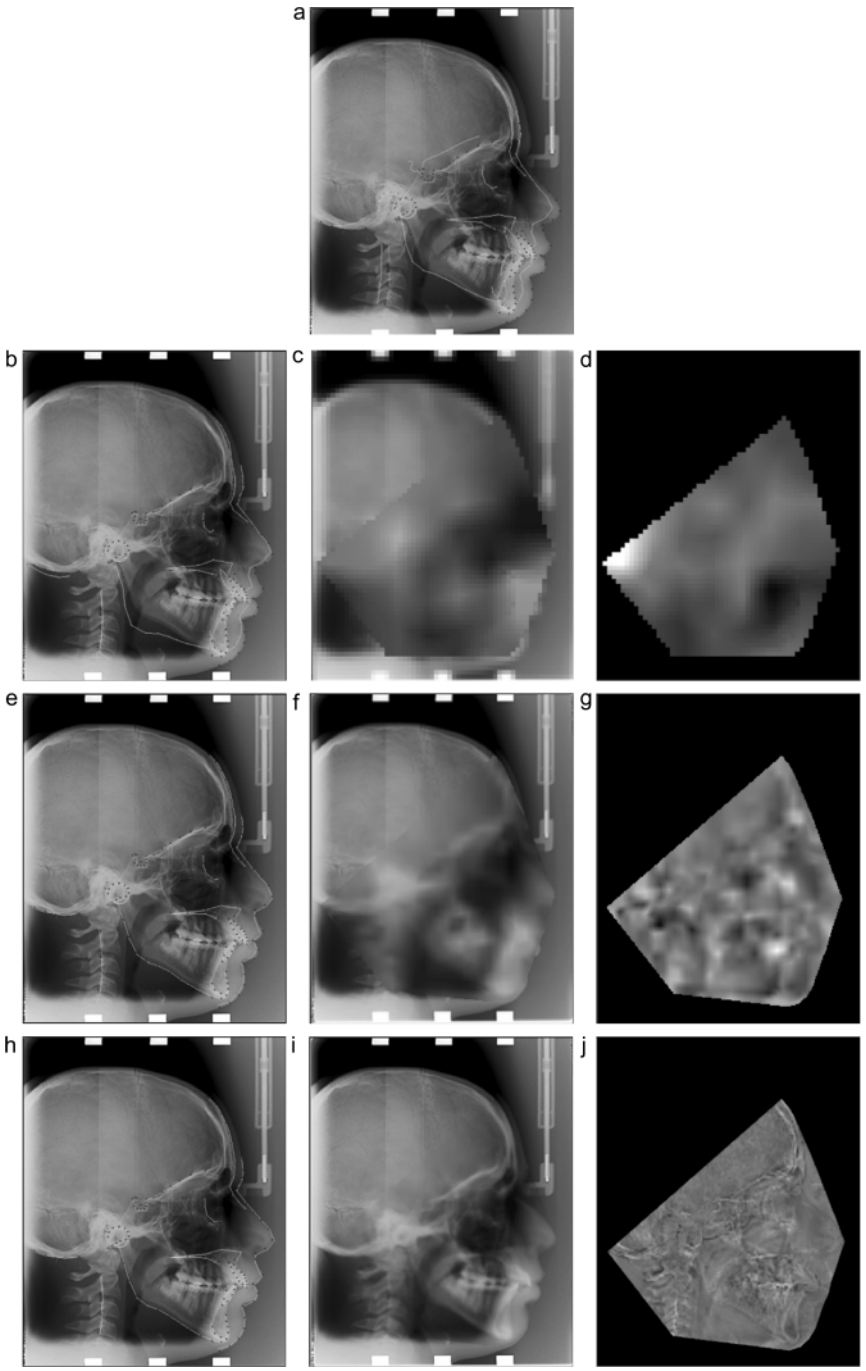

Figure 3 Multi-resolution active appearance model (AAM) search. (a) Initial positioning of the AAM; (b–g) AAM search through different resolution levels; (h–j) final convergence of the AAM and image.

faster and from further away than searching at a single resolution (Cootes *et al.*, 2001; Stegmann, 2004).

Model and method evaluation

The accuracy of each of the 60 templates was tested on five cephalograms that had not been used in the training phase, according to the leave-five-out methodology. For each template, training was undertaken on 55 cephalograms and testing on the five remaining cephalograms. In this way, performance of the AAM method was tested 300 times and its accuracy then estimated as the average error of all landmark detections.

Error in automatic landmark identification was calculated as the Euclidean, *x*- and *y*-axis distance from their manually determined position (gold standard). The bisecting line from the image of the cephalostat through the centre of the machine ear rod was defined as the *y*-axis, and the line perpendicular

to the *y*-axis through the centre of the machine ear rod as the *x*-axis. Thus, it was possible to evaluate if automatic landmark identification followed a certain envelope pattern, similar to manual detection. The identification of points subspinale (A) or supramentale (B), for example, is prone to error in the perpendicular rather than in the horizontal plane (Liu *et al.*, 2000).

Statistical analysis

Statistical evaluation using the Shapiro–Wilk test, frequency histogram, and normal probability plot confirmed that the collected data were not normally (Gaussian) distributed (Stevens and D'Agostino, 1986). Therefore, non-parametric statistical analysis was applied to the data: the average landmarking errors of repeat measurements for each method were determined using the median value and 80th percentile as a measure of spread. Differences between methods were examined using the non-parametric Wilcoxon signed rank test (Conover, 1980; Turner and Weerakone, 2001). Statistical analyses were performed using the Analyse-it for Microsoft Excel, version 1.62 (Analyse-it Software, Ltd, Leeds, UK) and the Statistical Package for Social Sciences, version 13.00 (SPSS, Inc., Chicago, Illinois, USA).

Error of the method

Ten cephalograms were randomly selected and the gold standard for all the landmarks reassessed 1 month after the original recordings. As recommended by Houston (1983) and Battagel (1993), the error of the method was assessed for random error using Dahlberg's formula. A paired *t*-test was also performed to assess systematic error. Dahlberg's values demonstrated that random error ranged from 0.11 to 0.35 mm for manual landmark identification. A paired *t*-test of the repeated measures showed no systematic errors.

Results

Average Euclidean, *x*- and *y*-axis landmarking errors for manual, and automatic landmark identification are shown in Tables 2 and 3. Best recognition performances were for Apex inferior (Ap1l) and Supramental (B) and the lowest were for Porion (Po) and Articulare (Ar). These results were compared with the manual method (Table 4) and with those obtained in previous studies (Tables 5–7). The overall success rate for all landmark detection attempts for automatic recognition was 28 per cent within 1 mm, 61 per cent within 2 mm, and 95 per cent within 5 mm precision (radii).

Discussion

Unlike previously tested methods (edge tracking and the ASM approach) where the search is made around the current position of each point using models of the image texture in small regions around each landmark, the AAM manipulates a full model of appearance, which represents both shape variation and the texture of the region covered by the model. This was used to generate full synthetic images of the modelled objects. AAM then uses the difference between the current synthesized image and the target image to update its parameters (Cootes *et al.*, 1999). After initial testing of the system, the shape-based modification of the AAM algorithm was used, in which linear shape update is predicted from current texture error, since it was able to locate the points slightly more accurately than the original formulation.

The manual method for landmark detection is still considered the most accurate. Differences in accuracy between the manual and proposed method varied from 0.17 to 3.06 mm. It was negligible for three of the 17 landmarks

Table 2 Median and 80th percentile values of landmarking errors for manual landmark identification (in mm).

Cephalometric landmarks	Median (Euclidean)	Minimum	Maximum	80th percentile	Median (*x*-axis)	Median (*y*-axis)
Sella (S)	0.18	0.02	0.45	0.29	0.07	0.12
Nasion (N)	0.38	0.06	3.83	1.36	0.25	0.29
Porion (Po)	0.21	0.03	2.87	0.51	0.20	0.05
Orbitale (Or)	0.24	0.07	2.10	0.70	0.18	0.10
Subspinale (A)	0.92	0.09	16.62	3.30	0.16	0.55
Anterior nasal spine (ANS)	0.47	0.06	8.56	1.12	0.34	0.13
Posterior nasal spine (PNS)	0.40	0.07	2.52	0.78	0.29	0.15
Supramentale (B)	0.80	0.06	4.25	1.52	0.09	0.79
Pogonion (Pg)	0.23	0.00	1.16	0.51	0.04	0.22
Gnathion (Gn)	0.34	0.02	1.28	0.58	0.19	0.17
Menton (Me)	0.66	0.09	2.57	1.02	0.59	0.15
Gonion (Go)	0.83	0.09	4.67	1.62	0.45	0.65
Articulare (Ar)	0.36	0.06	4.93	1.49	0.21	0.28
Incision superior (Is1u)	0.14	0.03	1.48	0.22	0.10	0.07
Apex superior (Ap1u)	1.07	0.09	3.19	1.64	0.38	0.85
Incision inferior (Is1l)	0.19	0.02	2.94	0.30	0.10	0.12
Apex inferior (Ap1l)	0.90	0.22	4.88	2.37	0.44	0.70
X (average)	0.49	0.06	4.02	1.14	0.23	0.32

Table 3 Median and 80th percentile values of landmarking errors for automatic landmark identification (in mm).

Cephalometric landmarks	Median (Euclidean)	Minimum	Maximum	80th percentile	Median (*x*-axis)	Median (*y*-axis)
Sella (S)	1.87	0.12	9.13	3.27	1.24	0.91
Nasion (N)	1.42	0.04	13.34	2.66	0.94	0.99
Porion (Po)	3.27	0.14	9.46	4.69	2.12	1.73
Orbitale (Or)	2.22	0.22	6.44	3.81	1.59	1.05
Subspinale (A)	1.41	0.03	12.27	3.09	0.62	1.01
Anterior nasal spine (ANS)	1.99	0.14	11.75	3.49	1.31	1.00
Posterior nasal spine (PNS)	1.56	0.02	8.76	2.46	0.81	0.92
Supramentale (B)	1.20	0.04	9.77	2.15	0.59	0.68
Pogonion (Pg)	1.33	0.17	9.54	2.41	0.71	0.84
Gnathion (Gn)	1.31	0.04	9.39	2.34	0.74	0.76
Menton (Me)	1.23	0.09	10.49	2.33	1.01	0.45
Gonion (Go)	2.13	0.13	9.94	3.58	1.15	1.15
Articulare (Ar)	2.31	0.36	6.96	3.56	1.31	1.28
Incision superior (Is1u)	1.34	0.09	15.19	2.22	0.84	0.85
Apex superior (Ap1u)	1.72	0.03	12.90	2.58	0.66	1.23
Incision inferior (Is1l)	1.24	0.06	10.45	2.56	0.93	0.72
Apex inferior (Ap1l)	1.07	0.07	12.48	1.93	0.62	0.70
X (average)	1.68	0.11	10.49	2.89	1.01	0.96

Table 4 Wilcoxon signed rank test comparing manual and automatic landmark identification.

Cephalometric landmarks	Difference (mm)	Wilcoxon test	*P*	
Sella (S)	1.70	1829	<0.0001	***
Nasion (N)	1.04	1506	<0.0001	***
Porion (Po)	3.06	1829	<0.0001	***
Orbitale (Or)	1.98	1816	<0.0001	***
Subspinale (A)	0.49	1074	0.2418	NS
Anterior nasal spine (ANS)	1.53	1435	0.0001	***
Posterior nasal spine (PNS)	1.16	1674	<0.0001	***
Supramentale (B)	0.40	1139	0.0991	NS
Pogonion (Pg)	1.10	1774	<0.0001	***
Gnathion (Gn)	0.97	1702	<0.0001	***
Menton (Me)	0.57	1547	<0.0001	***
Gonion (Go)	1.31	1645	<0.0001	***
Articulare (Ar)	1.94	1679	<0.0001	***
Incision superior (Is1u)	1.19	1819	<0.0001	***
Apex superior (Ap1u)	0.65	1266	0.0098	**
Incision inferior (Is1l)	1.05	1693	<0.0001	***
Apex inferior (Ap1l)	0.17	893	0.8713	NS

**$P < 0.01$; ***$P < 0.001$; NS, not significant.

(subspinale, supramentale, and apex inferior; Table 4). On average, for all the cephalometric landmarks, the proposed system had a precision of 1.68 mm, which is a considerable improvement with regard to other complementary automatic systems (Table 5; Rudolph *et al.*, 1998; Hutton *et al.*, 2000; Liu *et al.*, 2000; Grau *et al.*, 2001). However, care must be taken when comparing and interpreting such results, since the training data and validation data differs for all published studies.

Taking into account the nature of multivariate linear regression, it is anticipated that using a larger number of sample images for model training and building will lead to a better prediction model and consequently more accurate results. Therefore, the real impact of increasing the number of training images (potentially capturing more variations of morphological structures of the human skull and quality of cephalograms) on the performance of the proposed method requires further investigation. Considering that the accepted normal range of error for most cephalometric measurements is approximately ±2 mm and that inter-expert variability can vary up to 5 mm (Liu *et al.*, 2000), these results justify the potential of the studied method for clinical application. The AAM approach provides the opportunity for building more robust and precise systems for automatic landmark detection, as suggested by Hutton *et al.* (2000).

The magnitude of error in landmark identification depends on the position of the landmark. If the landmark is in a clear border of the craniofacial structure, such as sella (S) or pogonion (Pg), the error will be smaller. On the other hand, if the landmark is located on poorly defined structures which have a low signal to noise ratio, with many craniofacial structures overlying each other, such as porion (Po) and orbitale (Or), the error will be larger (Baumrind and Frantz, 1971). Experienced clinicians may be able to infer the position of landmarks from their background knowledge of cephalometry, even poorly defined ones, whereas the automatic systems are still unable to compete in this capacity. However, the results of the present research showed a significant improvement in locating low-contrast landmarks over other methods (Table 7), except the approaches presented by Grau *et al.* (2001) and Liu *et al.* (2000), but their systems were tested on a very small number of low resolution radiographs, making the results statistically unreliable. This can be attributed

Table 5 Comparison of the average automatic landmarking errors (mm) in different studies.

Cephalometric landmarks	Rudolph *et al.* (1998)	Hutton *et al.* (2000)	Liu *et al.* (2000)	Grau *et al.* (2001)	Present study
Sella (S)	5.06	5.5	0.94	1.92	1.87
Nasion (N)	2.57	5.6	2.32	1.40	1.42
Porion (Po)	5.67	7.3	2.43	—	3.27
Orbitale (Or)	2.46	5.5	5.28	1.92	2.22
Subspinale (A)	2.33	3.3	4.29	0.90	1.41
Anterior nasal spine (ANS)	2.64	3.8	2.90	0.75	1.99
Posterior nasal spine (PNS)	—	5.0	—	1.13	1.56
Supramentale (B)	1.85	2.6	3.69	—	1.20
Pogonion (Pg)	1.85	2.7	2.53	0.95	1.33
Gnathion (Gn)	—	2.7	1.74	1.44	1.31
Menton (Me)	3.09	2.7	1.90	0.48	1.23
Gonion (Go)	—	5.8	4.53	1.10	2.13
Articulare (Ar)	—	—	—	—	2.31
Incision superior (Is1u)	2.02	2.9	2.36	0.84	1.34
Apex superior (Ap1u)	2.17	2.9	—	0.89	1.72
Incision inferior (Is1l)	2.46	3.1	2.86	0.90	1.24
Apex inferior (Ap1l)	2.67	3.9	—	0.54	1.07
X (average)	2.83	4.08	2.91	1.08	1.68

Table 6 Comparison of the overall success rate of all landmark detection attempts, within 1, 2, and 5 mm precision (radii) of the active shape model (ASM) and active appearance model (AAM) approach.

	Present study		Hutton *et al.* (2000) automatic ASM %
	Manual %	Automatic AAM %	
<1 mm	72	28	13
<2 mm	87	61	35
<5 mm	98	95	74

to the fact that cephalometric images are very rich in subtle grey-level variations and in such cases an appearance model can represent both the shape and texture variability seen in a training set, better than edge-tracking methods.

The pattern of errors for most landmarks was similar to that found with manual tracing. Distribution of detection attempts for landmarks located on edges follows the shape of the border of the craniofacial structures. Landmarks lying on vertical borders, such as nasion (Na), are more accurately located in the horizontal dimension as opposed to the vertical dimension (Figure 4). Similarly, landmarks lying on horizontal edges are more accurately located in the vertical dimension. This is in accordance with the findings of Forsyth and Davis (1996).

Example of failure

Figure 5 shows an example where the AAM failed to locate boundaries correctly on images used for testing. In this case, the subject showed a more extreme shape variation from the mean. Due to this variation, the model was not able to locate the outer boundaries. This is because the model only samples the image under its current location and within variation limits. There is not always sufficient information to drive the model outward to the correct outer boundary. This can be overcome by modelling the whole surface of the radiograph or by using a larger number of sample images for model training that will capture even these extreme variations. Alternatively, it may be possible to combine different methods and include explicit searching outside the current patch, for instance by searching along normal to current boundaries as in the ASM (Cootes and Taylor, 2001).

Conclusions

Based on the obtained results, the following conclusions were made:

1. The AAM approach can adequately represent the average shape and texture variations of craniofacial structures on digital radiographs. As such it can successfully be implemented for automatic localization of cephalometric landmarks.
2. An increase in overall precision and detection of low-contrast cephalometric landmarks was achieved in comparison with other automatic systems.

Considering the established potentials and advantages of the AAM, it is expected that by creating more precise statistical models of shape and texture (based on a larger number of radiographs), and refining the AAM algorithm in the final adaptation phase, it will be possible to use it as a completely automatic system for automatic detection of cephalometric landmarks.

Table 7 Comparison of mean landmarking errors (mm) of low (L)- and high (H)-contrast cephalometric landmarks.

	Rudolph *et al.* (1997)		Rudolph *et al.* (1998)		Liu *et al.* (2000)		Hutton *et al.* (2000)		Grau *et al.* (2001)		Present study	
	L	H	L	H	L	H	L	H	L	H	L	H
Mean error	4.30	2.65	3.85	2.56	2.88	2.91	5.02	3.52	1.32	0.91	2.03	1.47
Difference	1.65		1.29		−0.03		1.50		0.41		0.56	

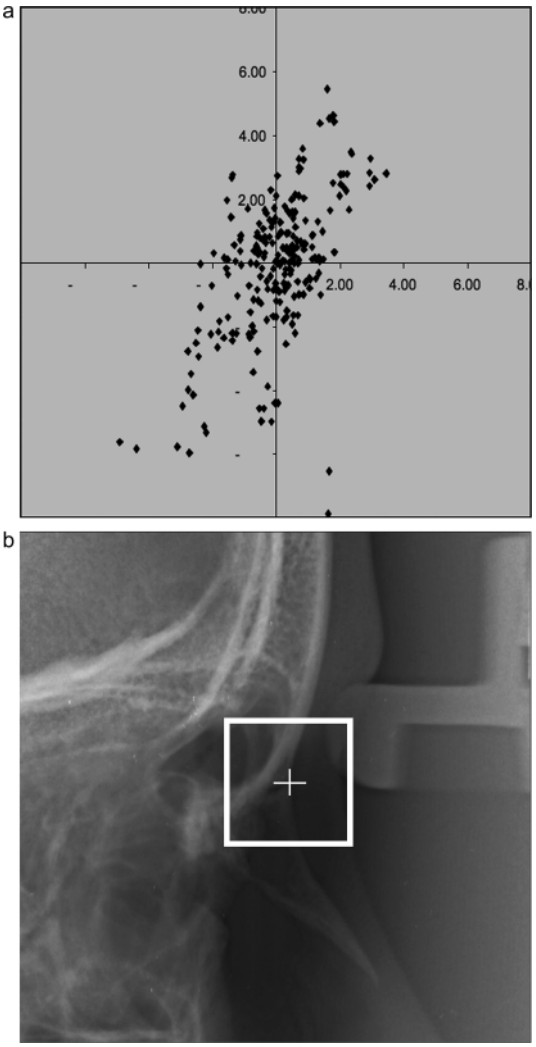

Figure 4 Distribution of automatic detection attempts (a) for the landmark nasion (N) (b).

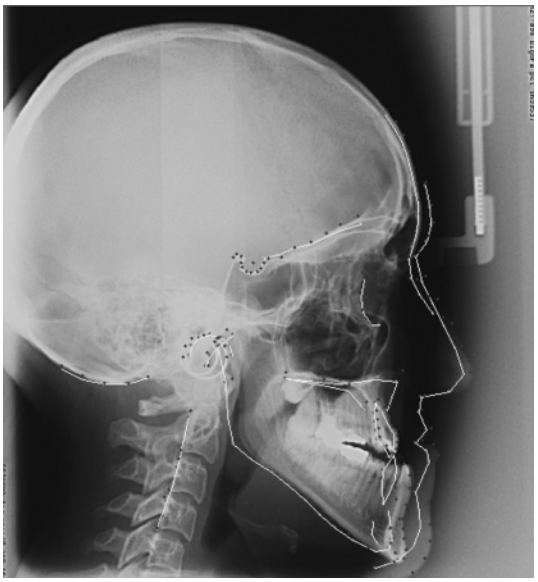

Figure 5 An example of a search failure, where the active appearance model did not locate the correct outer boundaries of the lower jaw.

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References

Battagel J M 1993 A comparative assessment of cephalometric errors. European Journal of Orthodontics 15: 305–314

Baumrind S, Frantz R C 1971 The reliability of head film measurements. 1. Landmark identification. American Journal of Orthodontics 60: 111–127

Baumrind S, Miller D M 1980 Computer-aided head film analysis: the University of California San Francisco method. American Journal of Orthodontics 78: 41–65

Broadbent H B 1931 A new X-ray technique and its application to orthodontia. Angle Orthodontist 1: 45–66

Cardillo J, Sid-Ahmed M A 1994 An image processing system for locating craniofacial landmarks. IEEE Transactions on Medical Imaging 13: 275–289

Ciesielski V, Vic A, Innes A, John S, Mamutil J 2003 Genetic programming for landmark detection in cephalometric radiology images. International Journal of Knowledge-Based and Intelligent Engineering Systems 7: 164–171

Cohen A M, Linney A D 1986 A low cost system for computer-based cephalometric analysis. British Journal of Orthodontics 13: 105–108

Cohen A M, Ip H H, Linney A D 1984 A preliminary study of computer recognition and identification of skeletal landmarks as a new method of cephalometric analysis. British Journal of Orthodontics 11: 143–154

Conover W J 1980 Practical non-parametric statistics, 2nd edn. Wiley, New York

Cootes T F, Taylor C J 2001 Statistical models of appearance for medical image analysis and computer vision. In: Proceedings of the Conference on Medical Imaging. SPIE Proceedings Series, San Diego, 4322: 236–248

Cootes T F, Edwards G J, Taylor C J 1999 Comparing active shape models with active appearance models. In: Proceedings of the 10th British Machine Vision Conference 1: BMVA Press, Nottingham, pp. 173–182

Cootes T F, Edwards G J, Taylor C J 2001 Active appearance models. IEEE Transactions on Pattern Analysis and Machine Intelligence 23: 681–685

Davis D 1994 Knowledge-based cephalometric analysis: a comparison with clinicians using interactive computer methods. Computers and Biomedical Research 27: 10–28

Davis D N, Taylor C J 1991 A blackboard architecture for automating cephalometric analysis. Journal of Medical Informatics 16: 137–149

Desvignes M, Romaniuk B, Demoment R, Revenu M, Deshayes M J 2000 Computer assisted landmarking of cephalometric radiographs. In: Proceedings of the Fourth IEEE Southwest Symposium on Image Analysis and Interpretation. IEEE Computer Society, SSIAI, Austin, pp. 296–300

El-Feghi I, Sid-Ahmed M A, Ahmadi A 2004 Automatic localization of craniofacial landmarks for assisted cephalometry. Pattern Recognition 37: 609–621

Forsyth D B, Davis D N 1996 Assessment of an automated cephalometric analysis system. European Journal of Orthodontics 18: 471–478

Grau V, Alcañiz M, Juan M C, Monserrat C, Knoll C 2001 Automatic localization of cephalometric landmarks. Journal of Biomedical Informatics 34: 146–156

Hofrath H 1931 Die Bedeutung der Röntgenfern und Abstandsaufnahme für die Diagnostik der Kieferanomalien. Fortschritte der Kieferorthopdiäe 1: 232–258

Houston W J B 1983 The analysis of errors in orthodontic measurements. American Journal of Orthodontics 83: 382–390

Houston W J B, Mahler R E, McElroy D, Sherriff M 1986 Sources of error in measurements from cephalometric radiographs. European Journal of Orthodontics 8: 149–151

Hutton T J, Cunningham S, Hammond P 2000 An evaluation of active shape models for the automatic identification of cephalometric landmarks. European Journal of Orthodontics 22: 499–508

Innes A, Ciesielski V, Mamutil J, Sabu J 2002 Landmark detection for cephalometric radiology images using pulse coupled neural networks. In: Proceedings of the International Conference on Artificial Intelligence. CSREA Press, Las Vegas, 2: 511–517

Kamoen A, Dermaut L, Verbeeck R 2001 The clinical significance of error measurement in the interpretation of treatment results. European Journal of Orthodontics 23: 569–578

Lévy-Mandel A D, Venetsanopoulos A N, Tsotsos J K 1986 Knowledge-based landmarking of cephalograms. Computers and Biomedical Research 19: 282–309

Liu J K, Chen Y T, Cheng K S 2000 Accuracy of computerized automatic identification of cephalometric landmarks. American Journal of Orthodontics and Dentofacial Orthopedics 118: 535–540

Miller R L, Dijkman D J, Riolo M L, Moyers R E 1971 Graphic computerization of cephalometric data. Journal of Dental Research 50: 1363–1369

Nimkarn Y, Miles P G 1995 Reliability of computer-generated cephalometrics. International Journal of Adult Orthodontics and Orthognathic Surgery 10: 43–52

Parthasarathy S, Nugent S T, Gregson P G, Fay D F 1989 Automatic landmarking of cephalograms. Computers and Biomedical Research 22: 248–269

Ren J *et al.* 1998 A knowledge-based automatic cephalometric analysis method. Proceedings of the 20th International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Computer Society, Hong Kong, 20: 723–727

Richardson A 1981 A comparison of traditional and computerized methods of cephalometric analysis. European Journal of Orthodontics 3: 15–20

Romaniuk B, Desvignes D, Revenu M, Deshayes M J 2002 Linear and non-linear model for statistical localization of landmarks. In: Proceedings of the 16th International Conference on Pattern Recognition 4: IEEE Computer Society, Washington, p. 40393

Rudolph D J, Coggins J M, Moon H 1997 Investigation of filter sets for supervised pixel classification of cephalometric landmarks by spatial spectroscopy. International Journal of Medical Informatics 47: 183–191

Rudolph D J, Sinclair P M, Coggins J M 1998 Automatic computerized radiographic identification of cephalometric landmarks. American Journal of Orthodontics and Dentofacial Orthopedics 113: 173–179

Sanei S, Sanaei P, Zahabsaniei M 1999 Cephalogram analysis applying template matching and fuzzy logic. Image and Vision Computing 18: 39–48

Savage A W, Showfety K J, Yancey J 1987 Repeated measures analysis of geometrically constructed and directly determined cephalometric points. American Journal of Orthodontics and Dentofacial Orthopedics 91: 295–299

Stegmann M 2000 Active appearance models: theory, extensions and cases. Thesis, University of Denmark, Lingby, pp. 37–39

Stegmann M 2004 Generative interpretation of medical images. Thesis, University of Denmark, Lingby

Stevens M A, D'Agostino R B 1986 Goodness of fit techniques. Marcel Dekker, Inc., New York

Turner P J, Weerakone S 2001 An evaluation of the reproducibility of landmark identification using scanned cephalometric images. Journal of Orthodontics 28: 221–229

Uchino E, Yamakawa T 1995 High speed fuzzy learning machine with guarantee of global minimum and its application to chaotic system identification and medical image processing. In: Proceedings of the Seventh International Conference on Tools with Artificial Intelligence. IEEE Computer Society, Herndon, pp. 242–249

Vučinić P 2006 Primena metode aktivnog modela oblikovanja u oblasti automatske identifikacije rendgenkefalometrijskih tacaka (Application of active appearance models for the automatic cephalometric landmarks recognition). Thesis, University of Novi Sad, Serbia

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Accuracy of cephalometric landmarks on monitor-displayed radiographs with and without image emboss enhancement

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SUMMARY The aim of this study was to evaluate the accuracy of some commonly used cephalometric landmarks of monitor-displayed images with and without image emboss enhancement. The following null hypothesis was tested: there is no improvement in landmark detection accuracy between monitor-displayed images, with and without image embossing enhancement.

Forty lateral cephalometric radiographs, taken from the data files of subjects were used in this study. A purpose-made software allowed recording of the cephalometric points and then, with the help of algorithms based on cellular neural networks, to transfer the previously processed radiographs into an embossed image. Five observers recorded 22 landmarks on the displayed images from the two image modalities, i.e. monitor-displayed radiograph (mode A) and monitor-displayed embossed radiograph (mode B). The positions of the landmarks were recorded and saved in the format of *x* and *y* co-ordinates and as Euclidean distance. The mean errors and standard deviation of landmarks location according to the two modalities were compared with the 'best estimate' for each landmark and the values were calculated for each of the 22 landmarks. One-way analysis of variance was then used to evaluate any statistically significant differences.

Euclidean distance mean errors were higher for the embossed images (except for Po) than for the unfiltered radiographs. These differences were all statistically significant ($P<0.05$) except for Or, Po, PM, Co, APOcc, and PPOcc. On the *x* and *y* co-ordinates, the accuracy of the cephalometric landmark detection improved on the embossed radiograph but only for a few points (Or on *x* axis and Po, PM, Co, and APOcc on *y* axis), as these were not statistically significant. The use of radiographic enhancement techniques, such as embossing, does not improve the level of accuracy for cephalometric points detection. Unless more precise algorithms are designed, this feature should not be used for clinical or research purposes.

Introduction

Image enhancements are a collection of processing techniques that seek to improve the visual appearance of digital images or transform the image into one more amenable to human and machine analysis. Enhancement techniques include: window and level selection, gamma correction, contrast manipulation, edge enhancement, subtraction, colourization, and embossing or three-dimensioanl (3D) reconstruction (Kogutt *et al.*, 1988; Crozier 1999; Menig 1999).

Embossing is the process of creating a 3D image starting from a two-dimensional (2D) image. Applying an embossing filter to an image often results in an image resembling paper or metal embossing of the original image, hence the name. The image obtained has sharpened edges and is graphically pleasing (Wiesemann *et al.*, 2006).

The use of enhancement techniques has proved to be beneficial in some radiographic applications (Jackson *et al.*, 1985; Kogutt *et al.*, 1988; Wiesemann *et al.*, 2006).

On this assumption, several software programs for cephalometric analyses have included sophisticated algorithms for image enhancement and facilitation of points for identification.

However, the use of enhancement algorithms for cephalometry has been questioned. In fact, even if they reduce random errors associated with landmark identification, the validity of the landmark may not be correct because of the introduction of systematic errors caused by the post-processing algorithms (Forsyth *et al.*, 1996; Menig, 1999).

Some of the drawbacks in these image enhancement techniques have been described, such as the enlarging tool (Jackson *et al.*, 1985; Kogutt *et al.*, 1988) and the edge enhancement technique (Forsyth *et al.*, 1996; Menig, 1999). However, little data are available on the clinical usefulness of digital cephalograms with emboss enhancement, even if it is perceived to improve clarity of cephalometric anatomical landmarks (Wiesemann *et al.*, 2006).

If enhancements are intended to reduce errors, increase accuracy, and simplify the process of extracting information, the enhanced images must provide perceptual information more suitable for locating landmarks than the original. Therefore, the aim of this study was to evaluate the accuracy of some commonly used cephalometric landmarks on monitor-displayed images with image emboss enhancement

and to compare findings with data obtained on the same monitor-displayed radiograph without any enhancement. The following null hypothesis was tested: there is no improvement in landmark detection accuracy between monitor-displayed images, with and without embossing.

Materials and methods

Forty lateral cephalometric radiographs, randomly selected from the data files of subjects attending the Department of Orthodontics, Catania University Hospital, were used in this study. The gender, type of occlusion, and skeletal pattern of the patients were not taken into consideration in the study design. The subjects were aged between 9 and 15 years of age (mean 13.9 years). Exclusion criteria were obvious malpositioning of the head in the cephalostat, unerupted or missing incisors and first molars, no unerupted or partially erupted teeth that would hinder landmark identification, patients with severe craniofacial deformities, and posterior teeth not in maximum intercuspation. Sample collection was approved by the University of Catania Research Ethics Committee and informed consent was obtained from each patient's parents before the study.

A power analysis suggested that a sample size of 40 radiographs was sufficient to evaluate significant differences in the accuracy of landmark detection with the two methods. In particular, the sample size ($N=40$) was chosen in such a way to obtain a power for the analysis of variance (ANOVA) test greater or equal to 0.8 for an estimated variance in landmark error equal to 0.1 mm and an effect size (difference between the mean with and without embossing) greater or equal to 0.065.

The cephalometric radiographs were scanned (Epson Expression 1680 Twain 2.10 Pro; Epson Italia S.p.A., Cinisello Balsamo, Italy) at a resolution of 300 dpi with 256 grey levels to transform the analogue image into a digital format using a scanner and stored in a PC (Intel Pentium IV, 3,2 GH with 2 GB RAM, 300 GB Hard Disk; ASUSTeK Computer Incorporated, Taipei, Taiwan) equipped with purpose-made software for cephalometric landmark recording. The software was designed and implemented in Borland C++ version 5.0 (Borland Software Corporation, Austin, Texas, USA) and allowed the recording of cephalometric points according to two modalities (mode A and mode B). Mode A consisted in landmarking the radiograph, which was shown on the screen without any kind of enhancement. In mode B, the software processed the same radiograph with algorithms based on cellular neural networks (CNNs; Giordano and Maiorana, 2007) and transformed it into an embossed image (Figure 1). The CNN is an unsupervised neural network that is computationally equivalent to a Turing machine and does not require training. By setting the values of two matrices, known as 'feedback' and 'control' templates, it is possible to implement any algorithm to manipulate the image (e.g. image filtering operations such as edge enhancement, embossing, morphological operations, etc.).

Prior to the study, the digitizer was checked for its accuracy according to a previous description (Macrì and Wenzel, 1993).

Twenty-two commonly used cephalometric landmarks were included in this analysis. Agreement between the five evaluators was reached on the definitions of landmarks before carrying out this study, and these written definitions for each landmark (Table 1) were given to evaluators. The observers were five orthodontists who were postgraduate trainers from the Orthodontic Department. The five observers recorded the 22 landmarks on the images displayed on the monitor from the two image modalities.

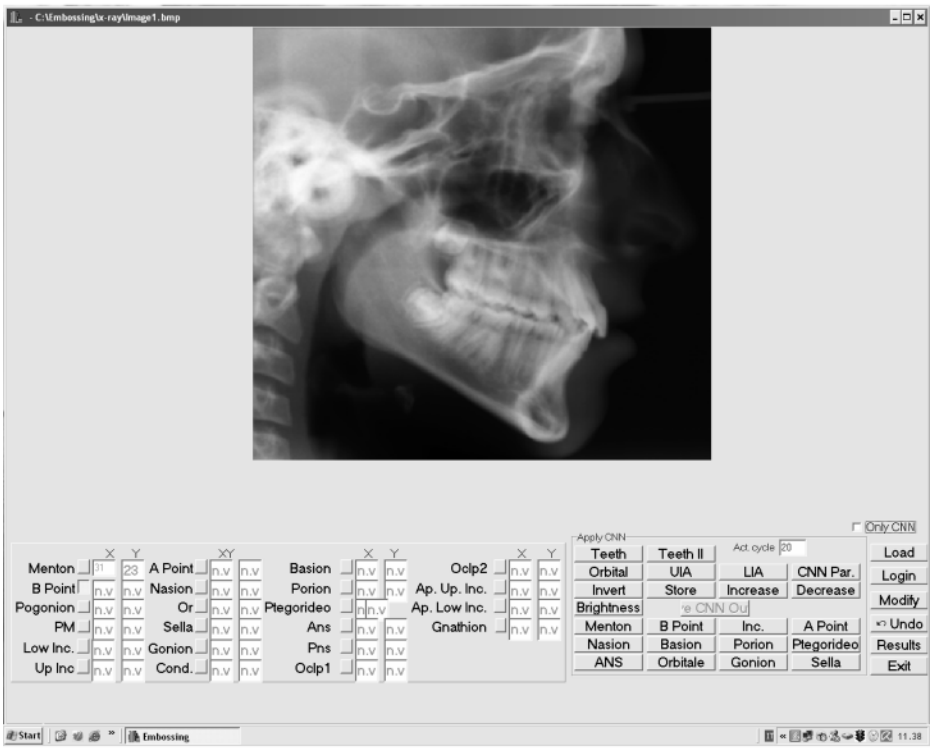
No more than 10 radiographs were traced in a single session to minimize errors due to examiner fatigue. Therefore, landmarking was carried out in eight sessions (40 images for mode A and 40 for mode B), with at least a 2 week interval between sessions. All recording sessions was performed in a dark room, the only available light being from the PC monitor. A 19 inch flat thin-film transistor screen (Samsung SyncMaster 913 V) set to an average resolution of 1280×1024 pixels, with bandwidths between 60 and 75 HZ, and a dot pitch of 0.294 mm, with standard setting: 80 per cent for contrast and 20 per cent for brightness. Landmark identification and recording directly on the monitor-displayed image was carried out with a mouse-controlled cursor. This cursor consisted of an arrow, and when a landmark was recorded, a red dot appeared on the screen over the selected pixel. The landmark position could be corrected until the operator was satisfied. Reference lines and perpendicular lines necessary to help identification appeared automatically on request. No time constraint was given to the users.

The positions of the landmarks were recorded and saved in the format of x and y co-ordinates with an origin fixed to one given pixel. For these monitor-displayed images, the construction of a x–y co-ordinate system was not necessary as the digital image consists of a pattern of rows and columns (the matrix) with an evenly spaced number of pixels in a known reference grid. The x and y co-ordinates were further analysed to evaluate the pattern of recording differences in the horizontal and vertical directions.

The mean x and y co-ordinate positions for each of 22 landmarks identified by the five observers, for the two modalities (mode A and mode B), were calculated and defined as the best estimate for that particular landmark in a given image. This best estimate was used to determine the inter-observer errors in both modalities, i.e. the digital image shown on the screen with and without image enhancement.

The mean distances in millimetres between the best estimate for each landmark and the mean of five locations identified by the five observers according to the two modalities were defined as inter-observer error. These were used as the variable determining accuracy for each landmark, with and without image enhancement.

a)



b)

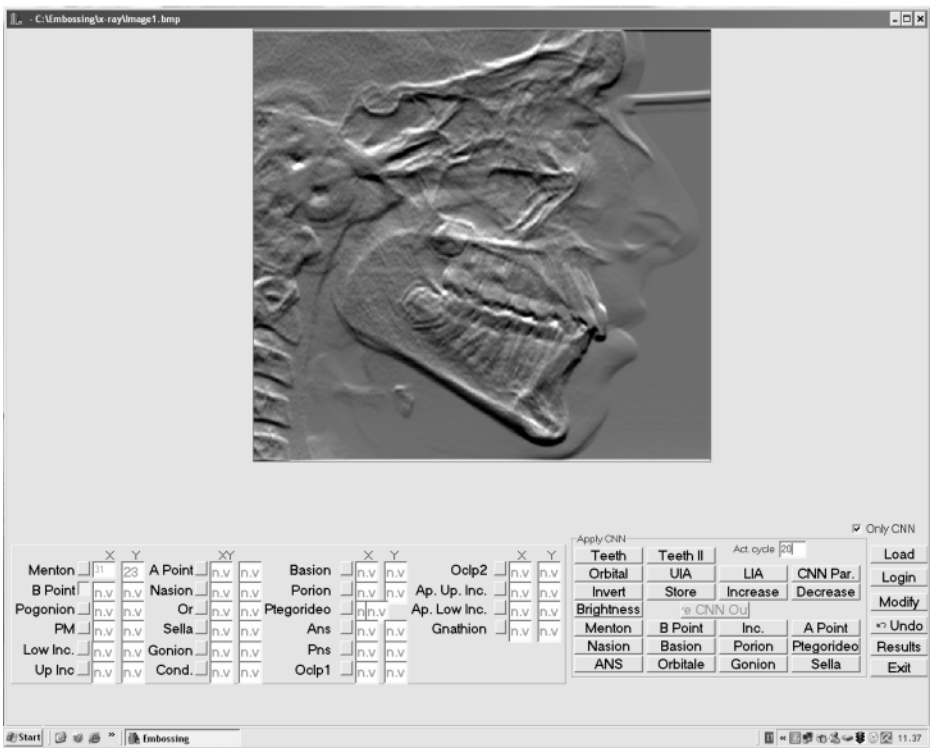


Figure 1 The interface of the landmarking tool: (a) an unfiltered and (b) an embossed radiograph.

Table 1 Definitions of landmarks.

Landmarks		
Name	Abbreviation	Definition
Nasion	Na	A point at the anterior limit of the nasofrontal suture.
Sella	S	Midpoint of the pituitary fossa as determined by inspection.
Orbitale	Or	A point located at the lowest point on the external border of the orbital cavity.
Porion	Po	A point located at the most superior point of the external auditory meatus.
Basion	Ba	The most inferior posterior point of the occipital bone at the anterior margin of the occipital foramen.
Pterygoid point	Pt	The intersection of the inferior border of the foramen rotundum with the posterior wall of the pterygomaxillary fissure.
Anterior nasal spine	ANS	Tip of anterior nasal spine.
Subspinale	A	The deepest point of the curve of the maxilla between ANS and the dental alveolus.
Posterior nasal spine	PNS	Tip of posterior nasal spine.
Supramentale	B	The deepest midline point on the mandible between infradentale and pogonion.
Protuberance menti or supra pogonion	PM	A point selected where the curvature of the anterior border of the symphysis changes from concave to convex.
Pogonion	Pg	Most anterior point on the midsagittal symphysis.
Gnathion	Gn	The most downward and forward point on the symphysis.
Menton	Me	The lowest point of the contour of the mandibular symphysis.
Gonion	Go	Intersection of the line connecting the most distal aspect of the condyle to the distal border of the ramus (ramus plane) and the line at the base of the mandible (mandibular plane).
Condylion	Co	The most postero-superior point on the outline of the mandibular condyle.
Upper incisor edge	UIE	Midpoint on the incisal edge of the most prominent upper central incisor.
Lower incisor edge	LIE	The incisal point of the most prominent mandibular incisor.
Upper incisor apex	UIA	The root apex of the most prominent upper incisor.
Lower incisor apex	LIA	The root apex of the most prominent lower incisor.
Anterior occlusal point	APOcc	The midpoint of the incisor overbite in occlusion.
Posterior occlusal point	PPOcc	The most distal point of the contact between the most posterior molar in occlusion.

Table 2 Mean error and standard deviation (SD), of the Euclidean distances (in millimetres), obtained from five observers' landmarking with (mode B) and without (mode A) enhancement from the 'best estimate' for each landmark.

Landmark	Mean error unfiltered image (A)	SD (A)	Mean error embossed image (B)	SD (B)	Difference (A − B)	*P*	One-way analysis of variance
Na	0.47	0.27	0.81	0.47	−0.34	0.00	*
S	0.33	0.15	0.46	0.21	−0.12	0.00	*
Or	1.52	0.86	1.57	0.86	−0.04	0.49	NS
Po	1.32	0.68	1.28	0.82	0.04	0.87	NS
Ba	0.91	0.48	1.21	0.62	−0.31	0.00	*
Pt	1.09	0.63	1.24	0.71	−0.16	0.05	*
ANS	0.55	0.31	0.68	0.35	−0.14	0.00	*
ANS	0.45	0.25	0.61	0.32	−0.17	0.00	*
PNS	0.72	0.45	1.17	0.63	−0.45	0.00	*
Ba	1.04	0.67	1.27	0.85	−0.24	0.00	*
PM	0.75	0.47	0.78	0.51	−0.03	0.63	NS
Po	0.51	0.31	0.63	0.35	−0.13	0.01	*
Gn	0.46	0.22	0.67	0.29	−0.21	0.00	*
Me	1.50	0.83	1.86	0.79	−0.36	0.00	*
Go	1.48	0.83	1.87	0.91	−0.39	0.00	*
Co	1.77	0.92	1.77	0.77	0.00	0.85	NS
UIE	0.24	0.12	0.71	0.42	−0.47	0.00	*
LIE	0.23	0.12	0.46	0.17	−0.23	0.00	*
UIA	0.94	0.49	1.08	0.58	−0.15	0.02	*
LIA	0.90	0.49	1.24	0.65	−0.34	0.00	*
APOcc	0.85	0.65	0.98	1.33	−0.13	0.57	NS
PPOcc	0.63	0.61	0.89	0.92	−0.25	0.15	NS

NS, not significant. *$P<0.05$.

Consequently, the accuracy of landmarks identification in each of the two modalities (monitor-displayed image with and without embossing) could be compared.

Statistical analysis

Mean errors and standard deviations of landmark location according to modes A and B were compared to the best

Table 3 Mean error and standard deviation (SD), on the *x* and *y* axes of the co-ordinate system (in millimetres), obtained from five observers' landmarking with (mode B) and without (mode A) enhancement from the 'best estimate' for each landmark.

Landmark	Mean error unfiltered image (A)	SD (A)	Mean error embossed image (B)	SD (B)	Difference (A − B)	*P*	One-way analysis of variance
NA X	0.23	0.18	0.34	0.24	−0.11	0.00	*
NA Y	0.40	0.30	0.74	0.55	−0.34	0.00	*
S X	0.17	0.11	0.30	0.22	−0.12	0.00	*
S Y	0.25	0.17	0.29	0.18	−0.04	0.02	*
Or X	1.43	0.90	1.33	0.95	0.10	0.34	NS
Or Y	0.41	0.30	0.70	0.42	−0.29	0.00	*
Po X	0.81	0.59	0.84	0.66	−0.04	0.95	NS
Po Y	1.01	0.63	0.97	0.75	0.05	0.87	NS
Ba X	0.46	0.32	0.88	0.66	−0.42	0.00	*
Ba Y	0.72	0.52	0.78	0.49	−0.06	0.15	NS
Pt X	0.39	0.31	0.42	0.31	−0.03	0.50	NS
Pt Y	0.94	0.69	1.12	0.75	−0.18	0.07	NS
ANS X	0.47	0.34	0.58	0.40	−0.12	0.00	*
ANS Y	0.22	0.15	0.26	0.17	−0.04	0.01	*
A X	0.20	0.14	0.29	0.19	−0.09	0.00	*
A Y	0.37	0.26	0.49	0.35	−0.12	0.00	*
PNS X	0.65	0.46	1.03	0.70	−0.39	0.00	*
PNS Y	0.20	0.14	0.51	0.32	−0.31	0.00	*
B X	0.20	0.15	0.23	0.17	−0.03	0.06	NS
B Y	1.08	0.73	1.29	0.90	−0.21	0.01	*
PM X	0.14	0.11	0.25	0.18	−0.11	0.00	*
PM Y	0.74	0.50	0.70	0.54	0.03	0.65	NS
Pg X	0.13	0.09	0.19	0.13	−0.05	0.00	*
Pg Y	0.48	0.34	0.57	0.38	−0.09	0.06	NS
Gn X	0.32	0.22	0.45	0.30	−0.13	0.00	*
Gn Y	0.27	0.20	0.40	0.30	−0.13	0.00	*
Me X	1.49	0.88	1.85	1.08	−0.36	0.00	*
Me Y	0.38	0.27	0.80	0.44	−0.42	0.00	*
Go X	0.81	0.56	0.89	0.57	−0.08	0.09	NS
Go Y	1.21	0.88	1.51	0.97	−0.30	0.00	*
Co X	0.96	0.61	0.99	0.63	−0.03	0.70	NS
Co Y	1.21	0.95	1.17	0.94	0.04	0.62	NS
UIE X	0.15	0.10	0.24	0.17	−0.09	0.00	*
UIE Y	0.16	0.13	0.63	0.45	−0.47	0.00	*
LIE X	0.13	0.09	0.23	0.17	−0.10	0.00	*
LIE Y	0.17	0.11	0.35	0.19	−0.18	0.00	*
UIA X	0.56	0.39	0.65	0.50	−0.09	0.09	NS
UIA Y	0.70	0.49	0.78	0.54	−0.08	0.15	NS
LIA X	0.61	0.45	0.91	0.64	−0.30	0.00	*
LIA Y	0.61	0.43	0.76	0.53	−0.15	0.01	*
APOcc X	0.43	0.54	0.56	0.97	−0.14	0.23	NS
APOcc Y	0.67	0.53	0.63	1.04	0.04	0.84	NS
PPOcc X	0.56	0.60	0.75	0.92	−0.19	0.29	NS
PPOcc Y	0.24	0.20	0.36	0.31	−0.12	0.04	*

NS, not significant. *$P<0.05$.

estimate for each landmark and values were calculated for each of the 22 landmarks, and differences were obtained. These were further analysed by ANOVA, to evaluate if they were statistically significant, in order to accept or reject the null hypothesis.

All statistical analyses were undertaken with the Statistical Package for Social Science (SPSS 16.0 release software Inc., Chicago, Illinois, USA).

Results

Table 2 reports, for each landmark, the Euclidean mean distance errors in millimetres and their standard deviations from the best estimate for each landmark, obtained for the five observers with and without image embossing. Table 3 shows the same data, but for each landmark co-ordinate (*x* and *y*).

The findings (Table 2) demonstrate that, in most instances, there were different mean distance errors between the embossed (mode B) and unfiltered (mode A) radiograph. The mean errors were higher for the embossed images (except for Po) than for the unfiltered radiograph. These differences were in most instances statistically significant ($P<0.05$).

The same pattern of errors was observed on the *x* and *y* co-ordinates, in fact accuracy on cephalometric landmark detection improved for the embossed radiograph only for a few points (Or on *x* axis and Po, PM, Co, and APOcc on *y*

axis) but these improvements were not statistically significant (Table 3). When comparing the mean distance errors between modes A and B, differences between the two methods were statistically significant ($P<0.05$) on the x co-ordinate for NA, S, Ba, ANS, A, PNS, PM, Pg, Gn, Me, UIE, LIE, and LIA and on the y co-ordinate for NA, S, Or, Po, ANS, A, PNS, B, Gn, Me, Go, UIE, LIE, LIA, and PPOcc.

Discussion

With the development of computer technology, it has become possible to 'capture' a radiographic image and to display this on a computer monitor as an array of small points (pixels), each with a particular shade of grey: the contrast and density of this image can be altered in the same way as a television picture. For example, it is possible to alter the radiograph image from negative to positive, manipulate contrast and brightness and alter the filter image. The perceived advantage of these techniques is that they can greatly facilitate landmark identification and therefore overall accuracy.

Some studies (Jäger *et al.*, 1989; Macrì and Wenzel, 1993; Wiesemann *et al.*, 2006) reported an improvement in image quality of digital cephalograms when using various digital enhancements and filtering techniques. However, this assumption is mostly based on observers' (raters') preferences of enhanced images over non-enhanced images and not if these enhancement affect the precision in landmark position identification.

Nevertheless, improved visual perception with manipulation of digital image does not necessarily mean an improved clinical performance. On this basis, the accuracy of landmark identification with and without the aid of emboss enhancement was evaluated in this study.

In most instances, embossing did not improve the accuracy of landmark detection both when considering Euclidean mean distance errors and errors from the x and y co-ordinate system. For several points, mean error differences were statistically significant. Higher mean errors from the 'best estimate' obtained for the embossed radiograph did not follow a specific pattern, as they were obtained both for points lying on edges and inside the skull. Therefore, it can be assumed that embossing filters introduce a random systematic error in the image (due, for example, to image distortion or edge erosion during processing), which negatively affects cephalometric point detection.

In the present study, several significant differences between the two image modalities, enhanced and non-enhanced images, were found. In all cases, an improvement of accuracy for emboss enhancement was observed. Thus, the null hypothesis is accepted.

Therefore, even though emboss enhancement is perceived to aid individual landmark clarity and also improve perception of overall image quality of cephalograms (Döler *et al.*, 1991; Wiesemann *et al.*, 2006), according to the findings of the present investigation, its use for clinical purposes cannot be recommended.

However, any enhancement techniques, as applied to cephalometry, have to be evaluated clinically.

Conclusions

The use of an embossing technique in cephalometry does not improve the level of accuracy of cephalometric point detection. Unless, more precise algorithms are designed, this feature should not be used for clinical and research purposes.

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References

Crozier S 1999 Is it time yet? Digital X-rays are here to stay, but how do you decide when to switch radiography systems? American Dental Association News, pp 28–32

Döler W, Steinhöfel N, Jäger A 1991 Digital image processing techniques for cephalometric analysis. Computers in Biology and Medicine 21: 23–33

Forsyth D B, Shaw W C, Richmond S, Roberts C T 1996 Digital imaging of cephalometric radiographs, Part 2: image quality. Angle Orthodontist 66: 43–50

Giordano D, Maiorana F 2007 A grid implementation of a cellular neural network simulator. 16th IEEE International Workshop on Enabling Technologies Infrastructure for Collaborative Enterprise (WETICE), Paris, France, pp. 217–222

Jackson P H, Dickson G C, Birnie D J 1985 Digital image processing of cephalometric radiographs: a preliminary report. British Journal of Orthodontics 12: 122–132

Jäger A, Döler W, Schormann T 1989 Digital image processing in cephalometric analysis. Schweizer Monatsschrift für Zahnmedizin 99: 19–23

Kogutt M S, Jones J P, Perkins D D 1988 Low-dose digital computed radiography in pediatric chest imaging. American Journal of Roentgenology 151: 775–779

Macrì V, Wenzel A 1993 Reliability of landmark recording on film and digital lateral cephalograms. European Journal of Orthodontics 15: 137–148

Menig J 1999 The DenOptix digital radiographic system. Journal of Clinical Orthodontics 33: 407–410

Wiesemann R B, Scheetz J P, Silveira A, Farman T T, Farman A G 2006 Cephalometric landmark clarity in photostimulable phosphor images using pseudo-colour and emboss enhancements. International Journal of Computer Assisted Radiology and Surgery 1: 105–112

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Mandibular dental arch changes associated with treatment of crowding using self-ligating and conventional brackets

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SUMMARY The purpose of this study was to investigate the effect of treatment of mandibular crowding with self-ligating and conventional brackets on dental arch variables. Fifty-six patients were selected from a pool of subjects satisfying the following inclusion criteria: non-extraction treatment in the mandibular or maxillary arches, eruption of all mandibular teeth, no spaces and an irregularity index greater than 2 mm in the mandibular arch, and no adjunct treatment such as etxra- or intraoral appliances. The patients were assigned to two groups: one group received treatment with the self-ligating bracket and the other with a conventional edgewise appliance, both with a 0.022 inch slot. Lateral cephalometric radiographs obtained at the beginning (T1) and end (T2) of treatment were used to assess the alteration in mandibular incisor inclination, and measurements of intercanine and intermolar widths were made on dental casts to investigate changes associated with the correction. The results were analysed with bivariate and multivariate linear regression analysis in order to examine the effect of the bracket systems on arch width or lower incisor inclination, adjusting for the confounding effect of demographic and clinical characteristics.

An alignment-induced increase in the proclination of the mandibular incisors was observed for both groups; no difference was identified between self-ligating and conventional brackets with respect to this parameter. Likewise, an increase in intercanine and intermolar widths was noted for both bracket groups; the self-ligating group showed a higher intermolar width increase than the conventional group, whereas the amount of crowding and Angle classification were not significant predictors of post-treatment intermolar width.

Introduction

Self-ligating brackets, first introduced in orthodontics several decades ago, have experienced a resurgence in the last 10 years with almost all major orthodontic companies offering a self-ligating appliance (Harradine, 2003). Several advantages such as faster wire engagement and disengagement, shorter treatment appointments, longer appointment intervals (Shivapuja and Berger, 1994; Berger and Byloff, 2001; Turnbull and Birnie, 2007), reduced treatment time and increased patient comfort (Eberting *et al.*, 2001; Harradine, 2001), reduced risk of enamel decalcification, and improved periodontal indices due to elimination of elastomeric modules have been reported. Along with the tentative advantageous features of self-ligating brackets, several controversial aspects on their mode of action and correction of malocclusions have been proposed.

The currently available accumulated evidence on the topic, however, is not supportive. A clinical trial that comparatively assessed the dental changes during the initial stages of non-extraction alignment of the mandibular arch found no difference between conventional and passive self-ligating brackets. Both bracket systems achieved alignment with a combination of dental arch expansion and lower incisor proclination (Pandis *et al.*, 2008). Similar findings have been reported by other investigators who examined the dental effects of conventional and self-ligating brackets at later stages of non-extraction and extraction orthodontic treatment (Scott *et al.*, 2008a,b; Fleming *et al.*, 2009).

Nonetheless, there is a lack of evidence on the post-treatment dental arch changes associated with treatment with conventional and self-ligating appliances as all the above-cited investigations followed the treatment of patients up to the stage of crowding alleviation. It was, therefore, the purpose of this study to assess the changes in mandibular incisor inclination and intercanine and intermolar widths after the completion of orthodontic treatment with self-ligating and conventional appliances.

Subjects and methods

Fifty-six patients were included in the study and they were followed until the end of orthodontic therapy. Selection of participants from a large pool of subjects was based on the following inclusion criteria: non-extraction treatment in the mandibular and maxillary arches; eruption of all mandibular teeth; no spaces in the mandibular arch; mandibular irregularity index greater than 2 mm; and no adjunct therapeutic intervention involving lip bumpers, maxillary expansion

Table 1 Demographic and clinical characteristics of study participants by bracket group.

Variable	Total *n* (mean ± SD or %)	Conventional *n* (mean ± SD or %)	Self-ligating *n* (mean ± SD or %)	*P*-value*
Age (years)	54 (13.8 ± 1.5)	27 (13.9 ± 1.4)	27 (13.6 ± 1.4)	NS
Gender				
Male	11 (20.4)	7 (26.0)	4 (14.8)	NS
Female	43 (79.6)	20 (74.0)	23 (85.2)	
Crowding (irregularity index)	54 (5.5 ± 2.3)	27 (5.5 ± 2.5)	27 (5.5 ± 2.2)	NS
Crowding				
Moderate	28 (51.8)	14 (51.8)	14 (51.8)	NS
Severe	26 (48.2)	13 (48.2)	13 (48.2)	
Angle Class				
I	32 (59.3)	18 (66.7)	14 (51.9)	NS
II	20 (37.0)	9 (33.3)	11 (40.7)	
III	2 (3.7)	0 (0.0)	2 (7.4)	

NS, not significant.
**P*-value for comparison of group means by *t*-test or differences in proportions by chi-square test.

appliances, or headgear. The demographics of the population studied are listed in Table 1. Complete records including cephalometric and panoramic radiographs radiographs with the use of the same cephalostat by the same operator; extraoral and intraoral photographs; and plaster models, prepared from alginate impressions.

The conventional edgewise group was bonded with the Roth prescription, 0.022 inch slot, (Microarch; GAC, Central Islip, New York, USA), and the self-ligating group received the low-incisor torque version of the Damon2, 0.022 inch slot appliances (Ormco, Glendora, California, USA). All first and second molars (where present) were bonded with bondable tubes. Bracket bonding, archwire insertion, as well as treatment were performed by the same clinician (NP).

The amount of crowding of the lower anterior dentition was assessed using the irregularity index (Little, 1975). Measurements were made on the initial casts by the same clinician using a fine-tip digital calliper, (Mitutoyo Digimatic NTD12-6”C; Mitutoyo Corp., Tokyo, Japan). Similarly, the irregularity index of patients was recorded and normalized in each bracket group in order to investigate the effect of bracket type at different crowding levels.

Archwire sequence was in most cases 0.016 inch CuNiTi 35°C (Ormco) ligated mainly with elastics and followed by a 0.020 inch medium Sentalloy archwire (GAC), 0.020 inch, and 0.018 × 0.025 inch stainless steel ligated with elastics for the conventional bracket group. In the self-ligating group, the archwire sequence involved a 0.014 inch CuNiTi Damon (Ormco) and 0.014 × 0.025 inch CuNiTi Damon (Ormco) and 0.016 × 0.025 inch stainless steel adapted to the dental archform created by the previous archwire (0.014 × 0.025 inch).

All patients were followed on a 4–8 week basis. At the end of treatment (T2), full records were taken. Changes in the intercanine and intermolar widths were recorded from dental casts, which were taken before treatment (T1) and at the stage of alignment (T2). Measurements were made with a digital calliper (Mitutoyo) and included the distance of the tips of the canines and the central groove of the molars.

Lateral cephalograms, traced by the same person (NP), were used to assess mandibular incisor inclination using the following angular measurements: lower incisor to mandibular plane (L1 to MP) and lower incisor to N–B line.

To assess intra-examiner reliability, eight plaster models and eight cephalometric radiographs were randomly selected. The cephalometric radiographs were re-traced and measurements of cephalometric variables were repeated. The intercanine and intermolar widths were re-measured on the dental casts. The reproducibility of the measurements was investigated with a paired *t*-test for each variable. Analysis revealed no statistical significance between the first and second measurements ($P > 0.05$).

Statistics

Descriptive statistics on the demographics of the study sample, clinical characteristics, cast, and cephalometric data were calculated. Data analysis was performed per protocol. Bivariate analysis of the bracket systems with different characteristics was performed with the use of the *t*- or chi-square test depending on the characteristic’s nature (numerical or categorical). Multivariate linear regression was used to examine the effect of the bracket system on arch width or lower incisor inclination adjusting for the confounding effect of demographic and clinical characteristics. A two-tailed *P*-value of 0.05 was considered statistically significant with a 95 per cent confidence interval. To conduct the statistical analysis, the Stata program version 10.1 (StataCorp, College Station, Texas, USA) was used.

Results

Figure 1 displays the adapted CONSORT patient flow chart.

The distribution of demographic variables of the populations including age, gender, irregularity index, and Angle classification are shown in Table 1; no discrimination with respect to these factors between the two population groups was noted.

In Table 2, the initial and final values of the angles used to determine the lower incisor position at T1 and T2 are listed for the entire sample as well as per bracket group. There was an overall increase in mandibular incisor proclination at T2; however, no difference between the two bracket groups was observed.

The results of intercanine and intermolar width changes (Table 3) suggest that the correction of crowding in both cases produced a small but statistically significant expansion in the mandibular arch. When the alterations in intercanine and intermolar widths between brackets were considered, the former did not show a change, whereas intermolar width was found to increase approximately 2.4 mm in the self-ligating compared with 1 mm in the conventional bracket group ($P < 0.05$).

The association of intermolar width with treatment system was further investigated as clinical and demographic characteristics were all mutually adjusted through multiple regression (Table 4). Patients with self-ligating brackets displayed a significantly larger intermolar width of 1.3 mm (95 per cent confidence interval: 0.3–2.3 mm) compared with the conventional bracket, even after adjusting for the effect of crowding severity and Angle classification, which were found not to be important factors in predicting the end of treatment intermolar width.

Discussion

The results of the present study suggest that correction of mandibular crowding at T2 was achieved through similar mechanisms with conventional and self-ligating brackets. These mechanisms involve incisor proclination and expansion of the dental arches and the results are in agreement with recent evidence (Pandis *et al*, 2008; Scott *et al.*, 2008a,b;

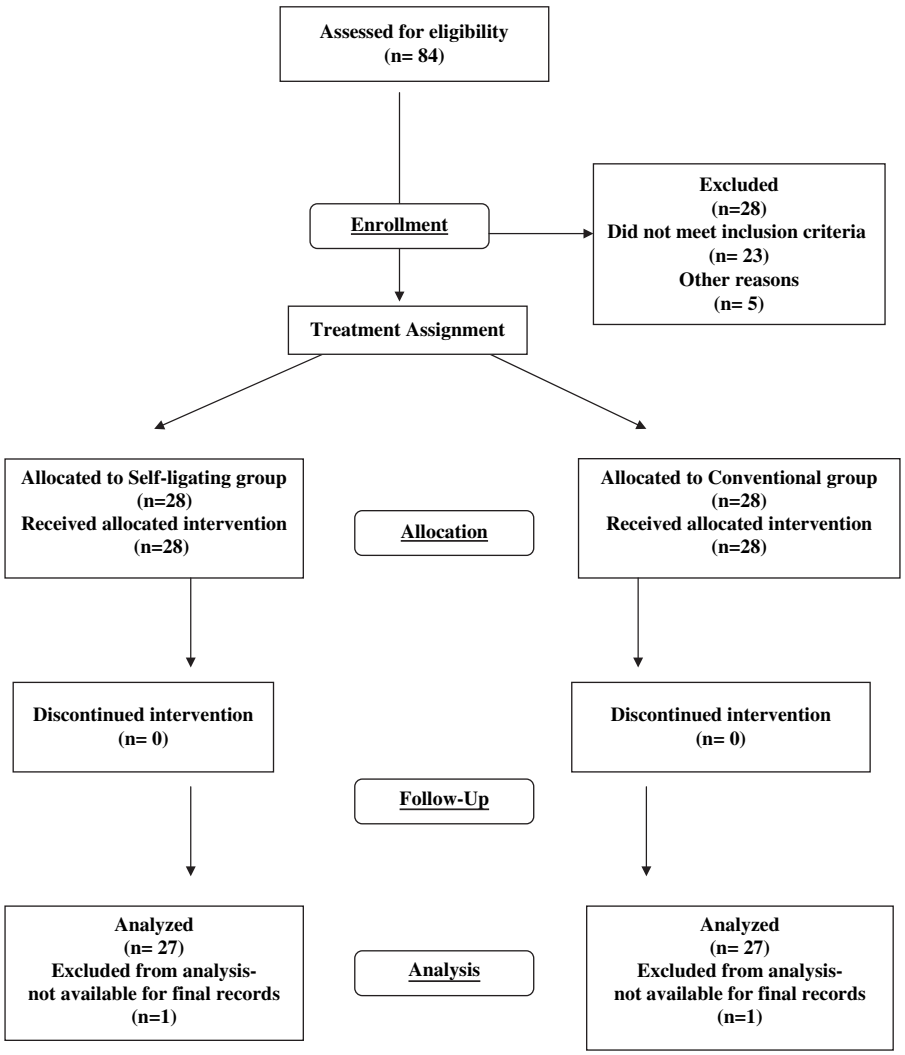

Figure 1 CONSORT flow chart for the study.

Table 2 Mandibular incisor inclination changes by bracket group.

Measurement (°)	Total n = 54 mean ± SD	Conventional n = 27 mean ± SD	Self-ligating n = 27 mean ± SD	P-value*
Initial L1–MP	92.5 ± 6.9	93.2 ± 5.8	91.8 ± 7.9	NS
Final L1–MP	96.9 ± 7.6	98.8 ± 6.7	94.9 ± 8.0	NS
Initial L1–NB	25.2 ± 5.8	25.0 ± 5.5	25.4 ± 6.3	NS
Final L1–NB	30.0 ± 5.9	30.7 ± 5.5	29.3 ± 6.3	NS

L1–MP, mandibular incisor to mandibular plane; L1–NB, Mandibular incisor to nasion-point B line; NS, not significant.
*P-value derived from t-test.

Table 3 Intercanine and intermolar width changes by bracket group.

Model measurement (mm)	Total n = 54 mean ± SD	Conventional n = 27 mean ± SD	Self-ligating n = 27 mean ± SD	P-value*,*
Initial intercanine width	25.4 ± 1.7	25.0 ± 1.5	25.8 ± 1.9	NS
Final intercanine width	27.1 ± 1.3	26.8 ± 1.2	27.4 ± 1.2	NS
Initial intermolar width	44.2 ± 2.6	44.2 ± 2.5	44.2 ± 2.6	NS
Final intermolar width	45.9 ± 1.9	45.2 ± 1.8	46.6 ± 1.7	<0.01

NS, not significant.
*P-value derived from t-test.
*Significance denotation applies to column comparisons (conventional versus self-ligating widths). Row comparisons (initial versus final widths) indicated that, overall, statistically significant differences were present between initial and final total widths (Paired t-test, P <0.001).

Table 4 Multiple regression-derived intermolar width change per indicated category of clinical predictors and corresponding 95 per cent confidence intervals (95% CIs) among the 54 study participants.*

Variable	Category	Parameter estimate	95% CI	P-value
Bracket	Conventional	Baseline		
	Self-ligating	1.3	0.3 to 2.3	0.01
Crowding	Moderate	Baseline		
	Severe	0.3	−0.7 to 1.3	NS
Angle Class	I	Baseline		
	II	−0.1	−1.1 to 0.9	NS
	III	1.6	−1.1 to 4.0	NS

NS, not significant.
*Values controlled for age and gender.

Fleming *et al.*, 2009). Interestingly, Franchi *et al.* (2006) employing low friction ligatures rather than sef-ligating brackets reported a similar increase in maxillary intermolar width when compared with conventional module ligation. That study also demonstrated 4 degrees buccal tipping of the molars. This finding may imply that molar expansion observed with self-ligating brackets is related to rolling or tipping of the molars rather than bodily movement or basal maxillary expansion.

Scott *et al.* (2008b) using study models at various stages of treatment found that alignment was associated with an increase in intercanine width, reduction in arch length, and proclination of mandibular incisors for both appliances, but the differences were not significant. That investigation included extraction cases, which may explain the associated arch length reduction and distal movement of canines into the wider section of the mandible. On the same topic, Fleming *et al.* (2009), employing a randomized control trial design, compared the effects of two pre-adjusted appliances on angular changes of the mandibular incisors and transverse mandibular arch changes over a minimum period of 30 weeks. The results indicated that bracket type had little effect on incisor inclination or intercanine, inter-first, and inter-second premolar dimensions. However, the self-ligating appliance produced more expansion in the molar region although this was small (0.9 mm). Such small changes in molar expansion of 1–2 mm will only result in an additional 0.27–0.58 mm increase in arch perimeter, which is also clinically insignificant (Germane *et al.*, 1991). Torque on the mandibular incisor brackets was −1 degree for the conventional and −6 degrees for the Damon group, whereas the final wire was 0.018 × 0.l025 for the conventional and 0.016 × 0.025 for the self-ligating group. Such a small variation is not expected to result in different tooth orientation as the free play exceeds, by a factor of 2, the difference in prescription (Sebanc *et al.*, 1984).

The actual arch space gain as a result of mandibular incisor proclination has not been unequivocally defined. Ricketts *et al.* (1982) proposed that 1 mm of incisor advancement produces 2 mm of arch length and 1 mm canine expansion produces 1 mm of arch length, whereas 1 mm of molar expansion results in an increase of 0.25 mm in arch length. Germane *et al.* (1991) developed a mathematical model and calculated the increases in arch length depending on the location of the expansion. They concluded that most arch

length is gained with a combination of incisor advancement and canine expansion compared with canine and molar expansion. They postulated that a 5 mm increase in arch length required approximately 5 mm of canine molar expansion or 4 mm of incisor advancement, or a combination of expansion and advancement. It was also shown that wide dental arches produce more arch length per millimetre of expansion compared with narrow arches and that proclination was less likely to arise where the labial segment was proclined at the outset, and expansion was unlikely to arise during levelling and alignment in wider arches. This finding has unfavourable implications for the average narrow arch shape, which in the vast majority of cases requires more expansion.

Moreover, excessive proclination may predispose to relapse and potential unfavourable periodontal sequelae in the form of loss of attachment, contributing to recession (Yared *et al.*, 2006). Even though this hypothesis has been disputed (Allais and Melsen, 2003; Ruf *et al.*, 1998), there is a possibility that proclined mandibular incisors retained with a fixed bonded appliance for long periods of time may predispose to attachment loss. Investigations, which rejected the involvement of incisor proclination in recession, did not consider the presence of a bonded appliance on the proclined teeth for long periods of time as in the case of fixed retention, which is usually advocated following correction of mandibular crowding. This factor may differentiate the effect of proclination, potentially inflicting additional changes in the periodontium.

Although overall expansion of the mandibular arch of the population treated in the study of Pandis *et al.* (2008) was found to be relatively small, the intermolar width in the Damon2 bracket group reached 1.5 mm above the value observed for conventional appliances. The use of preformed NiTi archwires at the initial stages of mechanotherapy precludes absolute control of the operator over the dimensions of the dental arch. It should be noted that the archwires used differed between the two bracket systems, in that the Damon 0.014 × 0.025 inch CuNiTi wire had a broader archform compared with the 0.020 inch Sentalloy archwire used with the conventional bracket. The difference in posterior expansion may thus be solely attributed to the differences in archwire form and cross-sectional thickness. Additionally, expansion with preformed arches in the order of 0.5–1 mm may be negligible and could be a spontaneous effect of treatment. Traditional assumptions on the intentional 'development of the arch', which are translated to substantially expanding the buccal segments, have been found to be highly unpredictable, probably depending on the axial inclination of the posterior teeth (Sandström *et al.*, 1988).

Maxillomandibular expansion has been the focus of a great deal of research over the past 30 years. However, the vast majority of evidence is concerned with expansion with the use of appliances in the mixed dentition stage and thus no direct extrapolation can be made when treating adolescents or adults with plain expanded archwires. It is interesting to note that McNamara *et al.* (2003) and Moussa *et al.* (1995) reported relapse with rapid maxillary expansion as high as 3 mm, whereas the use of quad-helix followed by edgewise appliances resulted in a decrease of 1.3 mm in intercanine and 1.5 mm in intermolar width.

Similarly, most changes associated with lip bumper therapy have been reported to be eliminated during fixed appliance therapy (McNamara *et al.*, 2003); during that stage, there is little or no overall change in mandibular arch depth, and only about 33 per cent of intercanine width and 60 per cent of intermolar width increases produced during the lip bumper phase are maintained. It has been reported, in a series of studies, that treatment with maxillomandibular expansion results in mandibular intercanine width decreases of 50 per cent of the treatment effect. Mandibular arch perimeter of the group that had been out of retention was approximately 4 mm deficient at the start of treatment; it increased 1.3 mm during treatment and decreased 1.5 mm post-treatment. Mandibular arch perimeter was approximately 2 mm deficient before treatment; it increased approximately 4 mm during treatment and decreased 3 mm post-retention (Buschang *et al.*, 2001; Ferris *et al.*, 2005; Buschang, 2006; Vargo *et al.*, 2007), whereas Heiser *et al.* (2008) showed a net mandibular intercanine width decrease in patients treated with or without extraction.

Even though expansion of the maxillary arch with the Haas type of expander has been shown to result in an increase in intercanine and intermolar width (Haas, 1980), the long-term outcome is unpredictable (Sandström *et al.*, 1988). Moreover, the results of these studies cannot be applied to fixed appliance treatment for reasons related to the age of patients, to the mechanotherapy used, and dental tipping. Therefore, the majority of evidence is supportive of the notion that expanding indiscriminately, especially in the absence of a crossbite, to accommodate dental width in a deficient arch length, results in relapse, the extent of which depends on a number of factors potentially including the appliance and age of the patient. Partial reversal and occasional total elimination of arch length gain has been shown in certain cases. In the light of the wealth of evidence on the topic, expansion of the mandibular dentition with archwires seems to be of limited long-term use, introducing also various retention concerns.

Especially molar expansion requires long-term retention with appliances, which necessitate the cooperation of the patient and thus present an unpredictable outcome. On the other hand, 2 mm expansion in the posterior segment of the arch yields a minimum increase in arch perimeter length (less than 1 mm; Germane *et al.*, 1992), whereas an intercanine width increase provides more favourable space gain, albeit showing a higher probability of relapse compared with expansion in the molar region.

Conclusions

There was an overall increase in the proclination of the mandibular incisors associated with alleviation of crowding

for both bracket groups; no difference was found between self-ligating and conventional brackets with respect to this parameter at the end of orthodontic treatment.

There was an overall increase in intercanine width at the end of treatment; however, no difference was noted between the conventional and self-ligating brackets. While intermolar width was also increased at the end of treatment for both bracket groups, nonetheless, there was a statistically significantly greater increase in the self-ligating group even after accounting for Angle classification and the variation in the amount of crowding.

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References

Allais D, Melsen B 2003 Does labial movement of lower incisors influence the level of the gingival margin? A case-control study of adult orthodontic patients. European Journal of Orthodontics 25: 343–352

Berger J L, Byloff F K 2001 The clinical efficiency of self-ligated brackets. Journal of Clinical Orthodontics 35: 304–308

Buschang P H 2006 Maxillomandibular expansion: short-term relapse potential and long-term stability. American Journal of Orthodontics and Dentofacial Orthopedics 129: S775–S779

Buschang P H, Horton-Reuland S J, Legler L, Nevant C 2001 Nonextraction approach to tooth size arch length discrepancies with the Alexander discipline. Seminars in Orthodontics 7: 117–131

Eberting J J, Straja S R, Tuncay O C 2001 Treatment time, outcome, and patient satisfaction comparisons of Damon and conventional brackets. Clinical Orthodontics and Research 4: 228–234

Ferris T, Alexander R G, Boley J, Buschang P H 2005 Long-term stability of combined rapid palatal expansion–lip bumper expansion therapy followed by full fixed appliances. American Journal of Orthodontics and Dentofacial Orthopedics 128: 310–325

Fleming P S, DiBiase A T, Sarri G, Lee R T 2009 Pain experience during initial alignment with a self-ligating and a conventional fixed orthodontic appliance system: a randomized controlled clinical trial. Angle Orthodontist 79: 46–50

Franchi L, Baccetti T, Camporesi M, Lupoli M 2006 Maxillary arch changes during leveling and aligning with fixed appliances and low-friction ligatures. American Journal of Orthodontics and Dentofacial Orthopedics 130: 88–91

Germane N, Lindauer S J, Rubenstein L K, Revere J H, Isaacson R J 1991 Increase in arch perimeter due to orthodontic expansion. American Journal of Orthodontics and Dentofacial Orthopedic 100: 421–427

Germane N, Staggers J A, Rubinstein L, Revere J T 1992 Arch length considerations due to the curve of Spee: a mathematical model. American Journal of Orthodontics and Dentofacial Orthopedics 102: 251–255

Haas A J 1980 Long-term posttreatment evaluation of rapid palatal expansion. Angle Orthodontist 50: 189–217

Harradine N W 2001 Self-ligating brackets and treatment efficiency. Clinical Orthodontics and Research 4: 220–227

Harradine N W 2003 Self-ligating brackets: where are we now? Journal of Clinical Orthodontics 30: 262–273

Heiser W, Richter M, Niederwanger A, Neunteufel N, Kulmer S 2008 Association of the canine guidance angle with maxillary and mandibular intercanine widths and anterior alignment relapse: extraction vs nonextraction treatment. American Journal of Orthodontics and Dentofacial Orthopedics 133: 669–680

Little R M 1975 The irregularity index: a quantitative score of mandibular anterior alignment. American Journal of Orthodontics 68: 554–563

McNamara J A, Baccetti T, Franchi L, Herberger T A 2003 Rapid maxillary expansion followed by appliances: a long term evaluation of changes in arch dimensions. Angle Orthodontist 73: 344–353

Moussa R, O'Reilly M T, Close J M 1995 Long-term stability of rapid palatal expander treatment and edgewise mechanotherapy. American Journal of Orthodontics and Dentofacial Orthopedics 108: 478–488

Pandis N, Polychronopoulou A, Eliades T 2008 Self-ligating vs. conventional brackets in the treatment of mandibular crowding: a prospective clinical trial of treatment duration and dental effects. American Journal of Orthodontics and Dentofacial Orthopedics 132: 208–215

Ricketts R M, Roth R H, Chaconas S J, Schulhof R J, Engel G A 1982 Orthodontic diagnosis and planning. Rocky Mountain Data Systems, Denver, pp. 194–200

Ruf S, Hansen K, Pancherz H 1998 Does orthodontic proclination of lower incisors in children and adolescents cause gingival recession? American Journal of Orthodontics and Dentofacial Orthopedics 114: 100–106

Sandström R A, Klapper L, Papaconstantinou S 1988 Expansion of the lower arch concurrent with rapid maxillary expansion. American Journal of Orthodontic and Dentofacial Orthopedics 94: 296–302

Scott P, DiBiase A T, Sherriff M, Cobourne M 2008a Alignment efficiency of Damon3 self-ligating and conventional orthodontic bracket systems: a randomized clinical trial. American Journal of Orthodontucs and Dentofacial Orthopedics 134: 470.e1–e8

Scott P, Sherriff M, DiBiase A T, Cobourne M T 2008b Perception of discomfort during initial orthodontic tooth alignment using a self-ligating or conventional bracket system: a randomized clinical trial. European Journal of Orthodontics 30: 227–232

Sebanc J, Brantley W A, Pincsak J J, Conover J P 1984 Variability of effective root torque as a function of edge bevel on orthodontic arch wires. American Journal of Orthodontics 86: 43–51

Shivapuja P K, Berger J 1994 A comparative study of conventional ligation and self-ligation bracket systems. American Journal of Orthodontics and Dentofacial Orthopedics 106: 472–480

Turnbull N R, Birnie D J 2007 Treatment efficiency of conventional vs self-ligating brackets: effects of archwire size and material. American Journal of Orthodontics and Dentofacial Orthopedics 131: 395–399

Vargo J, Buschang P H, Boley J C, English J D, Behrents R G, Owen III A H 2007 Short-term relapse after phase I maxillomandibular expansion. American Journal of Orthodontics and Dentofacial Orthopedics 131: 456–463

Yared K F G, Zenobio E G, Pacheco W 2006 Periodontal status of mandibular central incisors after orthodontic proclination in adults. American Journal of Orthodontics and Dentofacial Orthopedics 130: 6.e1–e8

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Resin-modified glass ionomer cements for bonding orthodontic retainers

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SUMMARY The aims of this study were to evaluate the shear bond strength (SBS), fracture mode, and wire pull out (WPO) resistance between resin-modified glass ionomer cement (RMGIC) and conventional orthodontic composite used as a lingual retainer adhesive. Forty lower human incisors were randomly divided into two equal groups. To determine the SBS, either Transbond-LR or Fuji Ortho-LC was applied to the lingual surface of the teeth by packing the material into cylindrical plastic matrices with an internal diameter of 2.34 mm and a height of 3 mm (Ultradent) to simulate the lingual retainer bonding area. To test WPO resistance, 20 samples were prepared for each composite where the wire was embedded in the composite material and cured, 20 seconds for Transbond-LR and 40 seconds for Fuji Ortho-LC. The ends of the wire were then drawn up and tensile stress was applied until failure of the resin. A Student's *t*-test for independent variables was used to compare the SBS and WPO data. Fracture modes were analyzed using Pearson chi-square test. Significance was determined at $P<0.05$.

The SBS values were 24.7 ± 9.2 and 10.2 ± 5.5 MPa and the mean WPO values 19.8 ± 4.6 and 11.1 ± 5.7 N for Transbond-LR and Fuji Ortho-LC, respectively. Statistical analysis showed that the SBS and WPO values of Transbond-LR and Fuji Ortho-LC were significantly different ($P < 0.001$). No significant differences were present among the groups in terms of fracture mode. However, the RMGIC resulted in a significant decrease in SBS and WPO; it produced sufficient SBS values on the etched enamel surfaces, when used as a bonded orthodontic retainer adhesive.

Introduction

Direct bonded lingual retainers are the most commonly preferred retention devices (Bearn, 1995) as they result in less relapse in the long term (Oesterle *et al.*, 2001). Besides various designs, their basic construction consists of a length of wire attached to the etched enamel with composite (Bearn, 1995). These composites include either conventional restorative or specific orthodontic bonding resins (Bearn, 1995).

Årtun (1984) investigated the potential caries and periodontal problems associated with long-term use of different types of bonded lingual retainers and concluded that, regardless of the type of wire involved in construction of the 3-3 retainers, there is a tendency for plaque and calculus to accumulate along the retainer wires, and for this tendency to increase with time. Plaque accumulation often promotes subsequent acid production leading to gingival problems, demineralization, and an alteration in the appearance of the enamel surface. In order to prevent demineralization or white spot lesions, research has focused mainly on protocols for fluoride intervention. The anti-cariogenic and remineralizing effects of long-acting fluoride release from conventional glass ionomer cements (GICs) can be predicted and there are also indications of a similar effect from resin-modified glass ionomer cements (RMGICs). The popularity of RMGICs has increased for direct bonding of orthodontic attachments (Kent *et al.*, 1973; Hotz *et al.*, 1977; Cook and Youngson, 1988; Jobalia *et al.*, 1997; Komori and Ishikawa, 1997; Bishara *et al.*, 1998a, 2000; Chung *et al.*, 1999; Flores *et al.*, 1999; Meehan *et al.*, 1999; Shammaa *et al.*, 1999; Graf and Jacobi, 2000; Owens and Miller, 2000; Sfondrini *et al.*, 2001; Valente *et al.*, 2002; Godoy-Bezerra *et al.*, 2006). Light-activated RMGIC have the advantages of GICs and the mechanical and physical properties of composite resins (Godoy-Bezerra *et al.*, 2006). Conventional GICs release fluoride, chemically bond to enamel (Kent *et al.*, 1973; Hotz *et al.*, 1977) and adhere to moist fields (Cook and Youngson, 1988).

Lower bond strengths compared with composite resins and higher bond strengths compared with GICs were reported for RMGICs in a number of studies (Jobalia *et al.*, 1997; Komori and Ishikawa, 1997; Chung *et al.*, 1999; Meehan *et al.*, 1999; Shammaa *et al.*, 1999; Owens and Miller, 2000; Sfondrini *et al.*, 2001). In contrast, RMGIC applications following 37 per cent phosphoric acid etch show comparable results with conventional orthodontic composites (Bishara *et al.*, 1998a, 2000; Flores *et al.*, 1999; Godoy-Bezerra *et al.*, 2006).

Lingual retainer fabrication requires meticulous work and the clinician often encounters problems with regard to isolation. The advantages of bonding to moist enamel surfaces and fluoride release are thought to be favourable properties of RMGICs in lingual retainer fabrication. No studies in the literature appear to have evaluated RMGICs as lingual retainer adhesives. The aim of this *in vitro*

study was to evaluate a conventional lingual retainer adhesive (Transbond-LR) and a widely used RMGIC (Fuji Ortho-LC) by means of shear bond strength (SBS) and wire pull out (WPO) tests.

For the purposes of this study, the null hypotheses assumed that there were no statistically significant differences in (1) bond strength, (2) failure site location, and (3) WPO values of materials bonded to enamel with RMGIC and a conventional lingual retainer adhesive system.

Materials and method

Bonding procedure

Forty freshly extracted human mandibular incisor teeth were used in this part of the study. Ethical approval for this research was obtained from the regional committee of Erciyes University. Teeth with hypoplastic areas, cracks, or irregularities of the enamel structure were excluded. The criteria for tooth selection dictated no pre-treatment with chemical agents such as alcohol, formalin, or hydrogen peroxide. The extracted teeth were stored in distilled water until use (maximum 1 month). The water was changed weekly to avoid bacterial growth. Callus and debris were removed with a scaler and the teeth were pumiced. The teeth were moulded in square acrylic blocks with the long axis perpendicular to the upper surface of the blocks. A 37 per cent phosphoric acid gel (3M Dental Products, St Paul, Minnesota, USA) was used for etching. Acid etching was performed for 15 seconds and washed for an additional 30 seconds. The enamel surface was dried with oil-free air until a frosty white appearance of the etched enamel was observed.

All bonding procedures were performed according to the manufacturer's instructions by one author (AB). In group I, Transbond XT Primer (3M Unitek, Monrovia, California, USA) was applied, while in group II, the etched enamel was wiped with cotton pellets in order to create a moist surface prior to application of the RMGIC. No primer or conditioners were used.

Transbond-LR (group I; 3M Unitek) and RMGIC (group II; Fuji Ortho-LC, GC Company, Tokyo, Japan) were added to the lingual surface by packing the material into cylindrical shaped plastic matrices with an internal diameter of 2.34 mm and a height of 3 mm (Ultradent, South Jordan, Utah, USA; Figure 1). Group I was considered as the control for group II. The adhesives were cured with a quartz tungsten halogen light source (Hilux 350, Express Dental Products, Toronto, Ontario, Canada). The curing times were 20 seconds for Transbond-LR and 40 seconds for Fuji Ortho-LC.

SBS testing

For SBS testing, the specimens were mounted in a universal testing machine (Hounsfield Test Equipment, Salford, Lancashire, UK). A notch-shaped apparatus (Ultradent) attached to a compression load cell at a crosshead speed of 0.5 mm/minute was applied to each specimen at the interface between the tooth and composite until failure occurred. The maximum load (N) was divided by the cross-sectional area of the bonded adhesive posts to determine bond strength in megapascals.

Fracture analysis

Fracture analyses were performed using an optical stereomicroscope at ×20 magnification (SZ 40, Olympus, Tokyo, Japan). The amount of adhesive remaining on the enamel surface was coded by one investigator (TU) who was blinded to group allocations. Failures were classified as cohesive if more than 80 per cent of the resin remained on the tooth surface, adhesive if less than 20 per cent of the resin remained on the tooth surface, or mixed if certain areas exhibited cohesive fractures and others adhesive fractures.

WPO testing

In order to perform the WPO test, 40 acrylic blocks, with a diameter of 25 mm and a height of 10 mm, were prepared in moulds. In each block, a hole, 4 mm in diameter and 3 mm

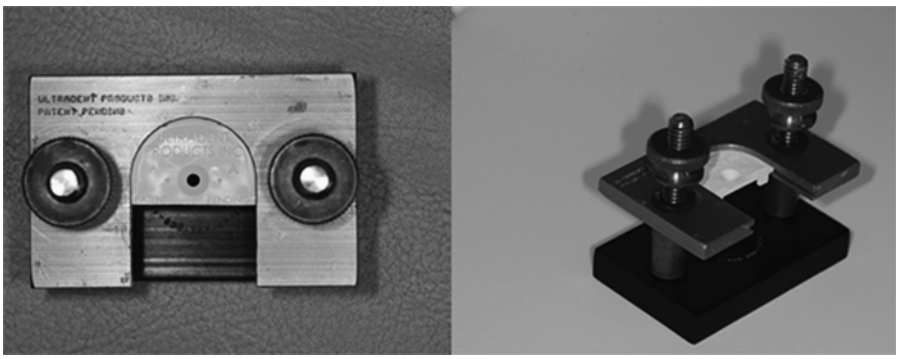

Figure 1 Apparatus for testing materials on the enamel surface.

in height, was drilled and a slot 0.6 mm wide and 1 mm deep was cut. Inclusion of the hole resulted in a clinically similar composite thickness and width, while the slot permitted the application of a standard 1 mm composite thickness over the wire. Similar to SBS testing, group I was prepared with Transbond-LR and group II with RMGIC.

Multistranded PentaOne® wire (Masel Orthodontics, Bristol, Pennsylvania, USA) 0.0215 inches in diameter was used in both groups. The wires were cut into 10 mm lengths. After insertion of the wires into the prepared slots, different resins were placed in the hole and cured. The curing was the same as for SBS testing.

The free ends of the wire were drawn up and bent with an orthodontic plier (Figure 2). The attachment arm of the tensile load cell of the universal testing machine was secured and the force applied at a crosshead speed of 0.5 mm/minute through the long axis of each sample. Data were recorded when the wires were pulled out from the resin.

Statistical analysis

All statistical analyses were performed with the Statistical Package for Social Sciences, version 13.0 for Windows 13.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics, including the mean, standard deviation, minimum, and maximum values, were calculated for the two groups. The normality test of Shapiro–Wilk and Levene's variance homogeneity test were applied to the bond strength data. The data were normally distributed, and there was homogeneity of variance between the groups. A Student's *t*-test for two independent variables was used to compare the SBS and WPO data of the two investigated adhesives. Fracture modes were analyzed using a Pearson chi-square test. Significance was predetermined at $P < 0.05$.

Results

Descriptive statistics and the results of the SBS testing are presented in Table 1. The Student's *t*-test revealed statistically significant differences in bond strength between the groups ($P < 0.001$). Thus, the first null hypothesis was rejected. Group I (24.7 ± 9.2 MPa) showed significantly higher scores compared with group II (10.2 ± 5.5 MPa).

The fracture patterns of the specimens are shown in Table 2. In general, a greater percentage of the fractures were adhesive at the tooth–composite interface (60 per cent in group I and 55 per cent in group II). There were no statistically significant differences between the groups ($\chi^2 = 0.110$). Therefore, the second null hypothesis of this study failed to be rejected ($P = 0.946$).

Descriptive statistics and the results of WPO testing are shown in Table 3. For pull out scores, there were significant differences between the groups ($P < 0.001$). The mean WPO forces for group I (19.8 ± 4.6 N) were higher than in group II (11.1 ± 5.7 N). The third null hypothesis was thus rejected.

Figure 2 Prepared block for wire pull out resistance test.

Table 1 Descriptive statistics and results of the *t*-test, comparing shear bond strength of the two groups tested.

Groups	*n*	Shear bond strength (MPa)				Significance
		Mean	SD	Minimum	Maximum	
Transbond-LR	20	24.7	9.2	11.6	44.2	***
Fuji Ortho-LC	20	10.2	5.5	2.3	23.2	

***$P < 0.001$.

Table 2 Modes of failure after shear bond testing.

Groups	*n*	Failures			Significance
		Adhesive	Cohesive	Mix	
Transbond-LR	20	12 (60%)	1 (5%)	7 (35%)	NS
Fuji Ortho-LC	20	11 (55%)	1 (5%)	8 (40%)	

NS, not significant.

Table 3 Descriptive statistics and the results of *t*-test, comparing wire pull out (WPO) values of two groups tested.

Groups	*n*	WPO test (N)				Significance
		Mean	SD	Minimum	Maximum	
Transbond-LR	20	19.8	4.6	11.0	29.0	***
Fuji Ortho-LC	20	11.1	5.7	4.0	25.0	

***$P < 0.001$.

Discussion

Bonded retainers with flexible spiral wires have been proposed for long-term retention (Zachrisson, 1977) and different wire and adhesive combinations have been mentioned (Bearn, 1995; Bearn *et al.*, 1997). Failure of a retainer bond results in a loss of retainer function and may,

if ignored, lead to relapse (Radlanski *et al.*, 2004). The most common failure type has been shown to be detachment at wire–composite interface (Bearn, 1995), but compound type failures are also described (Orsborn, 1983; Wasserstein and Brezniak, 1998). According to a review on lingual retainers (Bearn, 1995), the most appropriate materials for bonded lingual retainers have received little attention and studies are required for optimum wire–composite combinations.

The aim of this *in vitro* study was to evaluate RMGICs as an alternative for bonding lingual retainers. These materials are widely used in dentistry and orthodontics. They have higher adhesive properties compared with conventional GICs, can absorb or release fluoride (Newman *et al.*, 2001), and bond to moist environments eliminating the need to keep the teeth dry during bonding (Silverman *et al.*, 1995). These properties are advantageous for lingual retainer fabrication which is a technique-sensitive procedure and requires isolation, especially in the lower anterior segment. As lingual retainers are exposed to the oral cavity and are intended to serve in the mouth for a long period of time, fluoride release and uptake is thought to reduce the risk of decalcification.

Fuji Ortho-LC, which was the RMGIC of choice, is widely used and commercially available. Although bonding to moist enamel is possible with RMGIC, etching was performed because according to the manufacturer, when a higher bond strength is needed, conventional etching can be performed. In addition, the bond strength of RMGICs has been shown to be reduced by one-third to one-half without acid etching (Bishara *et al.*, 1998a). When maximum bond strength is needed and if water or saliva contamination is expected, Bishara *et al.* (1998a) advocated enamel surface treatment with 37 per cent phosphoric acid or 10 per cent polyacrylic acid. This can be the case particularly for lower bonded retainers. Phosphoric acid at a concentration of 37 per cent is preferred because etching with this concentration is shown to result in a comparable SBS to conventional orthodontic composites (Kent *et al.*, 1973; Hotz *et al.*, 1977; Cook and Youngson, 1988; Jobalia *et al.*, 1997; Komori and Ishikawa, 1997; Bishara *et al.*, 1998a, 2000; Chung *et al.*, 1999; Flores *et al.*, 1999; Meehan *et al.*, 1999; Shammaa *et al.*, 1999; Graf and Jacobi, 2000; Owens and Miller, 2000; Sfondrini *et al.*, 2001; Valente *et al.*, 2002; Godoy-Bezerra *et al.*, 2006). Furthermore, enamel etching is necessary if a strong bond is required (Silverman *et al.*, 1995).

In the present study, a statistically significant difference was found between the SBS values of the two adhesives tested. Fuji Ortho-LC specimens showed less favourable values compared with Transbond-LR. According to Reynolds (1979), adequate bond strength for clinical orthodontic needs varies between 5.9 and 7.8 MPa. In the present study, as the mean SBS value was 10.2 ± 5.5 MPa for group II, it is considered that Fuji Ortho-LC exhibited clinically acceptable SBS values. On the other hand, Schulz *et al.* (1985) related bond strength to the orthodontic force needed to move teeth in bone and suggested that an embedded wire or bracket should withstand forces of 0.5–4 N. The present results for both adhesives showed higher values than 0.5–4 N. However, clinical conditions may differ significantly *in vivo*. The present research was an *in vitro* study and the test conditions were not subjected to the rigours of the oral environment (Bishara *et al.*, 1998b). Heat and humidity conditions in the oral cavity are highly variable. Because of the differences between *in vivo* and *in vitro* conditions as well as the testing method, a direct comparison cannot be made with the findings of other studies.

Most orthodontic bonding studies have shown a mix or cohesive-type failure (Årtun and Bergland, 1984; Oliver, 1988). In those studies, after bond strength testing, a part of the composite resin remained on either the enamel surface or the bracket base, causing cohesive rather than adhesive failure between the enamel and composite resin. Because brackets were not used in the present study, more adhesive failures occurred and the actual bond strength between the enamel and composite could be measured. Similar to previous findings (Demir *et al.*, 2005; Malkoc *et al.*, 2005), it was considered that the higher percentage of adhesive failures confirmed the accuracy of the bond strength method.

The study design was adopted from the research of Bearn *et al.* (1997). The composite thickness over the wire was 1 mm as greater amounts of composite produce relatively small increases in detachment forces and offer little clinical benefit (Bearn *et al.*, 1997). This design was used to evaluate mean detachment forces both for Transbond-LR and Fuji Ortho-LC and these forces were interpreted as resistance to failure. The mean detachment values for Transbond-LR (19.8 ± 4.6 N) were higher than for Fuji Ortho-LC (11.1 ± 5.7 N) and the difference between the groups were statistically significant. Bearn *et al.* (1997) who compared six different composite resins, which were proposed as lingual retainer adhesives, via WPO tests reported scores of between 11.2 and 24.4 N. Transbond-LR in the present study showed higher detachment forces than those found by Bearn *et al.* (1997) with one exception, Concise with a detachment force of 24.4 N. On the other hand, Fuji Ortho-LC showed lower forces compared with the findings of Bearn *et al.* (1997). One major concern in this comparison is that increasing the wire diameter from 0.0175 to 0.0215 inches for PentaOne wire statistically increased the detachment force (Bearn *et al.*, 1997). Different from Bearn *et al.* (1997), PentaOne 0.0215 inch wire was used in this study. It can be assumed that samples prepared with Fuji Ortho-LC could result in lower WPO forces if 0.0175 inch PentaOne wire had been used.

The findings of this laboratory study may also encourage manufacturers to develop improved materials which at present are often marketed without adequate laboratory testing. With rapid advances in dental materials, newly developed products may overcome the shortcomings of RMGICs.

Conclusion

The RMGIC tested in this study resulted in lower bond strength values to etched enamel when compared with conventional lingual retainer adhesive but demonstrated SBSs which were within the range previously suggested for clinical acceptability.

There was no evidence to suggest a statistical difference between the failure characteristics of the groups.

RMGIC presented statistically lower WPO resistance values compared with the control composite, i.e. Transbond-LR.

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References

Årtun J 1984 Caries and periodontal reactions associated with long-term use of different types of bonded lingual retainers. American Journal of Orthodontics 86: 112–118

Årtun J, Bergland S 1984 Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. American Journal of Orthodontics 85: 333–340

Bearn R 1995 Bonded orthodontic retainers: a review. American Journal of Orthodontics and Dentofacial Orthopedics 108: 207–213

Bearn D R, McCabe J F, Gordon P H, Aird J C 1997 Bonded orthodontic retainers: the wire-composite interface. American Journal of Orthodontics and Dentofacial Orthopedics 111: 67–74

Bishara S E, Olsen M E, Damon P, Jakobsen J R 1998a Evaluation of a new light-cured orthodontic bonding adhesive. American Journal of Orthodontics and Dentofacial Orthopedics 114: 80–87

Bishara S E, VonWald L, Zamtua J, Damon P L 1998b Effects of various methods of chlorhexidine application on shear bond strength. American Journal of Orthodontics and Dentofacial Orthopedics 114: 150–153

Bishara S E, VonWald L, Laffoon J F, Jakobsen J R 2000 Effect of altering the type of enamel conditioner on the shear bond strength of a resin-reinforced glass ionomer adhesive. American Journal of Orthodontics and Dentofacial Orthopedics 118: 288–294

Chung C H, Cuozzo P T, Mante F K 1999 Shear bond strength of a resin-reinforced glass ionomer cement: an *in vitro* comparative study. American Journal of Orthodontics and Dentofacial Orthopedics 115: 52–54

Cook P A, Youngson C C 1988 An *in vitro* study of the bond strength of a glass ionomer cement in the direct bonding of orthodontic brackets. British Journal of Orthodontics 15: 247–253

Demir A, Malkoc S, Sengun A, Koyuturk A E, Sener Y 2005 Effects of chlorhexidine and povidone-iodine mouth rinses on the bond strength of an orthodontic composite. Angle Orthodontist 75: 392–396

Flores A R, Sae'z G, Barcelo F 1999 Metallic bracket to enamel bonding with a photopolymerizable resin-reinforced glass ionomer. American Journal of Orthodontics and Dentofacial Orthopedics 116: 514–517

Godoy-Bezerra J, Vieira S, Oliveria J H G, Lara F 2006 Shear bond strength of resin-modified glass ionomer cement with saliva present and different enamel pretreatments. Angle Orthodontist 76: 470–474

Graf I, Jacobi B E 2000 Bond strength of various fluoride-releasing orthodontic bonding systems. Journal of Orofacial Orthopedics 61: 191–198

Hotz P, McClean J W, Sced I, Wilson A D 1977 The bonding of glass ionomer cements to metal and tooth substrates. British Dental Journal 142: 41–47

Jobalia S B, Valente R M, de Rijk W G, BeGole E A, Evans C A 1997 Bond strength of visible light-cured glass ionomer orthodontic cement. American Journal of Orthodontics and Dentofacial Orthopedics 112: 205–208

Kent B E, Lewis G G, Wilson A D 1973 The properties of a glass ionomer cement. British Dental Journal 135: 322–326

Komori A, Ishikawa H 1997 Evaluation of a resin-reinforced glass ionomer cement for use as an orthodontic bonding agent. Angle Orthodontist 67: 189–196

Malkoc S, Demir A, Sengun A, Ozer F 2005 The effect on shear bond strength of different antimicrobial agents after acid etching. European Journal of Orthodontics 27: 484–488

Meehan M P, Foley T F, Mamandras A 1999 A comparison of the shear bond strengths of two glass ionomer cements. American Journal of Orthodontics and Dentofacial Orthopedics 115: 125–132

Newman G V, Newman R A, Sengupta A K 2001 Comparative assessment of light-cured resin-modified glass ionomer and composite resin adhesives*: in vitro* study of a new adhesive system. American Journal of Orthodontics and Dentofacial Orthopedics 119: 256–262

Oesterle L, Shellhart C, Henderson S 2001 Enhancing wire-composite bond strength of bonded retainers with wire surface treatment. American Journal of Orthodontics and Dentofacial Orthopedics 119: 625–631

Oliver R G 1988 The effect of different methods of bracket removal on the amount of residual adhesive. American Journal of Orthodontics and Dentofacial Orthopedics 93: 196–200

Orsborn D B 1983 Bonded lingual retainers. American Journal of Orthodontics 83: 218–220

Owens S E, Miller B H 2000 A comparison of shear bond strengths of three visible light-cured orthodontic adhesives. Angle Orthodontist 70: 352–356

Radlanski R J, Natalie D, Zain N D 2004 Stability of the bonded lingual wire retainer—a study of the initial bond strength. Journal of Orofacial Orthopedics 65: 321–335

Reynolds I R 1979 A review of direct orthodontic bonding. British Journal of Orthodontics 2: 171–178

Schulz R P, Mayhew R B, Oesterle L J, Pierson W P 1985 Bond strengths of three resin systems used with brackets and embedded wire attachments. American Journal of Orthodontics 87: 75–80

Sfondrini M F, Cacciafesta V, Pistorio A, Sfondrini G 2001 Effects of conventional and high-intensity light-curing on enamel shear bond strength of composite resin and resin-modified glass ionomer. American Journal of Orthodontics and Dentofacial Orthopedics 119: 30–35

Shammaa I *et al.* 1999 Comparison of bracket debonding force between two conventional resin adhesives and a resin-reinforced glass ionomer cement: an *in vitro* and *in vivo* study. Angle Orthodontist 69: 463–469

Silverman E, Cohen M, Demke R, Silverman M 1995 A new light-cured glass ionomer cement that bonds brackets to teeth without etching in the presence of saliva. American Journal of Orthodontics and Dentofacial Orthopedics 108: 231–236

Valente R M, Rijk W G, Drummond J L, Evans C A 2002 Etching conditions for resin-modified glass ionomer cement for orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 121: 516–520

Wasserstein A, Brezniak N 1998 Use of disclosing material to detect fixed retainer bond failures. Journal of Clinical Orthodontics 32: 598–599

Zachrisson B U 1977 Clinical experience with direct-bonded orthodontic retainers. American Journal of Orthodontics 71: 440–448

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Microleakage under orthodontic brackets bonded with the custom base indirect bonding technique

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SUMMARY The aim of this *in vitro* study was to compare microleakage of orthodontic brackets between enamel–composite and composite–bracket interfaces at the occlusal and gingival margins, bonded using indirect bonding systems with that of a conventional direct bonding method. Forty freshly extracted human maxillary premolar teeth were randomly divided into two groups. In group 1, the brackets were bonded to teeth directly according to the manufacturer's recommendations. Group 2 consisted of 20 teeth bonded indirectly with Transbond XT (3M-Unitek), as the adhesive, and Sondhi Rapid Set A/B Primer (3M-Unitek), a filled resin primer. After bonding, the specimens were further sealed with nail varnish, stained with 0.5 per cent basic fuchsine for 24 hours, sectioned and examined under a stereomicroscope, and scored for microleakage at the enamel–composite and composite–bracket interfaces from both the occlusal and gingival margins. Statistical analyses were performed using Kruskal–Wallis and Mann–Whitney *U*-tests with Bonferroni correction.

The gingival sides of group 1 displayed a higher median microleakage score than the occlusal side at the enamel–composite interface but this was not statistically significant ($P > 0.05$). All occlusal margins in both groups showed no microleakage under orthodontic brackets at the enamel–composite or composite–bracket interfaces. Comparisons of the microleakage scores between the direct and the indirect bonding groups at the enamel–composite and composite–bracket interfaces indicated no statistically significant microleakage differences at the gingival and occlusal margins ($P > 0.05$). The type of bonding method (direct versus indirect) did not significantly affect the amount of microleakage at the enamel–composite–bracket complex.

Introduction

The indirect bonding method was introduced by Silverman *et al.* (1972) to increase the accuracy of bracket placement. By placing the brackets on stone models before transferring to the mouth, orthodontists can visualize the tooth in three dimensions, allowing the brackets to be more precisely positioned on the teeth; this might decrease the need to reposition brackets later in treatment. Indirect bonding has several advantages when compared with the direct method. Accurate placement of brackets improves patient comfort and reduces chair time (Koo *et al.*, 1999; Sondhi, 1999). There are however disadvantages including technique sensitivity, the need for an additional set of impressions, increased laboratory time, and the risk of adhesive leakage to gingival embrasures (Sondhi, 1999).

Sondhi (1999) introduced a new resin with increased viscosity developed specifically for indirect bonding, which was designed to fill in any imperfections and decrease the incidence of bracket drift. It also exhibited a quicker setting time, which required less chair time holding the transfer tray and a minimum of excess resin around brackets after removal of the tray. This method is now used in many orthodontic clinics (Polat *et al.*, 2004).

Previous studies on dental composites have shown that many characteristics of the material, including hardness, tensile and compressive strength, and flexural modulus may vary when using different methods (e.g. curing mode, bonding technique). The polymerization shrinkage of the composite material may cause gaps between the adhesive and enamel surface and lead to microleakage, thus facilitating the formation of white spot lesions under the bracket (James *et al.*, 2003). Gap formation contributes to microleakage, permitting the passage of bacteria and fluids from the oral cavity (St Georges *et al.*, 2002). It is well documented that microleakage increases the likelihood of recurrent caries and post-operative sensitivity (James *et al.*, 2003).

James *et al.* (2003) investigated the increased risk of decalcification caused by microleakage around orthodontic brackets, while Arhun *et al.* (2006) assessed microleakage of a tooth–adhesive–bracket complex when metallic or ceramic brackets were bonded with a conventional and an antibacterial adhesive. Arhun *et al.* (2006) found that metallic brackets caused more leakage between the adhesive-bracket interface, which may lead to lower clinical shear bond strength and white spot lesions.

Uysal *et al.* (2008) evaluated microleakage under metallic and ceramic brackets bonded with orthodontic self-etching primer (SEP) systems and stated that SEP caused more leakage between the enamel–composite interfaces. Ramoglu

et al. (2009) compared microleakage of light-cured resin-modified glass ionomer cement (RMGIC) and conventional composite under orthodontic brackets and found that RMGIC had higher microleakage scores than conventional composites. Furthermore, Uysal *et al.* (2009a) investigated microleakage patterns of conventional glass ionomer cement (GIC), RMGIC, and poly-acid-modified composite (PAMC) for band cementation. They indicated that conventional GIC was associated with more microleakage than RMGIC and PAMC at both the cement–band and cement–enamel interfaces.

Ulker *et al.* (2009) assessed microleakage of a tooth–adhesive–bracket complex when adhesives were cured with high-intensity and conventional quartz–tungsten–halogen (QTH) lights and showed that high-intensity light units did not cause more microleakage than QTH. Uysal *et al.* (2009b) also investigated the effects of high-intensity curing lights on microleakage under orthodontic bands and found that a plasma arc curing light source is associated with more microleakage than a light-emitting diode and QTH at the cement–enamel interface.

No research in the literature has investigated the effect of indirect orthodontic bonding on microleakage under orthodontic brackets. Thus, the aim of this *in vitro* study was to compare microleakage of orthodontic brackets between the enamel–composite and composite–bracket interfaces at the occlusal and gingival margins when bonded with an indirect bonding system compared with a conventional direct bonding method. For the purpose of the present study, the null hypothesis assumed that there were no statistically significant differences between the microleakage of an enamel–composite–bracket complex with the direct or indirect bonding methods.

Materials and methods

Forty human premolars, extracted for orthodontic reasons with no decay, restorations, or infection, were collected. The extracted teeth were stored in distilled water until use (maximum 1 month). The specimens were randomly assigned to two equal groups on the basis of the bonding procedure. Immediately before bonding, the teeth were cleaned with a scaler and pumiced in order to remove soft tissue remnants, calculus, and plaque.

Group 1 was bonded directly according to the manufacturer's recommendations. A 37 per cent phosphoric acid gel (3M-Dental Products, St Paul, Minnesota, USA) was used to etch the 20 permolars for 15 seconds. The teeth were then rinsed with water from a 3-in-1 syringe for 30 seconds and dried with an oil-free air source for 20 seconds. After surface preparation, the liquid primer Transbond XT (3M-Unitek, Monrovia, California, USA) was applied to the etched surface. Standard edgewise metal premolar brackets (slot 0.022 inch; 3M-Unitek) with a base surface area of 12 mm^2 were bonded with Transbond XT (3M-Unitek) composite paste by direct bonding with a standard protocol.

Group 2 comprised 20 teeth bonded indirectly with Transbond XT, as the composite, and Sondhi Rapid Set A/B Primer (3M-Unitek), a filled resin primer. Before bonding the indirect samples, groups of five teeth were attached to a 0.040 inch stainless steel wire with sticky wax so that the interproximal surfaces of adjacent teeth were in contact. The wire was pre-bent to an approximate Dentec arch form (Daub *et al.*, 2006). A similar arch form template of boxing wax was luted to a flat surface, and the wire with the attached teeth was balanced on the top edge of the boxing wax template. The teeth were then mounted in cold cure acrylic. An alginate impression was made of the mounted teeth and poured in hard orthodontic stone (Snow White Stone, Heraeas Kulzer, Hanau, Germany). The working models were allowed to set overnight, and a layer of Al Cote separating medium (Dentsply Trubyte, York, Pennsylvania, USA) diluted with water at a 1:1 ratio was placed on each model and allowed to dry for 20 minutes. The brackets were placed on the working models with Transbond XT composite and the excess resin was removed with a hand instrument. The model was then placed into a Triad light curing unit (Dentsply Trubyte) at three angles to the light source and cured for a total of 10 minutes. A transfer tray was fabricated using a Biostar unit (Great Lakes Orthodontics, Tonawanda, New York, USA) to vacuform a 1 mm thick layer of Bioplast (Great Lakes Orthodontics), overlaid with a 1 mm thick layer of Biocryl (Great Lakes Orthodontics). The transfer tray was carefully removed from the working model and placed back into the Triad machine for 1 minute with the bracket bases facing the light source. The bracket bases were scrubbed with a toothbrush under running water and blown dry with oil-free air. The enamel in group 2 was prepared as for group 1. While the liquid primer Transbond XT applied to the etched surface in group 1, the Sondhi Rapid Set Primer was used in group 2. After etching and drying the teeth as described above, a thin layer of Sondhi Rapid Set Primer A was painted on each tooth and a thin layer of Sondhi Rapid Set Resin B was painted on the custom adhesive base of each bracket. The transfer tray was placed and held with finger pressure for 30 seconds and then left on the teeth without any pressure for 2 minutes before removal of the tray.

Microleakage evaluation

Prior to dye penetration, the apices of the teeth were sealed with sticky wax. The teeth were then rinsed in tap water, air-dried, and nail varnish was applied to the entire surface of the tooth except for approximately 1 mm away from the bracket margins. To minimize dehydration of the restorations, the teeth were replaced in water as soon as the nail polish dried. The teeth were immersed in a 0.5 per cent solution of basic fuchsine for 24 hours at room temperature. After removal from the solution, the teeth were rinsed in tap water and the superficial dye was removed with a brush and dried.

Four parallel longitudinal sections were made through the occlusal and gingival surfaces with a low-speed diamond saw (Isomet, Buehler, Lake Bluff, Illinois, USA) in the bucco-lingual direction according to Arhun *et al.* (2006). Each section was scored from both occlusal and gingival margins to the brackets at both the enamel–composite and the composite–bracket interfaces.

Microleakage was determined by direct measurement using an electronic digital calliper (Mitutoyo Miyazaki, Japan) recording the data to the nearest value as a range between 0.5 and 5 mm.

Statistical analysis

Both enamel–composite and composite–bracket interfaces were investigated at the gingival and occlusal sides. For each specimen, the microleakage scores of the gingival and occlusal sides were obtained by calculating the mean microleakage scores of each side measured from four sections. Statistical analysis was performed using Kruskal–Wallis and Mann–Whitney *U*-tests with Bonferroni correction (Statistical Package for Social Sciences, Version 13.0; SPSS Inc., Chicago, Illinois, USA). The level of significance was set at $P < 0.05$.

Results

Comparisons of the microleakage scores between the occlusal and the gingival sides for the enamel–composite and composite–bracket interfaces of two groups are shown in Table 1. The gingival sides of group 1 displayed higher median microleakage scores than the occlusal side at the enamel–composite interface, but this was not statistically significant ($P > 0.05$). For the occlusal margins in both groups, there was no microleakage under the orthodontic brackets at the enamel–composite or composite–bracket interfaces.

Descriptive values and comparisons of the microleakage scores for the two groups are shown in Table 2. At the enamel–composite interface, the microleakage scores for group 1 were higher than those of group 2; but this was not statistically significant ($P > 0.05$). Statistical comparisons of the microleakage scores between two groups at the enamel–composite and composite–bracket interfaces indicated that the type of bonding method did not significantly affect the amount of microleakage at the gingival or occlusal margins of the enamel–composite and composite–bracket interfaces. Therefore, the null hypothesis could not be rejected.

Table 1 Comparison of microleakage scores at the occlusal and gingival sides between the two different interfaces for direct and indirect bonding (Mann–Whitney *U*-test with Bonferroni correction).

Bonding type	Interface	*N*	Occlusal side descriptive values (mm)			Gingival side descriptive values (mm)			Statistical comparison	
			Percentiles			Percentiles				
			25th	50th (median)	75th	25th	50th (median)	75th	*P* value	
Direct bonding	Enamel–Composite	20	0	0	0	0	0.25	0.75	0.550	NS
	Composite–Bracket	20	0	0	0	0	0	0.25	0.194	NS
Indirect bonding	Enamel–Composite	20	0	0	0	0	0	0.25	0.540	NS
	Composite–Bracket	20	0	0	0	0	0	0.25	0.121	NS

NS, not significant.

Table 2 Comparison of microleakage scores between the two different bonding techniques at the enamel–composite and composite–bracket interfaces (Kruskal–Wallis test).

Interface	Composite	*N*	Descriptive values (mm)			Statistical comparison	
			Percentiles				
			25th	50th (median)	75th	*P* value	
Enamel–Composite	Direct bonding	20	0	0.25	0.375	0.073	NS
	Indirect bonding	20	0	0	0.25		
Composite–Bracket	Direct bonding	20	0	0	0.125	0.892	NS
	Indirect bonding	20	0	0	0.125		

N, sample size; NS, not significant.

Discussion

Several studies have investigated the bond strength values of orthodontic brackets bonded using the indirect method in comparison with the conventional direct method (Klocke *et al.*, 2003; Polat *et al.*, 2004; Daub *et al.*, 2006; Linn *et al.*, 2006). Klocke *et al.* (2003) noted that both the original and a modification of the technique of Thomas (1979) were able to produce bond strengths similar to direct bonding. Yi *et al.* (2003) also found no significant difference in bond strength between a light-cured, direct-bond control group and the Sondhi method. Polat *et al.* (2004) found no difference in bond strength between the light-cured direct bonded control and the Therma-Cure protocol, whereas the bond strengths for the Sondhi protocol were significantly lower. Linn *et al.* (2006) reported no statistically significant difference in bond strength between the Sondhi protocol, that is, using light-cured composite (Enlight LV) with a light-cured sealant (Ortho Solo), and a direct bonded light-cured group.

For restorative dentistry, microleakage is a phenomenon of the diffusion of organic or inorganic substances into a tooth through the interface between the restorative material and the tooth structure (De Almeida *et al.*, 2003). Microleakage increases the likelihood of recurrent carries and post-operative sensitivity (Gladwin and Bagby, 2004).

The polymerization shrinkage of the composite material may cause gaps between the composite and enamel interface and lead to microleakage, thus facilitating the formation of white spot lesions under the bracket surface area (James *et al.*, 2003). The potential for white spot lesion formation has been a clinical problem since fixed appliances were used (Zachrisson, 1977).

Several techniques have been introduced to assess microleakage around dental restorations. The easiest and most commonly used method involves exposure of the samples to a dye solution and then viewing cross-sections under a light microscope (Ozturk *et al.*, 2004). To evaluate the relevance of leakage testing, the effective size of oral bacteria must be considered. Because of the range of bacteria sizes, dyes such as methylene blue and fuchsine are realistic agents to identify the presence of a clinically relevant gap (Hanks *et al.*, 1994; Ferrari and Garcia-Godoy, 2002). Dye penetration was chosen for this study because it provided a simple, relatively cost-effective, quantitative and comparable method of evaluating the microleakage of different bracket bonding methods (Yap *et al.*, 1996; Ozturk *et al.*, 2004).

In vitro, microleakage is commonly assessed to detect bond failure at the enamel sealant interface through dye penetration. This failure can be due to polymerization shrinkage or different linear coefficients of thermal expansion from hard tooth substances and resin materials (Celiberti and Lussi, 2005). Thermal cycles are widely used to simulate temperature changes in the mouth, generating successive thermal stresses at the tooth-resin interface. Several investigations have indicated that an increase in the number of thermal cycles is not related to an increase in microleakage of restorations (Bedran-de-Castro *et al.*, 2004; Ulker, 2008). Therefore, thermocycling was not performed in this study.

It is well established that the type of cementing agent used for bonding has a bearing on microleakage (White *et al.*, 1992; Uysal *et al.*, 2008). It is also known that the composition and other characteristics of cementing agents determine the degree of leakage. Composite viscosity has been increased by fumed silica fibre (Sondhi, 1999). Sondhi Rapid Set adhesive contains approximately 5 per cent of a fine particle fumed silica fibre (Sondhi, 1999). Piwowarczyk *et al.* (2007) found that adhesive which contains fumed silica fibre results in smaller microleakage scores. Thus, it was expected that the direct bonding group in the present study would show higher microleakage scores than the indirect bonding group. This expectation was not true.

In the present study, it was observed that microleakage scores at the gingival margins were greater than at the occlusal margins when direct and indirect bonding methods were used between the enamel–composite and the composite–bracket interfaces. Arhun *et al.* (2006) indicated that microleakage scores obtained from the incisal and gingival margins of brackets demonstrated significant differences, implying increased microleakage at the gingival side. They interpreted these differences as being related to the curvature of the tooth anatomy, which may result in relatively thicker composite at the gingival margin. The findings of Uysal *et al.* (2008) and Ulker *et al.* (2009) were similar to those of Arhun *et al.* (2006) but the interpretation was different. They considered that lower or no microleakage scores at the occlusal than at the gingival side may be related to the curing method; as they applied the light from an occlusal direction.

Several studies have reported that indirect inlay composite restorations result in less microleakage than direct composite resins (Milleding, 1992; Hasanreisoglu *et al.*, 1996). The shrinkage produced by the polymerization process inherent in the composite resin is greater for direct insertion in a cavity when the direct technique is used than the shrinkage of the resinous cement layer used to fix the indirect inlay; this resulted in a greater magnitude of stress in the gingival wall, thus facilitating microleakage. Liberman *et al.* (1997) indicated that the indirect procedure resulted in a significant reduction in microleakage when compared with that produced by the semi-direct inlay technique. Alavi and Kianimanesh (2002) stated that, when bonding agents are correctly applied, there is no advantage with the indirect technique in small Class V cavities. From an orthodontic perspective, bonding of brackets is similar to this condition. In the present study, the microleakage scores of the direct bonding group were higher than in the indirect group; but this was not statically significant. The reason for the similar microleakage scores between the direct and indirect group may have been as a result of the use of a thin layer of composite.

Conclusions

Bonding of brackets by the direct or indirect method did not significantly affect the amount of microleakage at the enamel–composite–bracket complex.

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References

Alavi A A, Kianimanesh N 2002 Microleakage of direct and indirect composite restorations with three dentin bonding agents. Operative Dentistry 27: 19–24

Arhun N, Arman A, Cehreli S B, Arikan S, Karabulut E, Gulsahi K 2006 Microleakage beneath ceramic and metal brackets bonded with a conventional and an antibacterial adhesive system. Angle Orthodontist 76: 1028–1034

Bedran-de-Castro A K, Cardoso P E, Ambrosano G M, Pimenta L A 2004 Thermal and mechanical load cycling on microleakage and shear bond strength to dentin. Operative Dentistry 29: 42–48

Celiberti P, Lussi A 2005 Use of a self-etching adhesive on previously etched intact enamel and its effect on sealant microleakage and tag formation. Journal of Dentistry 33: 163–171

Daub J, Berzins D W, Linn B J, Bradley T G 2006 Bond strength of direct and indirect bonded brackets after thermocycling. Angle Orthodontist 76: 295–300

De Almeida J B, Platt J A, Oshida Y, Moore B K, Cochran M A, Eckert G J 2003 Three different methods to evaluate microleakage of packable composites in Class II restorations. Operative Dentistry 28: 453–460

Ferrari M, Garcia-Godoy F 2002 Sealing ability of new generation adhesive restorative materials placed on vital teeth. American Journal of Dentistry 15: 117–128

Gladwin M, Bagby M 2004 Clinical aspects of dental materials theory, practice, and cases. Lippincott Williams & Wilkins, Baltimore, pp. 47–57

Hanks C T, Wataha J C, Parsell R R 1994 Permeability of biological and synthetic molecules through dentine. Journal of Oral Rehabilitation 21: 475–487

Hasanreisoglu U, Sonmez H, Uctasli S, Wilson H J 1996 Microleakage of direct and indirect inlay/onlay systems. Journal of Oral Rehabilitation 23: 66–71

James J W, Miller B H, English J D, Tadlock L P, Buschang P H 2003 Effects of high speed curing devices on shear bond strength and microleakage of orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 123: 555–561

Klocke A, Shi J, Kahl-Nieke B, Bismayer U 2003 Bond strength with custom base indirect bonding techniques. Angle Orthodontist 73: 176–180

Koo B, Chung C, Vanarsdall R 1999 Comparison of accuracy of bracket placement between direct and indirect bonding techniques. American Journal of Orthodontics and Dentofacial Orthopedics 116: 346–351

Liberman R, Ben-Amar A, Herteanu L, Judes H 1997 Marginal seal of composite inlays using different polymerization techniques. Journal of Oral Rehabilitation 24: 26–29

Linn B J, Berzins D W, Dhuru V B, Bradley T G 2006 A comparison of bond strength between direct and indirect bonding methods. Angle Orthodontist 76: 289–294

Milleding P 1992 Microleakage of indirect composite inlays: an *in vitro* comparison with the direct technique. Acta Odontologica Scandinavica 50: 295–301

Ozturk N A, Usumez A, Ozturk B, Usumez S 2004 Influence of different light sources on microleakage of Class V composite resin restorations. Journal of Oral Rehabilitation 31: 500–504

Piwowarczyk A, Bender R, Ottl P, Lauer H C 2007 Long-term bond between dual-polymerizing cementing agents and human hard dental tissue. Dental Materials 23: 211–217

Polat O, Karaman A I, Buyukyilmaz T 2004 *In vitro* evaluation of shear bond strengths and *in vivo* analysis of bond survival of indirect-bonding resins. Angle Orthodontist 74: 405–409

Ramoglu S I, Uysal T, Ulker M, Ertas H 2009 Microleakage under ceramic and metallic brackets bonded with resin-modified glass ionomer. Angle Orthodontist 79: 138–143

Silverman E, Cohen M, Gianelly A A, Dietz V S 1972 A universal direct bonding system for both metal and plastic brackets. American Journal of Orthodontics 62: 236–244

Sondhi A 1999 Efficient and effective indirect bonding. American Journal of Orthodontics and Dentofacial Orthopedics 115: 352–359

St Georges A J, Wilder Jr D A, Perdigao J, Swift Jr J E 2002 Microleakage of Class V composites using different placement and curing techniques: an *in vitro* study. American Journal of Dentistry 15: 244–247

Thomas R 1979 Indirect bonding: simplicity in action. Journal of Clinical Orthodontics 13: 93–106

Ulker M 2008 Effect of artificial aging on bond strengths to dentin and on resin-dentin interface of self-etch adhesives (Micro tensile, SEM and TEM study). Thesis, University of Selcuk, Konya, Turkey

Ulker M, Uysal T, Ramoglu S I, Ertas H 2009 Microleakage under orthodontic brackets using high-intensity curing lights. Angle Orthodontist 79: 144–149

Uysal T, Ramoglu S I, Ertas H, Ulker M 2009a Different orthodontic band cements and the microleakage between cement/enamel and cement/band interfaces. American Journal of Orthodontics and Dentofacial Orthopedics In press

Uysal T, Ramoglu S I, Ulker M, Ertas H 2009b Effects of high curing lights on microleakage under orthodontic bands. American Journal of Orthodontics and Dentofacial Orthopedics In press

Uysal T, Ulker M, Ramoglu S I, Ertas H 2008 Microleakage under metallic and ceramic brackets bonded with orthodontic self-etching primer systems. Angle Orthodontist 78: 1089–1094

White S N, Sorensen J A, Kang S K, Caputo A A 1992 Microleakage of new crown and fixed partial denture luting agents. Journal of Prosthetic Dentistry 67: 156–161

Yap A, Stokes A N, Pearson G J 1996 An *in vitro* microleakage study of a new multi-purpose dental adhesive system. Journal of Oral Rehabilitation 23: 302–308

Yi G K, Dunn W J, Taloumis L J 2003 Shear bond strength comparison between direct and indirect bonded orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 124: 577–581

Zachrisson B U 1977 Clinical experience with direct-bonded orthodontic retainers. American Journal of Orthodontics 71: 440–448

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The effect of moisture on the shear bond strength of gold alloy rods bonded to enamel with a self-adhesive and a hydrophobic resin cement

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SUMMARY The aim of this *in vitro* study was to investigate the influence of enamel moisture on the shear bond strength (SBS) of a hydrophobic resin cement, Maximum Cure® (MC), and a self-adhesive resin cement, Multilink Sprint® (MLS), after etching of the enamel. Forty cylindrical gold alloy rods were used to simulate the Incognito® lingual bracket system. They were bonded to the enamel of 40 human teeth embedded in self-cured acrylic resin. Twenty were bonded with MC (10 on dry and 10 on wet enamel) and 20 with MLS (10 on dry and 10 on wet enamel). The SBS of MC and MLS was determined in a universal testing machine and the site of bond failure was defined by the adhesive remnant index (ARI). A Kruskal–Wallis test was performed followed by Games–Howell *post hoc* pairwise comparison tests on the SBS results ($P < 0.05$) and a chi-square test was used for the analysis of ARI scores ($P < 0.05$).

On dry enamel, no significant differences between MC (58 ± 5 MPa) and MLS (64 ± 13 MPa) were noted. On wet enamel, the adherence of MC (6 ± 8 MPa) and MLS (37 ± 13 MPa) significantly decreased but to a lesser extent for MLS. The ARI scores corroborated these results.

In conclusion, MC did not tolerate moisture. MLS was also affected but maintained sufficient adherence.

Introduction

Bonding of orthodontic brackets is a technique-sensitive procedure and moisture is cited as the most common cause for bond failure (Zachrisson, 1977; Hormati *et al.*, 1980; Xie *et al.*, 1993; Bishara *et al.*, 1998; Grandhi *et al.* 2001; Hobson *et al.*, 2001). The manufacturers of one of the most used lingual bracket systems, Incognito®, recommend bonding the brackets with a hydrophobic system, Maximum Cure® (MC), (Wiechmann, 2002). For clinical success, this material requires dry and isolated fields and enamel conditioning (Hormati *et al.*, 1980; Grandhi *et al.*, 2001; Rajagopal *et al.*, 2004). The practitioner must use spacers, salivary cotton rolls, and aspiration to decrease the humidity of the bonding environment and the risk of salivary contamination (Cacciafesta *et al.*, 1998, 2003). However, enamel contamination is difficult to control, especially in hard-to-reach areas such as the lingual surfaces of molars (Bishara *et al.*, 1998). Thus, it would be advantageous to be able to bond to enamel in a wet environment with less moisture-sensitive materials. Because of their composition, self-adhesive resin cements are potentially hydrophilic systems.

In a previous study within the field of restorative dentistry, it has been shown, under dry conditions, that acid etching of enamel with phosphoric acid prior to application of the self-adhesive resin cement significantly increased bond strength (De Munck *et al.*, 2004). No reported study has compared the shear bond strengths (SBS) of these materials under both dry and wet conditions.

The purpose of this research was to investigate the influence of enamel moisture on the adherence of a hydrophobic adhesive, MC commonly used in lingual orthodontics and Multilink Sprint® (MLS). It was hypothesized that the adherence to wet enamel of the self-adhesive resin cement would be reduced as compared with that to dry enamel but would be less affected than hydrophobic resin cement.

Materials and methods

Forty cylindrical gold alloy rods, 5 mm in length and with a plane base of 3 mm in diameter, were used to simulate Incognito® brackets. They were cast and covered, similar to Incognito® brackets, with the resin composite Phase II®, 'Reliance' after surface treatment of their base with Rocatec® (3M Espe, St Paul, Minnesota, USA). They were prepared in the Incognito® laboratory (3M Unitek, Bad Essen, Germany) and used within 15 days of fabrication. The composite was cleaned before bonding with an acetone-soaked cotton pledget. Forty freshly extracted human maxillary central incisors were collected, cleaned of soft tissue, and stored at 4°C in a solution of 1 per cent chloramine and used within 3 months. The criteria for tooth selection included intact buccal enamel, no pre-treatment with chemical agents, no cracks caused by extraction forceps, and no decay. The teeth had been extracted for reasons unrelated to the objectives of this study and with the patients' informed consent. The project

was approved by the scientific council of the Faculty of Dental Surgery, University of Paris-Descartes. These selected teeth had the greater portion of the roots removed using sandpaper (80 grit). The crowns were then roughened on their buccal surface with water-cooled sandpaper (800 grit) to expose the enamel in order to obtain a plane enamel surface (greater than 7 mm^2) on which the gold alloy rod could be bonded. Finally, the residual crowns were embedded in self-cured acrylic resin (Plexcil-Escil, Chassieu, France) in plastic cylinders with the flat enamel surface exposed. The flat surfaces were inspected under ×40 magnification to ensure that the enamel was intact and free of debris. The enamel surfaces were sandblasted for 5 seconds each from a distance of 1 cm, with 50 μm aluminum oxide powder (Al_2O_3), according to the manufacturer's protocol (Wiechmann, 2000; D'Arcangelo and Vanini, 2007), then rinsed and dried. The samples were randomly assigned to four groups, each consisting of 10 specimens.

Two adhesive systems were evaluated in the current study: the chemical compositions of which are detailed in Table 1.

MC: (control group) the hydrophobic resin cement recommended by the manufacturer;
MLS: a dual-curing, self-adhesive resin cement. The chemical compositions of the two cements are detailed in Table 1.

For each adhesive, two enamel surface conditions were studied: dry and wet. The various groups tested were group 1 for MC on dry enamel, group 2 for MC on wet enamel, group 3 for MLS on dry enamel, and group 4 for MLS on wet enamel. The teeth in groups 1 and 3 were conditioned with 37 per cent phosphoric acid (Scotchbond Etching, 3M Espe) for 30 seconds, followed by thorough washing for 10 seconds, and gentle drying with compressed air until a chalky white enamel appearance was obtained. The teeth in groups 2 and 4 were conditioned with 37 per cent phosphoric acid (Scotchbond Etching) for 30 seconds, followed by thorough washing for 10 seconds, and drying until a slightly shining, wet-appearing enamel surface was obtained. In groups 1 and 2, the two components of MC (part A and part B) were squeezed together and a thin coat of the resulting mix was applied on the tooth surface and on the rod. The rod was positioned on the enamel surface with sufficient pressure to express excess adhesive, which was removed from around the rod base with a cotton pledget. In groups 3 and 4, MLS was applied with an automix syringe on the tooth surface and on the rod. The rod was positioned on the enamel surface with sufficient pressure to express excess adhesive, which was removed from around the rod base with a probe and the material was light cured. The light source was a Demetron LC curing light (Kerr Corporation, Orange, California, USA) activated for 10 seconds of exposure on four areas around the sample to ensure sufficient irradiation of the cement with a total of 40 seconds exposure time. After bonding, all samples were set under a weight in a device that allowed the stabilization of the bonded rod during 5 minutes. The specimens were then stored in distilled water at 37°C for 24 hours and subsequently tested in shear mode.

The SBS was determined in a universal testing machine (LRX, Lloyd Instruments, Fareham, Hants, UK). The sample was immobilized in the device that has a sliding blade acting like a guillotine, giving a shearing fracture at the enamel–rod junction. A crosshead speed of 0.5 mm/minutes was chosen. The debonded specimens were observed using a binocular microscope (Olympus Europe SZH10, Hamburg, Germany) under ×40 magnification, and scoring was undertaken according to the adhesive remnant index (ARI; Årtun and Bergland, 1984). The ARI scores were used to define the site of bond failure between the enamel, the adhesive, and the rod base. ARI scores range from 0 to 3, that is score 0 = no adhesive remained on the tooth surface, score 1 = less than half of the adhesive remained on the tooth surface, score 2 = more than half of the adhesive remained on the tooth, and score 3 = all the adhesive remained on the tooth with a distinct impression of the rod base.

Statistical analysis

Each series of tests was carried out on 10 samples. The means and standard deviations for SBS were calculated. A Kruskal–Wallis test was performed followed by Games–Howell *post hoc* pairwise comparison tests on the SBS results. A chi-square test was used to determine significant differences in the ARI scores between the groups. Significance for all these tests was predetermined at $P < 0.05$. Statistical calculations were performed using the StatView® software for Windows Version 5.0 (SAS® Institute Inc, Cary, North Carolina, USA).

Table 1 Materials, manufacturers, batch number, and chemical composition.

Material	Manufacturer	Batch number	Composition (manufacturers' data)
Maximum Cure® (MC)	Reliance Orthodontic Products, Itasca, Illinois, USA	Part A 0610714; part B 0610713	MC part A: bisGMA, MMA, morpholinoethylmethacrylatehydrofluoride, amine; MC part B: bisGMA, MMA, benzoylperoxide
Multilink Sprint® (MLS)	Ivoclar Vivadent, Schaan, Liechtenstein	J22739	Base and catalyst: paste of dimethacrylates (24–26%), methacrylated phosphoric acid ester (<5%), inorganic fillers, ytterbiumtrifluoride, benzoylperoxide(<1%), stabilizers, pigments

Results

Shear bond strength

Table 2 presents the values of adherence obtained for the MC and MLS adhesive systems on dry and wet enamel. On dry enamel, there was no significant difference between MC (58 ± 5 MPa) and MLS (64 ± 13 MPa). On wet enamel, the adherence of MC (6 ± 8 MPa) and MLS (37 ± 13 MPa) decreased significantly, 90 ($P < 0.001$) and 41 ($P < 0.05$), per cent respectively.

Adhesive remnant index

The ARI scores for the four groups are listed in Table 3. The chi-square test indicated significant differences among the various groups. For groups bonded with MC, a lower frequency of failure at the enamel–adhesive interface was observed under dry than under wet conditions. No significant differences in debond location were found among the groups bonded with MLS under dry or wet enamel conditions. No significant differences in debond location were found between the two groups bonded with MLS and the group with MC under dry condition.

Discussion

The introduction of bonding materials less sensitive to moisture would be a welcome improvement because clinical conditions do not permit ideal isolation, in particular for lingual orthodontics.

This study intended to compare the bond strengths of a conventional hydrophobic resin cement and a self-adhesive resin cement after etching of the enamel under both dry and wet conditions. For both investigated systems, the adherence was reduced on wet enamel as compared with that on dry enamel but to a lesser extent for MLS. Thus, the hypothesis was confirmed.

On dry enamel, the MC adherence was high (58 MPa). This result is in agreement with the adherence value obtained in a previous study in restorative dentistry (De Munck *et al.*, 2004). The mechanism that explains this good bonding is well known. Etching of the enamel surface creates a superficial etching zone with an underlying porous zone. The inflow of the bonding agent into the porous zone results in the formation of resin tags, and micromechanical retention to etched enamel is established (Buonocore, 1955; Retief, 1978; Hitmi, 2004).

On wet enamel, a decrease of 90 per cent of MC adherence to dry enamel was observed. This reduction in bonding is also well known. It has been reported that the bond strength of resin composites to etched enamel is adversely affected by water contamination (Hormati *et al.*, 1980). Water contamination will prevent the bonding agent from sufficiently contacting the etched enamel surface, resulting in reduced bonding. Furthermore, hydrophobic monomers are unable to drive out water occupying the zones of demineralization of enamel and unable to infiltrate the surface zone of etched enamel (Hormati *et al.*, 1980). The weak diffusion of the monomer into the three-dimensional network of etched enamel results in a weak adhesion. This phenomenon was highlighted by Hitmi (2004) who observed, in an scanning electron microscopy study, infiltration defects of the hydrophobic resin, Concise®, into the stained thickness of enamel as well as the presence of a hiatus at the interface.

The range of ARI scores observed in the present study demonstrated that MC, used in a dry field, showed a significantly lower frequency of failure at the enamel–adhesive interface than on wet enamel. This finding corroborates the preceding explanations and is in agreement with the results of previous studies (Webster *et al.*, 2001; Cacciafesta *et al.*, 2003). On dry enamel, MLS adherence presented no significant difference as compared with MC. On wet enamel, the adherence values obtained with MLS decreased from 64 MPa to 37 MPa, that is relatively less than found for MC.

The results of the present study cannot be compared with those in the literature because self-adhesive resin cements have only recently been introduced (Hikita *et al.*, 2007; De Munck *et al.*, 2004) and no study has reported the influence of moisture on their adherence. The presence of hydrophilic groups (phosphate groups) in these self-adhesive resins may explain the moderate sensitivity to moisture. Thus, the use

Table 2 Means and standard deviations for shear bond strength (SBS) on dry and wet enamel of rods bonded with Maximum Cure® (MC) and Multilink Sprint® (MLS).

	SBS (MPa)	
	Dry enamel	Wet enamel
MC	58 ± 5[a]	6 ± 8[c]
MLS	64 ± 13[a]	37 ± 13[b]

Values with the same superscript letter are not significantly different at $P < 0.05$.

Table 3 Adhesive remnant index (ARI) scores for Maximum Cure® (MC) and Multilink Sprint® (MLS).

Group				ARI			
				0	1	2	3
1	MC	Dry	10[a]	4	2	2	2
2	MC	Wet	10[b]	10	0	0	0
3	MLS	Dry	10[a]	5	3	2	0
4	MLS	Wet	10[a]	6	2	2	0

Values with the same superscript letter indicates the classes to which each group was significantly associated ($P < 0.05$)

of MLS in a wet environment is a possible option because its adherence value of 37 MPa may be regarded as sufficient.

No significant difference in debond locations (ARI values) was found among the groups bonded with the self-adhesive cement under dry or wet enamel conditions and with MC on dry enamel. This confirms the moderate influence of moisture on MLS performance.

Limitations of the study

Investigation of hydrophilic adhesives is difficult (Klocke *et al.*, 2003) because the effectiveness of the materials may vary with the degree of moisture contamination (Grandhi *et al.*, 2001). There seems to be a limit as to how much of a wet field is acceptable, after which excessive surface moisture can result in a decrease in bond strength (Tay *et al.*, 1996). Furthermore, the present study does not truly reflect the oral environment because only the influence of water was tested. In the oral environment, saliva is more complex than water (Littlewood *et al.*, 2000). Many of its constituents may have additional effects on the resulting bond strength.

Conclusions

The findings of the present study have lead to the following conclusions:

1. MC is unable to tolerate moisture, which confirms the need to find alternative adhesives less sensitive to a moist environment.
2. MLS is also affected by moisture but maintains sufficient adherence in a moist environment; this should be clinically useful.

Clinical investigations are necessary to confirm the results obtained in this *in vitro* study.

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References

Årtun J, Bergland S 1984 Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pre-treatment. American Journal of Orthodontics and Dentofacial Orthopedics 85: 333–340

Bishara S, Olsen M, Damon P, Jakobsen J 1998 Evaluation of a new light-cured orthodontic bonding adhesive. American Journal of Orthodontics and Dentofacial Orthopedics 114: 80–87

Buonocore M G 1955 A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. Journal of Dental Research 34: 849–853

Cacciafesta V, Jost-Brinkman P G, Süssenberger U, Miethke R R 1998 Effects of saliva and water contamination on the enamel shear bond strength of a light-cured glass ionomer cement. American Journal of Orthodontics and Dentofacial Orthopedics 113: 402–407

Cacciafesta V, Sfondrini M F, De Angelis M, Scribante A, Klersy C 2003 Effect of water and saliva contamination on shear bond strength of brackets bonded with conventional, hydrophilic, and self-etching primers. American Journal of Orthodontics and Dentofacial Orthopedics 123: 633–640

D'Arcangelo C, Vanini L 2007 Effect of three surface treatments on the adhesive properties of indirect composite restorations. Journal of Adhesive Dentistry 9: 319–326

De Munck J, Vargas M, Van Landuyt K, Hikita K, Lambrechts P, Van Meerbeek B 2004 Bonding of an auto-adhesive luting material to enamel and dentin. Dental Materials 20: 963–971

Grandhi R K, Combe E C, Speidel T M 2001 Shear bond strength of stainless steel orthodontic brackets with a moisture-insensitive primer. American Journal of Orthodontics and Dentofacial Orthopedics 119: 251–255

Hikita K *et al.* 2007 Bonding effectiveness of adhesive luting agents to enamel and dentin. Dental Materials 23: 71–80

Hitmi L 2004 Etude et optimisation de l'adhésion à l'émail et à la dentine. Thesis, University of Paris VII

Hobson R S, Ledvinka J, Meechan J G 2001 The effect of moisture and blood contamination on bond strength of a new orthodontic bonding material. American Journal of Orthodontics and Dentofacial Orthopedics 120: 54–57

Hormati A A, Fuller J L, Denehy G E 1980 Effects of contamination and mechanical disturbance on the quality of acid etched enamel. Journal of the American Dental Association 100: 34–38

Klocke A, Shi J, Kahl-Nieke B, Bismayer U 2003 *In vitro* investigation of indirect bonding with a hydrophilic primer. Angle Orthodontist 73: 445–450

Littlewood S J, Mitchell L, Greenwood D C, Bubb N L, Wood D J 2000 Investigation of a hydrophilic primer for orthodontic bonding: an *in vitro* study. Journal of Orthodontics 27: 181–186

Rajagopal R, Padmanabhan S, Gnanamani J 2004 A comparison of shear bond strength and debonding characteristics of conventional, moisture-insensitive, and self-etching primers *in vitro*. Angle Orthodontist 74: 264–268

Retief D 1978 The mechanical bond. International Dental Journal 28: 18–27

Tay F R, Gwinnett A J, Wei S H Y 1996 The overwet phenomenon: a scanning electron microscopic study of surface moisture in the acid-conditioned, resin-dentin interface. American Journal of Dentistry 9: 43–48

Webster M J, Nanda R S, Duncanson M G, Khajotia S S, Sinha P K 2001 The effect of saliva on shear bond strengths of hydrophilic bonding systems. American Journal of Orthodontics and Dentofacial Orthopedics 119: 54–58

Wiechmann D 2000 Lingual orthodontics (Part 3): intraoral sandblasting and indirect bonding. Journal of Orofacial Orthopedics 61: 280–291

Wiechmann D 2002 A new bracket system for lingual orthodontic treatment. Part 1: theoretical background and development. Journal of Orofacial Orthopedics 63: 234–245

Xie J, Powers J M, McGuckin R S 1993 *In vitro* bond strength of two adhesives to enamel and dentin under normal and contaminated conditions. Dental Materials 9: 295–299

Zachrisson B J 1977 A posttreatment evaluation of direct bonding in orthodontics. American Journal of Orthodontics 71: 173–189

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Shear bond strength of fluoride-releasing orthodontic bonding and composite materials

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SUMMARY Several fluoride-releasing bonding materials are available for orthodontic bracket placement. These are supposed to prevent white spot lesions during therapy. The objectives of this *in vitro* study were to evaluate the shear bond strength (SBS) and failure mode of a recently introduced fluoride-releasing adhesive, as well as the comparison with established orthodontic adhesives. Sixty bovine mandibular incisors were randomly allocated to three groups (n = 20): stainless steel brackets were bonded with Transbond™ Plus Color Change Adhesive, Transbond™ XT, or Light Bond™. A universal testing machine was used to determine the SBS at a crosshead speed of 1 mm/minute. After debonding, the adhesive remnant index (ARI) was used to assess the adhesive remaining on the brackets.

One-way analysis of variance comparing the three experimental groups showed no differences between the bonding systems for mean SBS (P = 0.27). ARI scores showed more residual adhesive on the teeth bonded with the Transbond™ systems (P < 0.01). As the fluoride-releasing bonding system provided sufficient mean bond strength *in vitro* (19.9 MPa), it may be used as an additional prophylactic measure in orthodontic therapy. However, the clinical effectiveness of its fluoride release may be questionable, as the amount of fluoride required from a bonding material to be caries preventive is still unknown.

Introduction

Stable attachments are a prerequisite for successful orthodontic therapy. Since fixed appliances facilitate the retention of bacterial plaque (Sukontapatipark *et al.*, 2001) and hamper oral hygiene (Naranjo *et al.*, 2006), avoiding dental decay during treatment is a key issue in orthodontics (Sonis and Snell, 1989). In spite of advanced materials and treatment devices, fixed appliances still carry an elevated risk of distinctive white spot lesions adjacent to brackets (Sukontapatipark *et al.*, 2001). Their prevalence has been reported to be as high as 97 per cent (Boersma *et al.*, 2005). These lesions are precursors of enamel caries caused by demineralization of the tooth by organic acids produced by cariogenic bacteria (Featherstone, 2004). Hence, an ideal adhesive system should have sufficient bond strength to withstand untimely impact forces on bonded brackets and, at the same time, prevent decalcification. Based on the analysis of the cariostatic mechanisms of systemic and topical fluorides, the development of clinical procedures to establish and maintain low levels of free fluoride in the oral cavity for preventing dental decay has been suggested (Margolis and Moreno, 1990; Marinho *et al.*, 2003). Especially in orthodontic patients, fluoride delivery may be beneficial (Benson *et al.*, 2004). Various methods of administering fluoride during orthodontic treatment have been used, including toothpastes, mouth rinses, gels, and varnishes. In addition, materials have been introduced delivering fluoride during treatment such as fluoride-releasing composite bonding materials (resin modified), glass ionomer cements (GIC), compomers, slow-release fluoride devices, and fluoride-releasing elastomeric ligatures (Benson *et al.*, 2004).

Recently, Transbond™ Plus Color Change Adhesive (3M Unitek) was introduced. The adhesive contains a fluorosilicate glass as the fluoride source. The hydrophilic nature of the adhesive allows fluoride diffusion through the cured cross-linked matrix in an aqueous medium (Tzou and Darrell, 2007). Its pink colour may provide a visual aid for bracket positioning and excess removal of the adhesive. Upon light curing, the colour immediately fades. No original scientific data are available to date on the bond strength of this adhesive system.

The objectives of this *in vitro* study were to evaluate the shear bond strength (SBS) and failure mode of Transbond™ Plus Color Change Adhesive and to compare the results with those of two established orthodontic adhesives (Table 1). The null hypotheses were as follows:

1. There is no difference in SBS between the three tested adhesives.
2. There is no difference in the failure mode between the three groups in terms of the adhesive remnant index (ARI; Årtun and Bergland, 1984).

As the two tested fluoride-releasing adhesives were applied after different enamel preparations [conventional etching versus self-etching primer (SEP)], any influence of these procedures on SBS and failure mode was also evaluated.

Materials and methods

Sixty permanent incisors were extracted from lower bovine jaws obtained from a local abattoir. After manual removal of adherent soft tissue using a disposable scalpel, the teeth were stored in a disinfectant solution of 0.5 per cent chloramine-T at 6°C. The storage time varied from 7 to 9 weeks. The tooth surfaces were inspected for any gross evidence of fracture or caries. All teeth were cleansed with a brush and oil-free pumice at slow speed for 10 seconds and rinsed with tap water before random assignment to three groups of 20 specimens each. Sixty stainless steel maxillary lateral incisor brackets (0.018 inch Mini Diamonds, Ormco, Orange, California, USA) were used in this study. The area of the bracket base was 10.56 mm^2, ascertained by measuring the dimensions of six brackets.

Both Transbond™ Plus Color Change Adhesive and Transbond™ XT (3M Unitek) were used in combination with Transbond™ Plus SEP (3M Unitek). Following the removal of excess water on the tooth surface, the SEP was activated according to the product description and applied for 10 seconds. An air burst of 2 seconds was delivered using an oil- and moisture-free air source to achieve a thin film of the primer liquid. After applying one of the two adhesives, the paste was gently pressed onto the bracket base with a spatula. The bracket was then immediately placed onto the tooth surface.

The Light Bond™ group was treated with Gel Etching Agent and a sealant resin (Reliance Orthodontic Products). Subsequent to thorough air drying of the tooth surface, 37 per cent phosphoric acid was applied for 30 seconds. The tooth was rinsed with water for another 30 seconds and dried until the etched enamel appeared chalky white. A thin uniform coating of sealant resin was applied with a disposable brush, gently air-dried, and polymerized with an Ortholux light emitting diode curing light (3M Unitek) for 30 seconds. The application of the adhesive paste as well as the placement of the bracket was the same as for groups 1 and 2. The protocols are listed in Table 2.

When placing the brackets, a force of 300 g was applied for 5 seconds using a spring balance (Correx Tension Gauge, Haag-Streit AG, Koeniz, Switzerland) and ensuring a uniform thickness of the adhesive (Eliades and Brantley, 2000). Excess adhesive was removed with a scaler. The adhesive was light cured for 40 seconds (10 seconds each from the mesial, distal, cervical, and incisal). The efficiency of the lamp was tested prior to each curing cycle using the meter within the unit. A 0.018 × 0.025 inch stainless steel wire (Ormco) was laser welded to each bracket slot to minimize bracket deformation during the testing procedure (Klocke and Kahl-Nieke, 2006). The prepared specimens were then stored in water at 37°C. The bonded teeth were embedded in type 3 dental stone (Moldano blue; Heraeus

Table 1 Description of the bonding materials used.

Group	Test material	Type	Batch number	Manufacturer
1	Transbond™ Plus Self-Etching Primer	Fluoride-releasing self-etching primer	291 800C	3M Unitek Orthodontic Products, Monrovia, California, USA
	Transbond™ Plus Color Change Adhesive (capsules)	Fluoride-releasing resin-based composite adhesive	AG	
2	Transbond™ Plus Self-Etching Primer	Fluoride-releasing self-etching primer	291 800C	
	Transbond™ XT Light Cure Adhesive (capsules)	Resin-based composite adhesive	YA	
3	Gel Etching Agent	37% phosphoric acid	0703277	Reliance Orthodontic Products, Itasca, Illinois, USA
	Light Bond™ Sealant Resin	Fluoride-releasing sealant resin	0600609	
	Light Bond™ Adhesive Paste (capsules)	Fluoride-releasing resin-based composite adhesive	0704754	

Table 2 Bonding protocols according to the manufacturers' instructions.

	Transbond™ Plus Color Change Adhesive	Transbond™ XT	Light Bond™
Polishing	10 seconds	10 seconds	10 seconds
Enamel etching	–	–	30 seconds
Rinsing	–	–	30 seconds
Air drying	–	–	Until enamel is chalky white
Application of sealer	–	–	+
Etching and priming in one step	10 seconds	10 seconds	–
Air drying	2 seconds	2 seconds	+
Light curing	–	–	30 seconds
Application of composite and positioning of bracket	+	+	+
Light curing	40 seconds	40 seconds	40 seconds

Kulzer, Hanau, Germany) with the bracket bases aligned perpendicular to ensure a load application parallel to the bracket base. An incisal-to-cervical shear force was applied as close to the bracket–tooth interface as possible (Figure 1) by a chisel-shaped rod attached to the crosshead of a universal testing machine (Zwick, Ulm, Germany). Testing was performed approximately 3 hours after the preliminary bonding procedure at a crosshead speed of 1 mm/minute (Jonke *et al.*, 2008). The load at failure was recorded with the testXpert V11.0-software (Zwick) and calculated in megapascals (MPa) by dividing the shear force (Newton) by the area of the bracket base (square millimetres).

After debonding, the failure surfaces were examined using an optical stereomicroscope (Vision Engineering Ltd, Woking, Surrey, England) at a magnification of ×10. The mode of failure was assessed using the ARI (Årtun and Bergland, 1984), which defines the mode of bond failure between the enamel, adhesive, and bracket base (Table 3). Bonding, shear testing as well as ARI scoring were assessed by the same operator (BCP). The resulting bond strengths of the three groups were compared by a one-way analysis of variance (ANOVA). A chi-squared test was used to determine significant differences in the ordinal ARI scores. All statistical tests were run with a predetermined significance level of $\alpha = 0.05$.

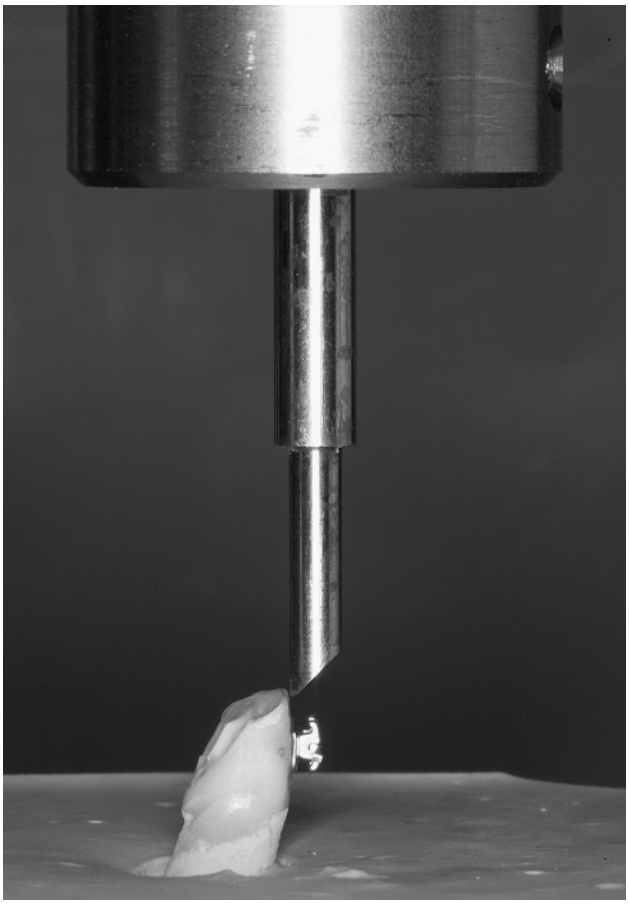

Figure 1 Experimental setting: a chisel-shaped rod applied the shear force as close to the bracket-tooth interface as possible. The bovine teeth were embedded in dental stone.

Table 3 Results of one-way analysis of variance comparing shear bond strengths between the groups.

Adhesive	Shear bond strength (MPa)			*n*
	Mean ± SD	Range	Median	
Transbond™ Plus Color Change	19.9 ± 4.3	10.0–24.1	21.7	20
Transbond™ XT	21.6 ± 5.3	9.5–29.7	22.0	20
Light Bond™	22.1 ± 3.9	9.5–26.8	23.3	20

Groups were not statistically different from each other ($F = 1.35$; $P = 0.27$).

Results

The results of the ANOVA comparing the SBS of orthodontic brackets to bovine teeth with the three adhesive systems are given in Table 3. There was little difference between the mean and median bond strengths values. There was no evidence suggesting a statistical difference in the mean SBS between the groups ($F = 1.35$; $P = 0.27$).

The distribution and results of the chi-squared analysis of the ARI scores are illustrated in Table 4. The chi-squared test showed a highly significant difference between ARI scores of the Transbond™ Plus and Light Bond™ groups. Fifty per cent of the enamel surfaces in the Light Bond™ group showed less than half of the adhesive remaining after debonding, indicating more failure at the enamel–adhesive interface. The Transbond™ Plus group showed more than half of the adhesive remaining in 95 per cent, indicating failure at the adhesive–bracket interface. Enamel fractures were not observed in any of the three groups.

Discussion

The null hypothesis that there is no difference in bond strength between the groups was accepted. The new fluoride-releasing Transbond™ Plus Color Change Adhesive provided the same high bond strength as Transbond™ XT. The latter has been used regularly as a control (Scougall Vilchis *et al.*, 2007). In the present study, both were used in combination with a fluoride-releasing SEP. Bond strengths of Transbond™ Plus Color Change Adhesive were also comparable with those obtained with the fluoride-releasing Light Bond™ adhesive, which was applied in combination with phosphoric acid and a sealant resin. With the introduction of this third well-established orthodontic adhesive, it was possible to assess the performance of a SEP versus conventional enamel conditioning. The acid etch

Table 4 Distribution and results of chi-squared test of adhesive remnant index (ARI) scores ($\chi^2 = 12.83$; $P < 0.05$).

Adhesive	ARI				*n*
	0	1	2	3	
Transbond™ Plus Color Change	—	1	12	7	20*
Transbond™ XT	2	3	11	4	20
Light Bond™	3	7	9	1	20*

0: no adhesive remaining on the tooth, failure between adhesive and enamel; 1: less than half of the adhesive left on the tooth; 2: more than half of the adhesive remaining on the tooth; 3: all adhesive left on the tooth with distinct impressions of the bracket mesh; failure between adhesive and bracket base.
*Groups were statistically different from each other ($P = 0.0034$).

regimen is widely presumed to produce the optimal bond of composite resin to enamel (House *et al.*, 2006). In fact, this system also showed the highest mean bond strength in this study, a finding, however, which was not statistically significant. Still, the number of steps required with this bonding process and the moisture sensitivity of the technique identified the need for the development of SEPs (House *et al.*, 2006). Originally designed for restorative dentistry, Transbond™ Plus SEP proved to be reliable and compatible with both adhesives tested in the setting of this study. Numerous *in vitro* (Hirani and Sherriff, 2006; Ritter *et al.*, 2006; Vicente and Bravo, 2006; Vicente *et al.*, 2006; Faltermeier *et al.*, 2007; Turk *et al.*, 2007) and *in vivo* (Cal-Neto *et al.*, 2006; Dos Santos *et al.*, 2006; Manning *et al.*, 2006) studies have assessed the bond strengths or rather bond failures of this particular fluoride-releasing SEP versus the conventional etch and prime regimen and found the SEP to perform as well, if not better.

Bond strengths recorded *in vivo* are significantly lower than those achieved *in vitro* due to deterioration of the adhesive in the oral environment (Murray and Hobson, 2003). Moreover, essential factors such as stresses arising from an activated archwire coupled with occlusal loads, critical pH, and temperature variations cannot be replicated in a laboratory investigation (Eliades and Brantley, 2000). The fact that the load was applied as close to the bracket–tooth interface as possible resulted in high values in this study. For shear testing, a significant influence of the distance of force application from the enamel surface is evident (Thomas *et al.*, 1999). The absence of thermocycling of the bonded teeth may also have contributed to these results. These facts confirm that inter-study comparisons of SBS are not appropriate because discrepancies in load location or debonding force angulation cannot be excluded (Klocke and Kahl-Nieke, 2006). However, several bond strength studies (D'Attilio *et al.*, 2005; Korbmacher *et al.*, 2006; Scougall Vilchis *et al.*, 2007) based their conclusions on comparisons with values suggested to be adequate for clinical use by previous investigators. Although they are frequently cited, these proposed stress values are not evidence based (Thind *et al.*, 2006). Inconsistencies in tooth selection, storage conditions, enamel preparation, bonding, or testing were not taken into account (Eliades and Brantley, 2000). As no standardized and widely used bond strength assessment protocol exists, an intra-study comparison with a control group seems to be a sensible approach.

In spite of the similar bond strengths in this study, analysis of ARI scores showed a statistically significant disparity. Thus, the null hypothesis that there would be no difference in ARI scores between the groups was rejected. Although the measured bond strengths appeared to be high, no enamel fractures were detected. Within the SEP groups, the majority of ARI scores were 2 and 3, indicating failure primarily at the bracket–adhesive interface. Therefore, the adhesive bond to enamel and the cohesive strength of the adhesive were higher than the adhesive bond to the bracket base. Almost the converse was true for the conventional etch group, which differed significantly from the Transbond™ Plus SEP combination. *In vitro* investigations of the locus of bond failure in comparisons of the performance of SEP and conventional etching have not produced a consensus view, i.e. whereas a large number of SEP studies showed bond failure to occur most frequently at the enamel–adhesive interface, others demonstrated that SEP produces a locus of bond failure similar to that of conventional etching (Thind *et al.*, 2006). In this study, the application of adhesive resin following acid etching resulted in far less adhesive remaining on the enamel surface after *in vitro* debonding. This has been observed only in a few previous studies (Bishara *et al.*, 2001; Thind *et al.*, 2006). Less residual adhesive after debonding would be beneficial for the clean-up procedure at the end of treatment: it would save both time and prevent iatrogenic enamel loss (Årtun and Bergland, 1984). However, the locus of bond failure is determined by a complex combination of contributory factors including the direction of the force applied, enamel pre-treatment, the adhesive itself, and the bracket type (Katona, 1997).

Excess composite around the bracket base is the critical site for plaque accumulation due to its rough surface and the shrinkage gap at its periphery (Sukontapatipark *et al.*, 2001). The initial pink colour of Transbond™ Plus Color Change Adhesive may provide a visual aid for minimizing excess composite around the bracket base. The adhesive contains a dye that photobleaches when exposed to light. The incorporation of a fluoride source in the adhesive resin is intended to prevent white spot lesions. Bonding materials capable of preventing the undesirable effect of white spot formation, while maintaining adequate bond strength, would be an advance. Although white spots were shown to fade partially after removal of fixed appliances (Van der Veen *et al.*, 2007), the overriding objective should be to prevent their development during orthodontic treatment. GIC would also satisfy the requirement of fluoride release

(Benson *et al.*, 2004; Eliades, 2006), but as conventional GIC show disadvantages such as requiring mixing and relatively low fracture strength, they are of limited use in stressed areas (Glasspoole *et al.*, 2001a). On the other hand, there is evidence suggesting that resin-modified GIC may provide adequate bond strengths *in vitro* (Movahhed *et al.*, 2005) and clinically (Summers *et al.*, 2004).

Albeit the caries-preventive effects of fluoride are well known (Ten Cate and Featherstone, 1991), the exact amount of fluoride release from a dental material to be clinically effective is still unclear (Erickson and Glasspoole, 1995; Ten Cate, 2004). A single report (Tzou and Darrell, 2007) dealing with the fluoride release rate of Transbond™ Plus Color Change Adhesive indicated that the release rate dropped to half the initial level after 1 week and to one-third after 4 weeks. A statistically significant degree of enamel protection was found when comparing fluoride-releasing materials (GIC, fluoride-releasing composites) with non-fluoride control materials (Sonis and Snell, 1989; Glasspoole *et al.*, 2001b). However, the data of two recent systematic reviews are controversial (Benson *et al.*, 2004; Derks *et al.*, 2004). While Benson *et al.* (2004) found evidence suggesting that a daily fluoride mouth rinse or fluoride-releasing bonding materials (especially GIC) reduced the severity of white spots, Derks *et al.* (2004) claimed that only the use of chlorhexidine or fluoride-containing toothpaste inhibited caries. Thus, the clinical use of a compliance-free fluoride-releasing bonding system may be regarded as an additional prophylactic measure in orthodontic therapy since its bond strength appears to be sufficient.

Laboratory testing based on recommendations in the literature (Eliades and Brantley, 2000; Klocke and Kahl-Nieke, 2006) is a necessity for the initial evaluation of bonding systems, although physical adhesive properties can only be clarified to a certain extent by *in vitro* approaches (Korbmacher *et al.*, 2006). Bovine enamel is a valid alternative to human enamel for SBS testing (Lopes *et al.*, 2003; Saleh and Taymour, 2003; Titley *et al.*, 2006; Krifka *et al.*, 2008), but the actual performance of a system has to be assessed in the environment for which it was intended (Eliades and Brantley, 2000).

Conclusions

1. The fluoride-releasing Transbond™ Plus Color Change Adhesive performed as well as the two established adhesives, Transbond™ XT and Light Bond™, in terms of SBS.
2. The fluoride-containing SEP used in combination with Transbond™ Plus Color Change Adhesive or Transbond™ XT showed sufficient bond strength compared with conventional etching combined with Light Bond™.
3. Comparison of the ARI scores indicated that there was significantly more residual adhesive remaining on teeth treated with the SEP.
4. These *in vitro* findings should be carefully extrapolated to the clinical setting.

Future clinical trials are needed for evidence-based recommendations on the optimal caries-preventive strategy since the amount of fluoride released from a bonding material to be clinically effective is still unknown.

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References

Årtun J, Bergland S 1984 Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. American Journal of Orthodontics 85: 333–340

Benson P E, Parkin N, Millett D T, Dyer F E, Vine S, Shah A 2004 Fluorides for the prevention of white spots on teeth during fixed brace treatment. Cochrane Database of Systematic Reviews 3: CD003809

Bishara S E, VonWald L, Laffoon J F, Warren J J 2001 Effect of a self-etch primer/adhesive on the shear bond strength of orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 119: 621–624

Boersma J G, van der Veen M H, Lagerweij M D, Bokhout B, Prahl-Andersen B 2005 Caries prevalence measured with QLF after treatment with fixed orthodontic appliances: influencing factors. Caries Research 39: 41–47

Cal-Neto J P, Miguel J A, Zanella E 2006 Effect of a self-etching primer on shear bond strength of adhesive precoated brackets *in vivo*. The Angle Orthodontist 76: 127–131

D'Attilio M, Traini T, Di Iorio D, Varvara G, Festa F, Tecco S 2005 Shear bond strength, bond failure, and scanning electron microscopy analysis of a new flowable composite for orthodontic use. The Angle Orthodontist 75: 410–415

Derks A, Katsaros C, Frencken J E, van 't Hof M A, Kuijpers-Jagtman A M 2004 Caries-inhibiting effect of preventive measures during orthodontic treatment with fixed appliances. A systematic review. Caries Research 38: 413–420

Dos Santos J E, Quioca J, Loguercio A D, Reis A 2006 Six-month bracket survival with a self-etch adhesive. The Angle Orthodontist 76: 863–868

Eliades T 2006 Orthodontic materials research and applications: part 1. Current status and projected future developments in bonding and adhesives. American Journal of Orthodontics and Dentofacial Orthopedics 130: 445–451

Eliades T, Brantley W A 2000 The inappropriateness of conventional orthodontic bond strength assessment protocols. European Journal of Orthodontics 22: 13–23

Erickson R L, Glasspoole E A 1995 Model investigations of caries inhibition by fluoride-releasing dental materials. Advances in Dental Research 9: 315–323

Faltermeier A, Behr M, Müssig D 2007 A comparative evaluation of bracket bonding with 1-, 2-, and 3-component adhesive systems. American Journal of Orthodontics and Dentofacial Orthopedics 132: 144. e1–e5

Featherstone J D 2004 The continuum of dental caries—evidence for a dynamic disease process. Journal of Dental Research 83: C39–C42

Glasspoole E A, Erickson R L, Davidson C L 2001a A fluoride-releasing composite for dental applications. Dental Materials 17: 127–133

Glasspoole E A, Erickson R L, Davidson C L 2001b Demineralization of enamel in relation to the fluoride release of materials. American Journal of Dentistry 14: 8–12

Hirani S, Sherriff M 2006 Bonding characteristics of a self-etching primer and precoated brackets: an *in vitro* study. European Journal of Orthodontics 28: 400–404

House K, Ireland A J, Sherriff M 2006 An *in-vitro* investigation into the use of a single component self-etching primer adhesive system for orthodontic bonding: a pilot study. Journal of Orthodontics 33: 116–124

Jonke E, Franz A, Freudenthaler J W, Koenig F, Bantleon H P, Schedle A 2008 Cytotoxicity and shear bond strength of four orthodontic adhesives. European Journal of Orthodontics 30: 495–502

Katona T R 1997 Stresses developed during clinical debonding of stainless steel orthodontic brackets. The Angle Orthodontist 67: 39–46

Klocke A, Kahl-Nieke B 2006 Effect of debonding force direction on orthodontic shear bond strength. American Journal of Orthodontics and Dentofacial Orthopedics 129: 261–265

Korbmacher H M, Huck L, Kahl-Nieke B 2006 Fluoride-releasing adhesive and antimicrobial self-etching primer effects on shear bond strength of orthodontic brackets. The Angle Orthodontist 76: 845–850

Krifka S, Börzsönyi A, Koch A, Hiller K A, Schmalz G, Friedl K H 2008 Bond strength of adhesive systems to dentin and enamel-human vs. bovine primary teeth *in vitro*. Dental Materials 24: 888–894

Lopes M B, Sinhoreti M A, Correr Sobrinho L, Consani S 2003 Comparative study of the dental substrate used in shear bond strength tests. Pesquisa Odontológica Brasileira 17: 171–175

Manning N, Chadwick S M, Plunkett D, Macfarlane T V 2006 A randomized clinical trial comparing 'one-step' and 'two-step' orthodontic bonding systems. Journal of Orthodontics 33: 276–283

Margolis H C, Moreno E C 1990 Physicochemical perspectives on the cariostatic mechanisms of systemic and topical fluorides. Journal of Dental Research 69: 606–613

Marinho V C, Higgins J P, Logan S, Sheiham A 2003 Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. Cochrane Database of Systematic Reviews 4: CD002782

Movahhed H Z, Øgaard B, Syverud M 2005 An *in vitro* comparison of the shear bond strength of a resin-reinforced glass ionomer cement and a composite adhesive for bonding orthodontic brackets. European Journal of Orthodontics 27: 477–483

Murray S D, Hobson R S 2003 Comparison of *in vivo* and *in vitro* shear bond strength. American Journal of Orthodontics and Dentofacial Orthopedics 123: 2–9

Naranjo A A, Triviño M L, Jaramillo A, Betancourth M, Botero J E 2006 Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. American Journal of Orthodontics and Dentofacial Orthopedics 130: 275. e17–e22

Ritter D E, Ritter A V, Bruggeman G, Locks A, Tulloch J F 2006 Bond strengths and adhesive remnant index of self-etching adhesives used to bond brackets to instrumented and uninstrumented enamel. American Journal of Dentistry 19: 47–50

Saleh F, Taymour N 2003 Validity of using bovine teeth as a substitute for human counterparts in adhesive tests. Eastern Mediterranean Health Journal 9: 201–207

Scougall Vilchis R J, Yamamoto S, Kitai N, Hotta M, Yamamoto K 2007 Shear bond strength of a new fluoride-releasing orthodontic adhesive. Dental Materials Journal 26: 45–51

Sonis A L, Snell W 1989 An evaluation of a fluoride-releasing, visible light-activated bonding system for orthodontic bracket placement. American Journal of Orthodontics and Dentofacial Orthopedics 95: 306–311

Sukontapatipark W, el-Agroudi M A, Selliseth N J, Thunold K, Selvig K A 2001 Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. European Journal of Orthodontics 23: 475–484

Summers A, Kao E, Gilmore J, Gunel E, Ngan P 2004 Comparison of bond strength between a conventional resin adhesive and a resin-modified glass ionomer adhesive: an *in vitro* and *in vivo* study. American Journal of Orthodontics and Dentofacial Orthopedics 126: 200–206

Ten Cate J M 2004 Fluorides in caries prevention and control: empiricism or science. Caries Research 38: 254–257

Ten Cate J M, Featherstone J D 1991 Mechanistic aspects of the interactions between fluoride and dental enamel. Critical Reviews in Oral Biology and Medicine 2: 283–296

Thind B S, Stirrups D R, Lloyd C H 2006 A comparison of tungsten-quartz-halogen, plasma arc and light-emitting diode light sources for the polymerization of an orthodontic adhesive. European Journal of Orthodontics 28: 78–82

Thomas R L, de Rijk W G, Evans C A 1999 Tensile and shear stresses in the orthodontic attachment adhesive layer with 3D finite element analysis. American Journal of Orthodontics and Dentofacial Orthopedics 116: 530–532

Titley K C, Childers S, Kulkarni G 2006 An *in vitro* comparison of short and long term bond strengths of polyacid modified composite resins to primary human and bovine enamel and dentine. European Archives of Paediatric Dentistry 7: 246–252

Turk T, Elekdag-Turk S, Isci D 2007 Effects of self-etching primer on shear bond strength of orthodontic brackets at different debond times. The Angle Orthodontist 77: 108–112

Tzou S, Darrell J 2007 Transbond™ Plus Color Change Adhesive: on-demand convenience with fluoride release, moisture tolerance and color change features. Orthodontic Perspectives XIV 1: 21–23

Van der Veen M H, Mattousch T, Boersma J G 2007 Longitudinal development of caries lesions after orthodontic treatment evaluated by quantitative light-induced fluorescence. American Journal of Orthodontics and Dentofacial Orthopedics 131: 223–228

Vicente A, Bravo L A 2006 Direct bonding with precoated brackets and self-etching primers. American Journal of Dentistry 19: 241–244

Vicente A, Bravo L A, Romero M 2006 Self-etching primer and a non-rinse conditioner versus phosphoric acid: alternative methods for bonding brackets. European Journal of Orthodontics 28: 173–178

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Factors affecting the shear bond strength of metal and ceramic brackets bonded to different ceramic surfaces

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SUMMARY The aims of this study were to evaluate the shear bond strength (SBS) of metal and ceramic brackets bonded to two different all-ceramic crowns, IPS Empress 2 and In-Ceram Alumina, to compare the SBS between hydrofluoric acid (HFA), phosphoric acid etched, and sandblasted, non-etched all-ceramic surfaces. Ninety-six all-ceramic crowns were fabricated resembling a maxillary left first premolar. The crowns were divided into eight groups: (1) metal brackets bonded to sandblasted 9.6 per cent HFA-etched IPS Empress 2 crowns; (2) metal brackets bonded to sandblasted 9.6 per cent HFA-etched In-Ceram crowns; (3) ceramic brackets bonded to sandblasted 9.6 per cent HFA-etched IPS Empress 2 crowns; (4) ceramic brackets bonded to sandblasted 9.6 per cent HFA-etched In-Ceram crowns; (5) metal brackets bonded to sandblasted 37 per cent phosphoric acid-etched IPS Empress 2 crowns; (6) metal brackets bonded to sandblasted 37 per cent phosphoric acid-etched In-Ceram crowns; (7) metal brackets bonded to sandblasted, non-etched IPS Empress 2 crowns; and (8) metal brackets bonded to sandblasted, non-etched In-Ceram crowns. Metal and ceramic orthodontic brackets were bonded using a conventional light polymerizing adhesive resin. An Instron universal testing machine was used to determine the SBS at a crosshead speed of 0.1 mm/minute. Comparison between groups was performed using a univariate general linear model and chi-squared tests.

The highest mean SBS was found in group 3 (120.15 ± 45.05 N) and the lowest in group 8 (57.86 ± 26.20 N). Of all the variables studied, surface treatment was the only factor that significantly affected SBS ($P < 0.001$). Acid etch application to sandblasted surfaces significantly increased the SBS in groups 1, 2, 5, and 6. The SBS of metal brackets debonded from groups 1, 3, and 5 were not significantly different from those of groups 2, 4, and 6. All debonded metal brackets revealed a similar pattern of bond failure at the adhesive–restorative interface. However, ceramic brackets had a significantly different adhesive failure pattern with dominant failure at the adhesive–bracket interface. Ceramic fractures after bracket removal were found more often in groups 1–4. No significant difference in ceramic fracture was observed between the IPS Empress 2 and In-Ceram groups.

Introduction

Most adult patients usually present with restored teeth. Dental ceramic is widely used to restore missing or damaged teeth. Various types of ceramics have been developed. These vary in chemical composition, method of manufacture, and physical properties. All-ceramic restorations, also known as 'free metal restorations', are among the most recent types of ceramics. These include conventional powder and slurry, castable, machinable, pressable, and infiltrated ceramics (Rosenblum and Schulman, 1997). The IPS Empress system (Ivoclar-Vivadent, Şchaan, Liechtenstein) is supplied in a form of feldspathic ingots, which are made up of micro-leucite crystals that are produced by controlled crystallization in a glass containing nucleating agents. These ingots are heated and subsequently pressed in a mould using an alumina plunger to form an all-ceramic restoration.

In-Ceram ceramics (Vita Zahnfabrik, H. Rauter GmbH & Co. KG, Bad Säckingen, Germany) are fabricated by an infiltrated molten glass matrix in a porous core composed of aluminium oxide or spinel. The glass-infiltrated core is subsequently veneered with feldspathic porcelain. Restorations fabricated by aluminium oxide infiltrated cores are considered the strongest all-ceramic restoration (Rosenblum and Schulman, 1997).

Ceramic is an inert material. It does not adhere chemically to any of the currently available bonding resins. Conventional acid etching is ineffective in the preparation of ceramic surfaces for mechanical retention of brackets and orthodontic attachments (Zachrisson *et al.*, 1996). It is important to prepare ceramic surfaces prior to bonding. Numerous approaches have been reported in the literature. These can be classified into three major groups, namely mechanical, chemical, or a combination. Mechanical alteration of porcelain surfaces to increase bond strength has been achieved by sandblasting (Zachrisson *et al.*, 1996; Cochran *et al.*, 1997; Kocadereli *et al.*, 2001). However, it has been shown that although roughening of porcelain surfaces significantly increases bond strength, it also results in a higher incidence of porcelain fracture associated with debonding (Kao *et al.*, 1988).

Numerous types of acid etching solution with variable concentrations have been developed. These include hydrofluoric acid (HFA) gel (Zachrisson *et al.*, 1996; Kocadereli *et al.*, 2001), acidulated phosphate fluoride (APF; Major *et al.*, 1995), and phosphoric acid gel and solutions (Yen *et al.*, 1993). The most commonly used ceramic acid etchant is a 9.6 per cent HFA gel (Stangel *et al.*, 1987). A 2–4 minute application of HFA gel on ceramic surface has been advocated (Zachrisson *et al.*, 1996; Zachrisson, 2000). However, HFA is a strong acidic solution that should be applied with extreme caution avoiding contact with the soft tissues (Zachrisson *et al.*, 1996; Bourke and Rock, 1999; Larmour *et al.*, 2006; Turk *et al.*, 2006).

Due to the potential toxicity of HFA, Nelson and Barghi (1989) suggested that application of 1.23 per cent AFF for 10 minutes results in an effective bond strength similar to HFA applied for 1 minute. On the other hand, etching ceramic surfaces with 37 per cent phosphoric acid was reported to produce a clinically acceptable bond strength comparable with that produced by the application of HFA (Yen *et al.*, 1993; Bourke and Rock, 1999; Larmour *et al.*, 2006).

Silane coupling agents have been reported to enhance bond strength to porcelain surfaces (Newman *et al.*, 1984; Wood *et al.*, 1986; Kao *et al.*, 1988; Winchester, 1991; Bourke and Rock, 1999; Kocadereli *et al.*, 2001). The silane reacts with the silica within the porcelain and the organic groups of the bonding resin, thus forming a bridge between the two materials (Newman *et al.*, 1984; Kern and Thompson, 1994).

The aims of this study were to evaluate the shear bond strength (SBS) of metal and ceramic brackets bonded to two different all-ceramic crowns, IPS Empress 2 and In-Ceram Alumina, to compare the SBS between HFA, phosphoric acid etched, and non-etched all-ceramic surfaces; compare the SBS between IPS Empress 2 and In-Ceram crowns, to investigate the mode of adhesive failure after debond; and evaluate the integrity of the ceramic crowns after debond.

Materials and methods

Ninety-six all-ceramic crowns resembling maxillary first premolars were fabricated utilizing a silicone index. Two types of ceramic crowns were prepared; lucite-based IPS Empress 2 crowns ($n = 48$) and glass infiltrated In-Ceram alumina crowns veneered with VM7 feldspathic porcelain ($n = 48$).

The IPS Empress 2 and the In-Ceram ceramics were divided into groups of 12 crowns as follows:

1: metal brackets bonded to HFA-etched IPS Empress 2 crowns.
2: metal brackets bonded to HFA-etched In-Ceram crowns.
3: ceramic brackets bonded to HFA-etched IPS Empress 2 crowns.
4: ceramic brackets bonded to HFA-etched In-Ceram crowns.
5: metal brackets bonded to phosphoric acid-etched IPS Empress 2 crowns.
6: metal brackets bonded to phosphoric acid-etched In-Ceram crowns.
7: metal brackets bonded to sandblasted non-etched IPS Empress 2 crowns.
8: metal brackets bonded to sandblasted non-etched In-Ceram crowns.

Ceramic crowns were deglazed by aluminium oxide sandblasting with 50 μm abrasive powder with a microetcher at 80 psi for 2 seconds through a nozzle at a distance of 10 mm and an angle of 45 degrees. After sandblasting, the crowns surfaces were cleaned with water and dried with oil free compressed air.

In the first four groups, the surfaces were etched with 9.6 per cent HFA gel for 2 minutes while in groups 7 and 8, the crowns were etched with 37 per cent phosphoric acid gel for 1 minute. The acid was rinsed away with water and dried with oil free compressed air. In groups 5 and 6, no acid etch was used. This was followed by a silane coupling agent. Tranbond XT primer (3M/Unitek, Monrovia, Bohemia, California, USA) was applied to the etched surfaces in a thin film. Transbond XT adhesive paste was applied to the bracket base (Ominarch metal brackets and Allure Ceramic brackets, 0.022 inch Roth prescription, GAC International Inc., New York, USA) and the bracket was positioned and pressed firmly on the ceramic crowns. Excess adhesive was removed from around the bracket base using a probe and the adhesive was light cured for 40 seconds. The composite resin (Transbond XT) was light cured using a light emitting diode (Ultra-Lite 5 Turbo, Rolence enterprise Inc., Hsin-Chuang City, Taiwan). The light was applied on the interproximal surfaces of the bracket for 10 seconds each. A 1 cm long 0.017 × 0.025 inch rectangular stainless steel archwire was ligated into the orthodontic bracket slot.

All crowns were cemented with glass ionomer cement (universal glass ionomer cement, Super Dent, Westbury, New York, USA) on dies prepared by clear autopolymerizing polymethyl metahacrylate acrylic resin (PMMA; Acrylic Melliodent, Heraeus Kulzer, GmbH, Ettlingen, Germany). Thereafter, the specimens were embedded in custom-made specimen blocks in clear PMMA. The resin covered the occlusal surface of the all-ceramic crowns with the test surface exposed.

After polymerization, the specimens were transferred to a water bath at 37°C for 24 hours. Subsequently, they were thermocycled from 5 to 55°C and back to 5°C 500 times. The exposure in each bath was 60 seconds and the transfer time between baths 20 seconds.

The specimens were mounted on Universal Testing Machine (Instron 1195, Instron Limited, High Wycombe, Buckinghamshire, UK) with the tensile load applied parallel to the buccal surface of the restoration in a gingivo-occlusal direction. The machine had an upper jaw that was mounted to

a movable crosshead and a lower jaw mounted on the base. The crosshead moved at fixed rate of 1 mm per minute at a full scale of 200 Newton (N) until failure occurred. The force required to debond the brackets was recorded in Newton.

After bond failure, the different groups were masked and the bracket bases and ceramic surfaces were examined visually by a single operator (IAAA) to determine the amount of composite resin remaining according to the modified Adhesive Remnant Index (ARI; Årtun and Bergland, 1984; Bishara *et al.*, 1999). According to Bishara *et al.* (1999), the ARI scale ranges from 1 to 5:

1. All adhesive remaining on the enamel with the impression of the bracket base.
2. More than 90 per cent of the adhesive remaining on the enamel surface.
3. Less than 90 per cent but more than 10 per cent of the adhesive remaining on the enamel surface.
4. Less than 10 per cent of the adhesive remaining on the enamel surface.
5. No adhesive remaining on the enamel surface.

In order to evaluate the type of bond failure at the bracket adhesive interface in each test group, the debonded bracket bases were examined using scanning electron microscopy (SEM; FEI, Quanta 200, Göteborg, Sweden).

Damage to the ceramic surface which may have occurred during shear bond testing was recorded using the Porcelain Fracture Index (PFI; Bourke and Rock, 1999). The index is divided into four scores as follows:

0. ceramic surface intact or in the same condition as before the bonding procedure;
1. surface damage limited to glaze layer or very superficial ceramic;
2. surface damage which features significant loss of ceramic requiring restoration of the defect by composite resin or replacement of the restoration;
3. surface damage where the core material has been exposed due to the depth of the cohesive failure.

Method error

Ten randomly selected crowns were re-examined by the same examiner after a period of 1 week, and the kappa test was applied to test intra-examiner reliability. Kappa values were above 92 per cent for the ARI and PFI.

Statistical analysis

The mean and standard deviation (SD) of each group were calculated. Comparison between groups was performed using a univariate general linear model with SBS as the dependent variable and the type of bracket, type of porcelain surface, and surface treatment as fixed variables. Bonferroni *post hoc* multiple comparisons were used. Comparison between the different adhesives and modes of failure was carried out using the chi-square test.

Results

The mean and SD of the SBS of the different groups are shown in Table 1. The highest mean SBS was 120.15 ± 45.05 N which was recorded in group 3 when ceramic brackets were bonded using HFA on the IPS impress ceramic surface, whereas the lowest mean SBS was 57.86 ± 26.19 N which was recorded in group 8 when metal brackets were bonded to In-Ceram ceramic without acid etching. The only factor which significantly affected the SBS was surface treatment (Tables 2 and 3). The SBS of the HFA etched, phosphoric acid etched, and sandblasted non-etched groups

Table 1 Means and standard deviation (SD) of the shear bond strength (SBS) (N) of the different surface-treated ceramic crowns.

Group	Description	SBS, mean ± SD
1	Metal brackets, HFA, IPS Empress 2	101.70 ± 52.94
2	Metal brackets, HFA, In-Ceram	106.82 ± 34.83
3	Ceramic brackets, HFA, IPS Empress 2	120.15 ± 45.05
4	Ceramic brackets, HFA, In-Ceram	115.18 ± 32.57
5	Metal brackets, phosphoric acid, IPS Empress 2	110.30 ± 36.97
6	Metal brackets, phosphoric acid, In-Ceram	87.00 ± 37.11
7	Metal brackets, sandblasted non-etched IPS Empress 2	59.72 ± 27.33
8	Metal brackets, sandblasted non-etched In-Ceram	57.86 ± 26.20

HFA, hydrofluoric acid.

Table 2 *F* and *P* values for the effect of the studied variables on shear bond strength.

Variable	*F* values	*P* values
Type of bracket	1.547	0.217
Type of porcelain	1.203	0.276
Surface treatment	11.137	***
Type of bracket × type of porcelain	0.219	0.641
Type of porcelain × surface treatment	0.947	0.392

****P* < 0.001.

Table 3 Means, standard error (SE) and 95% confidence interval (CI) of shear bond strength (N) of the different variables used in this study.

Variable	Type	Mean ± SE	95% CI
Type of bracket	Metal	87.23 ± 4.35	78.60–95.87
	Ceramic	100.64 ± 9.82	81.14–120.15
Type of porcelain	IPS-Empress	99.80 ± 7.56	84.78–114.82
	In-Ceram	88.08 ± 7.56	73.06–103.09
Surface treatment	Hydrofluoric acid	110.96 ± 5.39	100.25–121.67
	Phosphoric acid	105.35 ± 9.34	86.80–123.90
	Sandblasted-non-etched	65.50 ± 9.10	47.43–83.57

were 110.96 ± 5.39 N, 105.35 ± 9.34 N, and 65.50 ± 9.10 N, respectively. Bonferroni multiple comparisons tests (Table 4) revealed a significant difference in SBS between the HFA-etched groups and the sandblasted non-etched groups ($P \leq 0.001$) and between the phosphoric acid-etched and sandblasted non-etched groups ($P < 0.001$).

The pattern of bond failure using the ARI in the different groups is shown in Tables 5 and 6. SEM of the bracket bases of the different tested groups is shown in Figure 1a–h. There were statistically significant differences in ARI scores between metal and ceramic brackets debonded from HFA-etched IPS Empress 2 (groups 1 and 3; $P < 0.01$) and In-Ceram (groups 2 and 4; $P < 0.05$). ARI scores recorded for groups 1 and 3 did not differ significantly from those recorded in groups 2 and 4 with a similar bonding protocol.

The results of PFI in the different groups is shown in Tables 7 and 8. The highest incidence of cohesive ceramic fracture (67 per cent of crowns fractured) was observed while attempting to debond metal brackets from HFA-etched In-Ceram group (group 2). IPS Empress 2 crowns showed lower rates of ceramic fracture (groups 1, 3, 5, and 7).

Table 4 Mean differences, standard errors, and *P* values for the shear bond strength (N) of the surface treatment variable using Bonferroni multiple comparisons test.

Groups	Mean difference ± standard error	*P* value
Hydrofluoric acid and phosphoric acid	12.31 ± 9.34	0.572
Hydrofluoric acid and sandblasted non-etched	52.17 ± 9.09	0.000***
Phosphoric acid and sandblasted non-etched	39.86 ± 10.57	0.001***

***$P \leq 0.001$.

Table 5 Adhesive Remnant Index (ARI) scores for the tested groups (see Table 1).

	ARI scores				
Group	1	2	3	4	5
1	0	0	0	3	9
2	0	0	0	2	10
3	5	0	3	0	4
4	4	0	0	0	8
5	0	0	0	1	11
6	0	0	0	0	12
7	1	0	1	1	9
8	0		1	1	10

Discussion

In the present study, IPS Empress 2 and In-Ceram crowns were divided into groups containing 12 crowns fabricated by a single operator simulating the maxillary left first premolar. A minimum of 10 specimens is recommended to perform SBS testing (Fox *et al.*, 1994). However, a sample size greater than 10 per group is recommended for bond strength testing of natural teeth where variations in tooth shape exist (Eliades and Brantley, 2000). Maxillary premolar teeth are the teeth most frequently extracted as an integeral part of orthodontic therapy. Therefore, the premolar tooth form was selected to allow clinical simulation and to compare the outcome of the present study with previously reported investigations (Barbosa *et al.*, 1995; Bourke and Rock, 1999; Kocadereli *et al.*, 2001).

In this study, the SBS of HFA-etched crowns were significantly higher than those in the non-etched groups. This is in agreement with the findings of Al Edris *et al.* (1990) where a threefold increase in bond strength was found after the application of HFA to sandblasted ceramic surfaces. However, other authors found similar bond strength between HFA-etched and sandblasted non-etched groups (Zachrisson *et al.*, 1996; Bourke and Rock, 1999; Kocadereli *et al.*, 2001) while others observed higher values with sandblasting than with acid etching (Schmage *et al.*, 2003; Turk *et al.*, 2006; Karan *et al.*, 2007). Karan *et al.* (2007) compared the effect of sandblasting alone with that of sandblasting and HFA etching on three ceramic groups, namely feldspathic, lucite-based ceramics, and lithium disilicate ceramics. They

Table 6 Levels of significance for the Adhesive Remnant Index scores for the tested groups (see Table 1).

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Group 1			**	*				
Group 2			**	*				
Group 3	**	**			**	**		*
Group 4	*	*				*		
Group 5			**					
Group 6			**					
Group 7								
Group 8			*					

*$P < 0.05$, **$P < 0.01$.

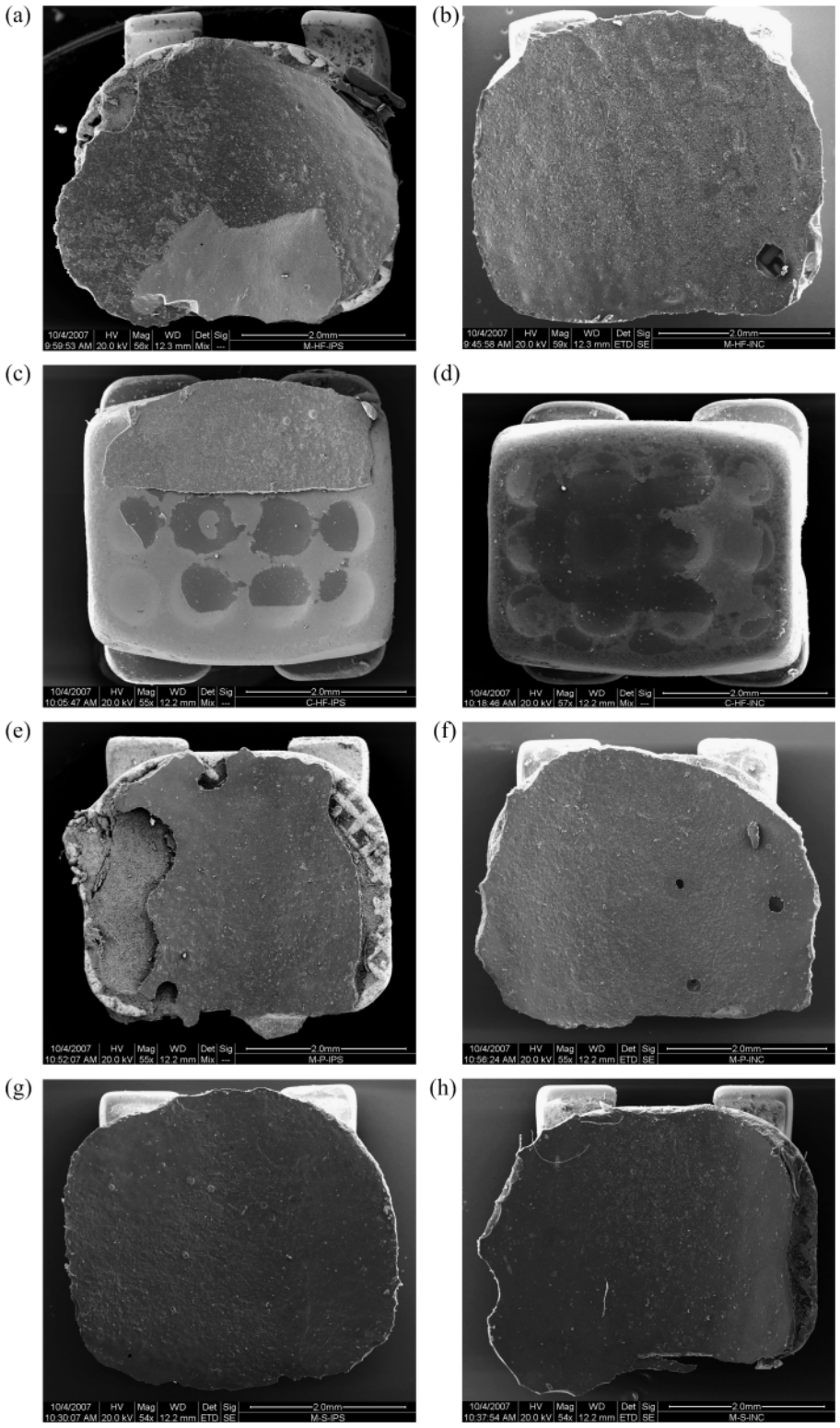

Figure 1 Scanning electron photomicrographs of (a) metal bracket debonded from hydrofluoric acid (HFA)-etched IPS Empress 2 crown; (b) metal bracket debonded from HFA-etched In-Ceram crown; (c) ceramic bracket debonded from HFA-etched IPS Empress 2 crown; (d) ceramic bracket debonded from HFA-etched In-Ceram crown; (e) metal bracket debonded from phosphoric acid-etched IPS Empress 2 crown; (f) metal bracket debonded from phosphoric acid-etched In-Ceram crown; (g) metal bracket debonded from sandblasted non-etched IPS Empress 2 crown; (h) metal bracket debonded from sandblasted non-etched In-Ceram crown.

reported that the SBS of the acid-etched groups was lower than those of the sandblasted non-etched groups.

In this study, it was found that the phosphoric acid-etched groups had similar bond strengths to those etched with HFA. This is in agreement with Nebbe and Stein (1996), Bourke and Rock (1999), Pannes *et al.* (2003) and Larmour *et al.* (2006) but in contrast to Ajlouni *et al.* (2005).

In the present investigation, metal and ceramic brackets had similar SBS values. This is in agreement with the findings of Willems *et al.* (1997).

No significant differences were found with all surface preparation techniques between the IPS Empress 2 and In-Ceram ceramic groups. However, Turk *et al.* (2006) reported that lithium disilicate had a higher SBS than feldspathic porcelain restorations. Moreover, Abu Alhaija and Al-Wahadni (2007) observed significant differences between feldspathic and lithium disilicate ceramic restorations, with a higher mean SBS reported in the feldspathic porcelain group. This may be due to differences in the processing methods and the molecular structure of the two all-ceramic restorations.

In the present study, a high incidence of adhesive bond failure (scores 4 and 5) was observed for all metal bracket groups. In the ceramic bracket groups, mainly cohesive bond failures (score 1) were observed. These findings are similar to those reported by Willems *et al.* (1997).

Table 7 Porcelain Fracture Index (PFI) for the tested groups (see Table 1).

PFI scores				
Group	0	1	2	3
Group 1	5	4	3	0
Group 2	2	2	3	5
Group 3	5	5	2	0
Group 4	6	2	2	2
Group 5	10	1	0	1
Group 6	6	0	0	6
Group 7	12	0	0	0
Group 8	10	0	2	0

It was also observed that neither the type of ceramic materials nor surface conditioning protocol affected the ARI scores within the groups. These results are similar to the findings of Zachrisson *et al.* (1996), Bourke and Rock (1999), and Turk *et al.* (2006) who all reported adhesive type bond failures.

There was a significant difference in ceramic cohesive failure pattern between the different methods of surface preparation. More ceramic fractures were present in the HFA etch IPS Empress 2 and In-Ceram crowns (groups 1–4) compared with those present within sandblasted specimens, whereas phosphoric acid etched and sandblasted IPS Empress 2 and In-Ceram crowns (groups 5–8) were comparable. This finding is in agreement with those of Bourke and Rock (1999). Larmour *et al.* (2006) reported a similar amount of ceramic cohesive failure among both phosphoric and HFA-etched groups, while Karan *et al.* (2007) found that sandblasting lucite-based IPS Empress revealed more ceramic fractures than HFA-etched surfaces.

Although *in vitro* bond strength studies are useful to provide information about new adhesive materials and bonding techniques, *in vitro* bond strength data should be interpreted with caution. A major drawback of *in vitro* bond strength studies is the difficulty in simulating the complex nature of the oral environment. Variations in temperature, stresses, humidity, acidity, and plaque are impossible to reproduce in the laboratory.

Conclusions

1. Both metal and ceramic brackets bonded to HFA-etched IPS Empress 2 and In-Ceram crowns resulted in a similar SBS.
2. The type of surface treatment was the only factor that significantly affected SBS.
3. The pattern of bond failure of metal brackets was at the adhesive–restorative interface, whereas for the ceramic brackets it was at the adhesive–bracket interface.
4. The greatest incidence of ceramic fracture after debonding was observed in the HFA-etched IPS Empress 2 and In-Ceram groups.

Table 8 Levels of significance for the Porcelain Fracture Index scores for the tested groups (see Table 1).

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Group 1						**	**	
Group 2					**		***	*
Group 3							**	
Group 4							*	
Group 5		**						
Group 6	**							*
Group 7	**	***	**	*		**		
Group 8		*				*		

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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References

Abu Alhaija E S, Al-Wahadni A M 2007 Shear bond strength of orthodontic brackets bonded to different ceramic surfaces. European Journal of Orthodontics 29: 386–389

Ajlouni R, Bishara S E, Oonsombat C, Soliman M, Laffoon J 2005 The effect of porcelain surface conditioning on bonding orthodontic brackets. Angle Orthodontist 75: 858–864

Al Edris A, Al Jabr A, Cooley R L, Barghi N 1990 SEM evaluation of etch patterns by three etchant on three porcelains. Journal of Prosthetic Dentistry 64: 734–739

Årtun J, Bergland S 1984 Clinical trials with crystal growth conditioning as an alternative to acid-etch pretreatment. American Journal of Orthodontics 85: 333–340

Barbosa V L, Almeida M A, Chevitarese O, Keith O 1995 Direct bonding to porcelain. American Journal of Orthodontics and Dentofacial Orthopedics 107: 159–164

Bishara S E, VonWald L, Olsen M E, Laffoon J F 1999 Effect of time on the shear bond strength of glass ionomer and composite orthodontic adhesives. American Journal of Orthodontics and Dentofacial Orthopedics 116: 616–620

Bourke B M, Rock W P 1999 Factors affecting the shear bond strength of orthodontic brackets to porcelain. British Journal of Orthodontics 26: 285–290

Cochran D, O'Keefe K L, Turner D T, Powers J M 1997 Bond strength of orthodontic composite cement to treated porcelain. American Journal of Orthodontics and Dentofacial Orthopedics 111: 297–300

Eliades T, Brantley W A 2000 The inappropriateness of conventional orthodontic bond strength assessment protocols. European Journal of Orthodontics 22: 13–23

Fox N A, McCabe J F, Buckley J G 1994 A critique of bond strength testing in orthodontics. British Journal of Orthodontics 21: 33–43

Kao E, Boltz K, Johnston W 1988 Direct bonding of orthodontic brackets to porcelain veneer laminates. American Journal of Orthodontics and Dentofacial Orthopedics 94: 458–468

Karan S, Buyukilmaz T, Toroglu M S 2007 Orthodontic bonding to several ceramic surfaces: are there acceptable alternatives to conventional methods? American Journal of Orthodontics and Dentofacial Orthopedics 132: 144.e7–144.e14

Kern M, Thompson V P 1994 Sandblasting and silica coating of a glass-infiltrated alumina ceramic: volume loss, morphology and changes in the surface composition. Journal of Prosthetic Dentistry 71: 453–461

Kocadereli I, Canay S, Akça K 2001 Tensile bond strength of ceramic orthodontic brackets bonded to porcelain surfaces. American Journal of Orthodontics and Dentofacial Orthopedics 119: 617–620

Larmour C J, Bateman G, Stirrups D R 2006 An investigation into the bonding of orthodontic attachments to porcelain. European Journal of Orthodontics 28: 74–77

Major P W, Koehler J R, Manning K E 1995 24-hour shear bond strength of metal orthodontic brackets bonded to porcelain using various adhesion promoters. American Journal of Orthodontics and Dentofacial Orthopedics 108: 322–329

Nebbe B, Stein E 1996 Orthodontic brackets bonded to glazed and deglazed porcelain surfaces. American Journal of Orthodontics and Dentofacial Orthopedics 109: 431–436

Nelson E, Barghi N 1989 Effect of APF etching time on resin bonded porcelain. Journal of Dental Research 68: 271 (abstract)

Newman S M, Dressler K B, Grenadier M R 1984 Direct bonding of orthodontic brackets to esthetic restorative materials using a silane. American Journal of Orthodontics 86: 503–506

Pannes D D, Bailey D K, Thompson J Y, Pietz D M 2003 Orthodontic bonding to porcelain: a comparison of bonding systems. Journal of Prosthetic Dentistry 89: 66–69

Rosenblum M A, Schulman A 1997 A review of all-ceramic restorations. Journal of the American Dental Association 128: 297–307

Schmage P, Nergiz I, Herrmann W, Ozcanqzcan M 2003 Influence of various surface conditioning methods on the bond strength of metal brackets to ceramic surfaces. American Journal of Orthodontics and Dentofacial Orthopedics 123: 540–546

Stangel I, Nathanson S, Hsu C 1987 Shear strength of composite bond to etched porcelain. Journal of Dental Research 66: 1460–1465

Turk T, Sarac D, Sarac Y S, Elekdag-Turk S 2006 Effects of surface conditioning on bond strength of metal brackets to all-ceramic surfaces. European Journal of Orthodontics 28: 450–456

Willems G, Carels C E, Verbeke G 1997 *In vitro* peer/shear bond strength evaluation of orthodontic bracket base design. Journal of Dentistry 25: 271–278

Winchester L 1991 Direct orthodontic bonding to porcelain: an *in vitro* study. British Journal of Orthodontics 18: 299–308

Wood D P, Jordan R E, Way D C, Galil K A 1986 Bonding to porcelain and gold. American Journal of Orthodontics 89: 194–205

Yen T W, Blackman R B, Baez R J 1993 Effect of acid etching on the flexural strength of a feldspathic porcelain and a castable glass ceramic. Journal of Prosthetic Dentistry 70: 224–233

Zachrisson B U 2000 Orthodontic bonding to artificial tooth surfaces: clinical versus laboratory findings. American Journal of Orthodontics and Dentofacial Orthopedics 117: 592–594

Zachrisson Y O, Zachrisson B U, Buyukyilmaz T 1996 Surface preparation for orthodontic bonding to porcelain. American Journal of Orthodontics and Dentofacial Orthopedics 109: 420–430

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Bond strength of ceramic brackets bonded to enamel with amorphous calcium phosphate-containing orthodontic composite

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SUMMARY The aim of this *in vitro* study was to compare the shear bond strength (SBS) and failure modes of a conventional resin-based composite with a recently developed amorphous calcium phosphate (ACP)-containing orthodontic composite system. Forty freshly extracted human maxillary premolar teeth were randomly divided into two equal groups. Conventional composite (group 1; Transbond-XT®; 3M Unitek) and ACP-containing orthodontic composite (group 2; Aegis-Ortho®; Harry J. Bosworth Co.) were used for bonding ceramic orthodontic brackets. The SBS of these brackets were measured and recorded in megapascals (MPa). Adhesive remnant index (ARI) scores were determined after bracket failure. Data were analysed with a Student's *t*-test for two independent variables and Pearson's chi-square tests.

Statistical analysis showed that the bond strength of group 1 (mean: 36.7±6.8 MPa) was significantly higher than group 2 (mean: 24.2±5.4 MPa; $P<0.01$). Although a greater percentage of the fractures were cohesive at the composite interface (Score 1 + Score 2=70 per cent in group 1 and 90 per cent in group 2), statistically significant differences were observed between the groups ($P<0.05$). The ACP system is suitable for bonding ceramic orthodontic brackets due to the lower SBS values compared with conventional composite. The ACP-containing composite is recommended for use in clinical orthodontic practice in order to achieve lower decalcification scores under ceramic orthodontic brackets.

Introduction

White spot lesions are not only unaesthetic but they may also become irreversible and lead to lesions. For these reasons, white spot lesions are of concern to orthodontists, and advancements in orthodontic adhesive materials serve as one possible avenue to prevent this occurrence (Foster *et al.*, 2008).

Schumacher *et al.* (2007) developed a biologically active restorative material that may stimulate the repair of tooth structure through the release of components, including calcium and phosphate. This material contains amorphous calcium phosphate (ACP) as the bioactive filler encapsulated in a polymer binder (Skrtic *et al.*, 2003, 2004a; Antonucci and Skrtic, 2005). ACP has the properties of both a preventive and a restorative material that justify its use as a dental sealant, composite, and more recently, an orthodontic adhesive. ACP-filled composite resins have been shown to recover 71 per cent of the lost mineral content of decalcified teeth (Antonucci and Skrtic, 2005). One ACP-containing composite, Aegis Ortho (Harry J. Bosworth Co., Skokie, Illinois, USA), has been marketed for use as a light-cured orthodontic composite with similar properties to previously used resins. These materials encourage the formation of hydroxyl-apatite, which in turn can be used by the tooth for remineralization (Skrtic *et al.*, 1996). This can be maintained for a considerable time, offering a promising antagonist to demineralization, and can promote the prevention of future white spots throughout orthodontic treatment (Skrtic *et al.*, 2004b).

With metal brackets, the critical question for the clinician was whether the bond was too weak to withstand the forces applied during orthodontic treatment. With ceramic brackets, the concern was whether the bond was too strong for safe debonding (Bishara, 2000). Since ceramic brackets do not bend during debonding, it is necessary to break the adhesive force of the composite or the cohesive force between the bracket and adhesive system (Verstrynge *et al.*, 2004). Debonding forces can break the ceramic bracket or the adhesive system at the tooth/resin surface, which often creates cracks in susceptible enamel. Although attempts have been made to reduce the shear bond strength (SBS) of ceramic brackets by changing the composites, the etchants, or the etching times, no consistent methods have been found that would apply to all types of ceramic brackets (Chaconas *et al.*, 1991).

Recently, the remineralization potential (Skrtic *et al.*, 2003; Antonucci and Skrtic, 2005) and bond strengths (Dunn, 2007; Foster *et al.*, 2008) of ACP-containing materials used either with metallic bracket or lingual retainer composite (Uysal *et al.*, 2009b) have been investigated. In an *in vitro* study, Dunn (2007) concluded that metallic brackets bonded to teeth with an ACP-containing composite material failed at significantly lower forces than those bonded with an orthodontic resin-based

composite. Uysal *et al.* (2009b) reported significantly lower SBS values for Aegis Ortho when used as a lingual retainer composite. The aim of this study was to reduce the SBS values of ceramic brackets by changing the composite type, using a newly introduced composite, for minimizing the possible enamel fracture risks during debonding and to compare the SBS and failure modes of a conventional composite with a recently developed ACP-containing orthodontic composite system. The null hypothesis assumed that there were no statistically significant differences between (1) the SBS and (2) the site of bond failure of ceramic brackets bonded to enamel either with a conventional composite or with an ACP-containing orthodontic composite system.

Materials and methods

Forty human maxillary premolars were used in this study. All teeth were newly extracted for orthodontic reasons and presented no caries, cracks, or fissures. The criteria for tooth selection dictated no pre-treatment with a chemical agent, such as alcohol, formalin, or hydrogen peroxide, or any other form of bleaching. Their buccal surfaces were intact, and they had not been subjected to orthodontic or endodontic treatment.

Immediately after extraction, the teeth were cleaned of any residual tissue tags, washed under running tap water and stored in distilled water, which was changed weekly to avoid bacterial growth. The roots of the teeth were placed vertically in a self-cure acrylic resin so that the crowns were exposed, avoiding contact between the resin and crown. The buccal surfaces were cleaned and polished with a rubber cup and slurry with pumice and water, then rinsed with a water spray and finally dried with compressed air.

The enamel surfaces were acid etched with phosphoric acid gel (35 per cent acid etch; Harry J. Bosworth Co.) for 30 seconds, rinsed for 15 seconds with sterile water from an air/water syringe, and dried with oil- and moisture-free air. In all etched cases, a frosty white appearance of the enamel was present. Ceramic brackets (Clarity™, metal-reinforced ceramic bracket, 0.022 inch slot; 3M Unitek, Monrovia, California, USA) were bonded to the teeth using the bonding protocols recommended by the manufacturer. The average surface of the orthodontic bracket base according to the manufacturer was 14.54 mm^2.

Group 1 (control group): Transbond XT® (3M Unitek) primer was applied to the etched surface in a thin film and left uncured. Transbond XT® (3M Unitek) composite paste was applied to the bracket base, and the bracket was positioned on the tooth and pressed firmly into place. The excess composite was removed from around the bracket with a scaler.

Group 2: A thin layer of ACP-containing orthodontic composite (Aegis Ortho; Harry J. Bosworth Co.) was applied to the etched enamel. A thin layer of the composite was also applied to the base of the ceramic bracket and immediately pressed into the composite on the tooth surface. Following the manufacturer's recommendations, excess composite was not removed.

A quartz tungsten halogen light unit (Hilux 350; Express Dental Products, Toronto, Canada) with a 10 mm diameter light tip was used for curing the specimens from the mesial and distal for 10 seconds each (total time 20 seconds). The specimens were then stored in distilled water at 37°C for 24 hours before bond strength testing.

Debonding procedure

After completing the procedures, the embedded specimens were secured in a jig attached to the base plate of a universal testing machine (Hounsfield Test Equipment, Salford, Lancashire, UK). A chisel-edge plunger was mounted in the movable crosshead of the testing machine and positioned so that the leading edge was aimed at the enamel/composite interface. A crosshead speed of 0.5 mm/minute was used, and the maximum load necessary to debond the bracket was recorded. The force required to remove the brackets was measured in Newtons (N), and the SBS (1 MPa = 1 N/mm^2) was then calculated by dividing the force values by the bracket base area (14.54 mm^2).

Residual adhesive

After debonding, all teeth and brackets were examined under ×10 magnification (SZ 40; Olympus, Tokyo, Japan). The amount of adhesive remaining on the enamel surface was coded using the criteria proposed in the adhesive remnant index (ARI) of Årtun and Bergland (1984):

0 = no adhesive remains on the tooth surface
1 = less than half the adhesive remains on the tooth surface
2 = more than half the adhesive remains on the tooth surface
3 = all the adhesive remains on the tooth surface.

Statistical methods

All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS for Windows 13.0; SPSS, Chicago, Illinois, USA). Descriptive statistics, including the mean, standard deviation, minimum and maximum values, were calculated for the two groups. Shapiro–Wilks normality and Levene's variance homogeneity tests were applied to the SBS data. The data showed normal distribution, and there were homogeneity of variances among the groups. A Student's *t*-test was used to compare the SBS data of the two composites. Fracture modes were analysed using a Pearson's chi-square test. Significance was predetermined at $P < 0.05$.

Results

The descriptive statistics for each group are presented in Table 1. The mean difference between the SBS of groups

1 and 2 was −12.5 MPa, 95 per cent confidence interval, $t = 3.749$, df = 28, and $P = 0.001$. The results of the Student's *t*-test for independent samples revealed statistically significant differences in bond strength between the two groups ($P < 0.01$). Thus, the first null hypothesis was rejected. Statistical testing showed that the SBS of group 1 (mean: 36.7 ± 6.8 MPa) was significantly higher than that of group 2 (mean: 24.2 ± 5.4 MPa).

The failure modes of the specimens are shown in Table 2. Although a greater percentage of the fractures were cohesive at the composite interface (Score 1 + Score 2 = 70 per cent in group 1 and 90 per cent in group 2), statistically significant differences were found between the groups ($P < 0.05$). Therefore, the second null hypothesis of this study was rejected. A significant difference in ARI scores was observed between the groups.

Discussion

Many adult patients demand high quality orthodontic treatment with the use of ceramic brackets. On the other hand, many clinicians complain about the side effects of these brackets because of their higher bond strength. However, a review of the literature indicated that no research had been published that evaluated and compared the effect of the SBS of ceramic orthodontic brackets bonded with ACP composite.

The development and incorporation of ACP materials in dentistry is an alternative approach to reverse the effects of demineralization on enamel surfaces. Only a few articles have investigated the incorporation of calcium phosphates into orthodontic composites. Sudjalim *et al.* (2007) evaluated the effects of sodium fluoride (NaF) and 10 per cent casein phosphopeptide (CPP)-ACP on enamel demineralization adjacent to orthodontic brackets and found that application of CPP-ACP, NaF, or CPP-ACP/NaF can significantly prevent enamel demineralization when orthodontic composite resin is used for bonding. Recently, investigations related to a commercially available orthodontic ACP-containing composite were performed. Dunn (2007) and Foster *et al.* (2008) compared the SBS of metallic orthodontic brackets bonded to enamel using ACP-containing composite with that of brackets bonded with a conventional resin-based orthodontic composite and found low but acceptable bond strengths. The present results support the previous findings that the use of ACP-containing composite significantly decreases SBS values when compared with that of a conventional composite.

It should be noted that the SBS range for the ACP-containing composite (16.0–34.0 MPa) was lower than that of the Transbond XT® group (22.0–48.0 MPa), perhaps due to its lower maximum bond strength. This may partly account for its low standard deviation (Table 1). Nonetheless, the ACP-containing composite produced a consistent bond.

Reynolds (1975) suggested that a minimum bond strength of 5.9–7.8 MPa is adequate for bonding brackets. Tavas and Watts (1984) reported that shear/peel strengths of direct bonded adhesives should develop to 4 kg in 5 minutes and 6 kg in 24 hours. The SBS values of the different brackets used in this study were greater than this minimum requirement and were therefore within clinically acceptable ranges. Ceramic orthodontic brackets bonded with Aegis-Ortho® showed a lower bond strength than those bonded with Transbond XT®. These lower SBS values for ceramic brackets were considered acceptable. However, clinical conditions may differ significantly from an *in vitro* setting. Clinically, composites are subject to stresses, temperature fluctuations, variable electrolytes, microorganisms, and other factors that may affect their performance.

The sites of failure within the bracket–resin–enamel complex may occur within the bracket itself, between the bracket and the resin, within the resin, and between the tooth surface and the resin. Bond failure at the bracket-resin interface or within the resin is more desirable than at the resin–enamel interface because enamel fractures and cracks have been reported during bracket debonding especially with ceramic brackets (Bishara *et al.*, 1997). Earlier reports on the bond failure site showed that metal brackets consistently failed at the resin–bracket base interface, whereas ceramic brackets with chemically retained bases primarily failed at the resin–enamel interface (Joseph and Rossouw, 1990). For mechanically retained brackets, the most common failure site was the bracket–resin interface, and, on average, more than 50 per cent of the resin remained on the teeth after debonding

Table 1 Descriptive statistics and *t*-test results of the bond strength comparison of the two composites tested.

Groups tested	*n*	Bond strength (MPa)				Significance
		Mean	Standard deviation	Minimum	Maximum	
Transbond XT	20	36.7	6.8	22.0	48.0	**($P < 0.01$)
ACP-containing composite	20	24.2	5.4	16.0	34.0	

Table 2 Modes of failure after shear bond testing using the adhesive remnant index (ARI).

Groups tested	*N*	ARI scores				Significance
		0	1	2	3	
		n (%)	*n* (%)	*n* (%)	*n* (%)	
Transbond XT	20	4 (20)	6 (30)	8 (40)	2 (10)	*($P < 0.05$)
ACP-containing composite	20	2 (10)	15 (75)	3 (15)	0 (0)	

(Forsberg and Hagberg, 1992). In the present study, ARI scores were predominantly 1–2 in the subgroups, but the differences were statistically significant. These scores indicate that the mode of failure was primarily at the resin–resin interface, and the risk of enamel fracture is therefore decreased. The results of this study showed that, although higher bond strength values were obtained in the Transbond XT® group compared with those in the ACP-containing composite group, acceptable ARI scores were recorded for both composites. This can be desirable because of less damage or fracturing of the enamel during debonding of ceramic brackets.

ACP-containing composite may be an adjunct in the prevention of white spot lesions especially where compliance is lacking. Uysal *et al.* (2009a) recommended the use of ACP-containing orthodontic composite for any at-risk orthodontic patient because they found that using ACP-containing orthodontic composite for bonding orthodontic brackets successfully inhibited caries *in vitro*.

The findings of the present study suggest that ACP-containing composite can provide a lower but suitable bond strength to at least one orthodontic composite and minimally meet the bond strength recommendations of different authors (Reynolds, 1975; Tavas and Watts, 1984). Future *in vivo* studies, examining the efficacy of these composites in preventing white spot lesions, appear warranted.

Conclusion

Bearing in mind the shortcomings of an *in vitro* setting, it was concluded that:

1. ACP-containing Aegis-Ortho® composite resulted in a significant decrease in bond strength of ceramic orthodontic brackets to etched enamel surface. However, all bond strength values were within clinically acceptable ranges.
2. Although bonding brackets to enamel prepared with ACP-containing composite or a conventional method did not significantly alter the site of failure, ceramic brackets bonded with ACP-containing composite can be beneficial due to the bond failure location occurring generally between the resin–resin interface during debonding resulting in less damage to the enamel.

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References

Antonucci J M, Skrtic D 2005 Matrix resin effects on selected physicochemical properties of amorphous calcium phosphate composites. Journal of Bioactive and Compatible Polymers 20: 29–49

Årtun J, Bergland S 1984 Clinical trials with crystal growth conditioning as an alternative to acid-etching enamel pretreatment. American Journal of Orthodontics 85: 333–340

Bishara S E 2000 Ceramic brackets and the need to develop national standards. American Journal of Orthodontics and Dentofacial Orthopedics 117: 595–597

Bishara S E, Olsen M E, VonWald L 1997 Evaluation of debonding characteristics of a new collapsible ceramic bracket. American Journal of Orthodontics and Dentofacial Orthopedics 112: 552–559

Chaconas S J, Caputo A A, Niu G S L 1991 Bond strength of ceramic brackets with various bonding systems. Angle Orthodontist 61: 35–42

Dunn W J 2007 Shear bond strength of an amorphous calcium-phosphate-containing orthodontic resin cement. American Journal of Orthodontics and Dentofacial Orthopedics 131: 243–247

Forsberg C M, Hagberg C 1992 Shear bond strength of ceramic brackets with chemical or mechanical retention. British Journal of Orthodontics 19: 183–189

Foster J A, Berzins D W, Bradley T G 2008 Bond strength of an amorphous calcium phosphate-containing orthodontic adhesive. Angle Orthodontist 78: 339–344

Joseph V P, Rossouw E 1990 The shear bond strengths of stainless steel and ceramic brackets used with chemically and light-activated composite resins. American Journal of Orthodontics and Dentofacial Orthopedics 97: 121–125

Reynolds I R 1975 A review of direct orthodontic bonding. British Journal of Orthodontics 2: 171–178

Schumacher G E, Antonucci J M, O'Donnell J N R, Skrtic D 2007 The use of amorphous calcium phosphate composites as bioactive basing materials. Their effect on the strength of the composite/adhesive/dentin bond. Journal of the American Dental Association 138: 1476–1484

Skrtic D, Antonucci J M, Eanes E D 2003 Amorphous calcium phosphate-based bioactive polymeric composites for mineralized tissue regeneration. Journal of Research and National Institutes and Standards Technology 108: 167–182

Skrtic D, Antonucci J M, Eanes E D, Eidelman N 2004a Dental composites based on hybrid and surface-modified amorphous calcium phosphates. Biomaterials 25: 1141–1150

Skrtic D, Antonucci J M, McDonough W G, Lui D W 2004b Effect of chemical structure and composition of the resin phase on mechanical strength and vinyl conversion of amorphous calcium phosphate-based composites. Journal of Biomedical Materials Research 68A: 763–772

Skrtic D, Hailer A W, Takagi S, Antonucci J M, Eanes E D 1996 Quantitative assessment of the efficacy of amorphous calcium phosphate/methacrylate composites in remineralizing caries-like lesions artificially produced in bovine enamel. Journal of Dental Research 75: 1679–1686

Sudjalim T R, Woods M G, Manton D J, Reynolds E C 2007 Prevention of demineralization around orthodontic brackets *in vitro*. American Journal of Orthodontics and Dentofacial Orthopedics 131: 705.e1-9

Tavas M A, Watts D C 1984 A visible light activated direct bonding material: an *in vitro* comparative study. British Journal of Orthodontics 11: 33–37

Uysal T, Amasyali M, Koyuturk A E, Sagdic D 2009a Efficiency of amorphous calcium phosphate–containing orthodontic composite and resin modified glass ionomer on demineralization evaluated by a new laser fluorescence device. European Journal of Dentistry 3: 127–134

Uysal T, Ulker M, Akdogan G, Ramoglu S I, Yilmaz E 2009b Bond strength of amorphous calcium phosphate–containing orthodontic composite used as a lingual retainer adhesive. Angle Orthodontist 79: 117–121

Verstrynge A, Ghesquiere A, Willems G 2004 Clinical comparison of an adhesive precoated vs. an uncoated ceramic bracket system. Orthodontics and Craniofacial Research 7: 15–20

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Effects of long-term storage and thermocycling on bond strength of two self-etching primer adhesive systems

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SUMMARY The effects of 2 years of storage and 6000 thermocycles on the shear bond strength (SBS) of two self-etching adhesive systems were studied. Two self-etching primer (SEP) systems (Transbond Plus and Beauty Ortho Bond) and one etch and rinse system (Transbond XT) were used to bond brackets to 126 human premolars that were then stored in artificial saliva for 24 hours or 2 years and thermocycled in distilled water before SBS testing with a universal testing machine. The adhesive remnant index (ARI) scores were calculated. Data were compared by two-way analysis of variance and chi-square analysis. Enamel/adhesive interfaces were examined by scanning electron microscopy.

There was no significant difference in the mean SBS for the bonding materials among the three conditions. ARI scores showed that Transbond XT and Beauty Ortho Bond had less adhesive remaining on the teeth after ageing compared with storage for 24 hours. Specimens bonded with Beauty Ortho Bond showed leakage between the resin adhesive and enamel after ageing. Both SEP systems produced adequate SBS even after 2 years or 6000 times thermocycling. Thermocycling is an appropriate technique for determining the durability of orthodontic bracket bonding materials.

Introduction

After the concept of acid etching was introduced by Buonocore (1955), the direct bonding of orthodontic appliances to enamel with composite resin was introduced by Newman (1965) and is now widely accepted by most orthodontists (Eliades and Eliades, 2001; Eliades *et al.*, 2001). Bonding practices based on a self-etching primer (SEP), which combine etching and priming into a single step, are now being used in clinical orthodontics (Cehreli *et al.*, 2005; Arhun *et al.*, 2006; Bishara *et al.*, 2006; Faltermeier *et al.*, 2007; Scougall Vilchis *et al.*, 2007; Iijima *et al.*, 2008). In addition to saving time and reducing procedural errors, their lower etching ability, due to their higher pH compared with phosphoric acid, might minimize the potential for iatrogenic damage to enamel (Pashley and Tay, 2001; Zeppieri *et al.*, 2003).

Bracket-bonding failure sometimes occurs during the later stages of treatment due to heavy forces produced by an archwire or occlusal force. Bracket bond failure is not only frustrating for the practitioner but can also significantly affect treatment efficiency and have an economic impact on a practice (Northrup *et al.*, 2007). Although it has been demonstrated that the shear bond strengths (SBSs) of brackets bonded with SEP adhesive systems were similar to those with a conventional etch and rinse adhesive system (Scougall Vilchis *et al.*, 2007; Iijima *et al.*, 2008), most of these bonding studies measured short-term adhesive bond strength and did not extend the study period to encompass the duration of normal orthodontic treatment. Recently, Oesterle and Shellhart (2008) studied the effect of composite ageing for two conventional etch and rinse adhesive systems on SBS during a normal 24 month orthodontic treatment period and concluded that the SBS of orthodontic brackets increases from 30 minutes to 24 hours and then tends to decrease over the next 24 months.

The most commonly used artificial ageing technique is long-term water storage. Another widely used ageing technique is thermocycling. The International Organization for Standardization (ISO) TR 11450 standard (1994) indicates that a thermocycling regimen comprising 500 cycles in water between 5 and 55°C is an appropriate artificial ageing test, and many studies have been carried out following the ISO standard. However, this number of cycles is probably too low to achieve a realistic ageing effect (Gale and Darvell, 1999). Recent studies in orthodontics have used various number of thermocycles: approximately 1500 cycles between 10 and 50°C after 3 months of storage (Trites *et al.*, 2004), 500 cycles between 5 and 55°C (Bishara *et al.*, 2007), and 6000 cycles between 5 and 55°C (Faltermeier *et al.*, 2007).

The purpose of this study was to investigate the effects of long-term storage (2 years) and thermocycling (6000 iterations) on the SBS of two SEP adhesive systems. The null hypothesis tested was that the SBS of the self-etching adhesive systems would decrease with long-term storage and thermocycling.

Materials and Methods

One hundred and twenty-six non-carious human maxillary premolars were used in this study. The teeth, which had been extracted for orthodontic reasons, were randomly divided into nine groups of 14 specimens for measurement of SBS. Selection criteria included the absence of any visible decalcification or cracking of the enamel surface under a stereomicroscope (SEM; SMZ 1500, Nikon, Tokyo, Japan) at a magnification of ×10. The extracted teeth were stored in a 0.5 per cent chloramine solution at approximately 4°C. The buccal surfaces of all teeth were cleaned using non-fluoridated pumice. The teeth were also polished using a rubber cup, thoroughly washed, and dried using a moisture-free air source.

Group 1: Transbond XT etch and rinse adhesive system. The enamel surfaces were treated with 35 per cent phosphoric acid etching gel (Transbond XT Etching Gel, 3M Unitek) for 15 seconds, washed for 20 seconds, and dried with oil-free air stream. Table 1 lists the bonding materials used in the present study and Figure 1 is a flow chart of the bracket-bonding instructions. Transbond XT primer was applied to the etched surface, and metal upper premolar brackets (Victory Series, 3M Unitek), with a base area of 10.0 mm^2, were bonded with Transbond XT composite (3M Unitek).

Group 2: Transbond Plus SEP adhesive system. Transbond Plus SEP (3M Unitek) was applied and rubbed on the enamel surfaces for approximately 3 seconds. An air jet was lightly applied to the enamel, and the brackets were bonded with Transbond XT composite.

Group 3: Beauty Ortho Bond SEP adhesive system. Beauty Ortho Bond primers A and B (Shofu) were mixed. The solution was then rubbed onto the enamel surfaces for approximately 3 seconds. An air jet was briefly applied to the enamel, and the brackets were bonded with Beauty Ortho Bond Paste (composite).

Each bonding procedure was performed by the same operator (MI). The excess bonding material was removed with a small scaler. All samples were light cured for 20 seconds (Jetlite 3000, J.Morita USA Inc., Irvine, California, USA) (10 seconds from each proximal side).

After bonding, the specimens were stored in artificial saliva at 37°C for 24 hours (T1) or 2 years (T2). A third group (T3) was thermocycled between 5 and 55°C for 6000 cycles after 24 hours of storage at 37°C in distilled water. SBS was then measured. The specimens were fixed to a custom-fabricated acrylic resin block using Model Repair II (Densply-Sankin, Tokyo, Japan) and the block was fixed to a universal testing machine (EZ Test, Shimadzu, Kyoto, Japan). A knife-edged shearing blade was secured to the crosshead with the direction of force parallel to the buccal surface and the bracket base. Force was applied directly to the bracket–tooth interface. The brackets were debonded at a crosshead speed of 0.5 mm/minute.

After bond failure, the bracket bases and enamel surfaces were examined with a SEM at a magnification of ×10. The adhesive remnant index (ARI) scores were used to assess the amount of adhesive left on the enamel surface (Årtun and Bergland, 1984).

The interface morphology between the adhesive resin and the intact enamel was evaluated under a SEM (SSX-550, Shimadzu). After the SBS was determined, the specimens were cut with a slow-speed water-cooled diamond saw (Isomet, Buehler, Lake Bluff, Illinois, USA), so that they were divided into occlusal and cervical halves; one half was encapsulated for observation of the adhesive interface. The specimens were then polished using a series of abrasives, finishing with a 1 μm diamond paste to obtain a suitable polished surface. The encapsulated specimens were immersed in 6 M hydrochloric acid for 40 seconds and then dehydrated in a graded series of ethanol and water up to 100 per cent ethanol. All specimens were sputter coated with gold (SC-701AT, Sanyu Electron, Tokyo, Japan) and examined under a SEM operating at 15 kV.

Table 1 Materials and instruction employed in present study.

Material	Manufacturer	Components (lot no.)	Composition	pH[a]	Instructions
Transbond XT	3M Unitek, Monrovia, California, USA	Etching gel: (6GN); primer: (5CL); paste: (6TG)	35% phosphoric acid, tetraethylene-glycol dimethacrylate (TEGDMA), bisphenol-A-diglycidel methacrylate (Bis-GMA); Bis-GMA, TEGDMA, silane-treated quartz, amorphous silica, camphorquinone	1.39	Etch enamel 15 seconds; rinse and air-dry; apply thin coat primer; apply adhesive to bracket; 20 seconds light curing
Transbond Plus self-etching system	3M Unitek	Self-etching primer: (237956E); paste: (6TG)	Water, methacrylated phosphoric acid, esters, amino benzoate, camphorquinone, Bis-GMA, TEGDMA, silane treated quartz, amorphous silica, camphorquinone	1.85	Apply primer 3 seconds; gentle air-dry; apply adhesive to bracket; 20 seconds light curing
Beauty Ortho Bond self-etching system	Shofu, Kyoto, Japan	Primer A: (030602); primer B: (030602); paste: (120503)	Water, acetone, others, phosphoric acid monomer, ethanol, TEGDMA, surface pre-reacted glass-ionomer, filler, Bis-GMA, camphorquinone	2.20	Apply primer 3 seconds; gentle air-dry; apply adhesive to bracket; 20 seconds light curing

[a]Published values.

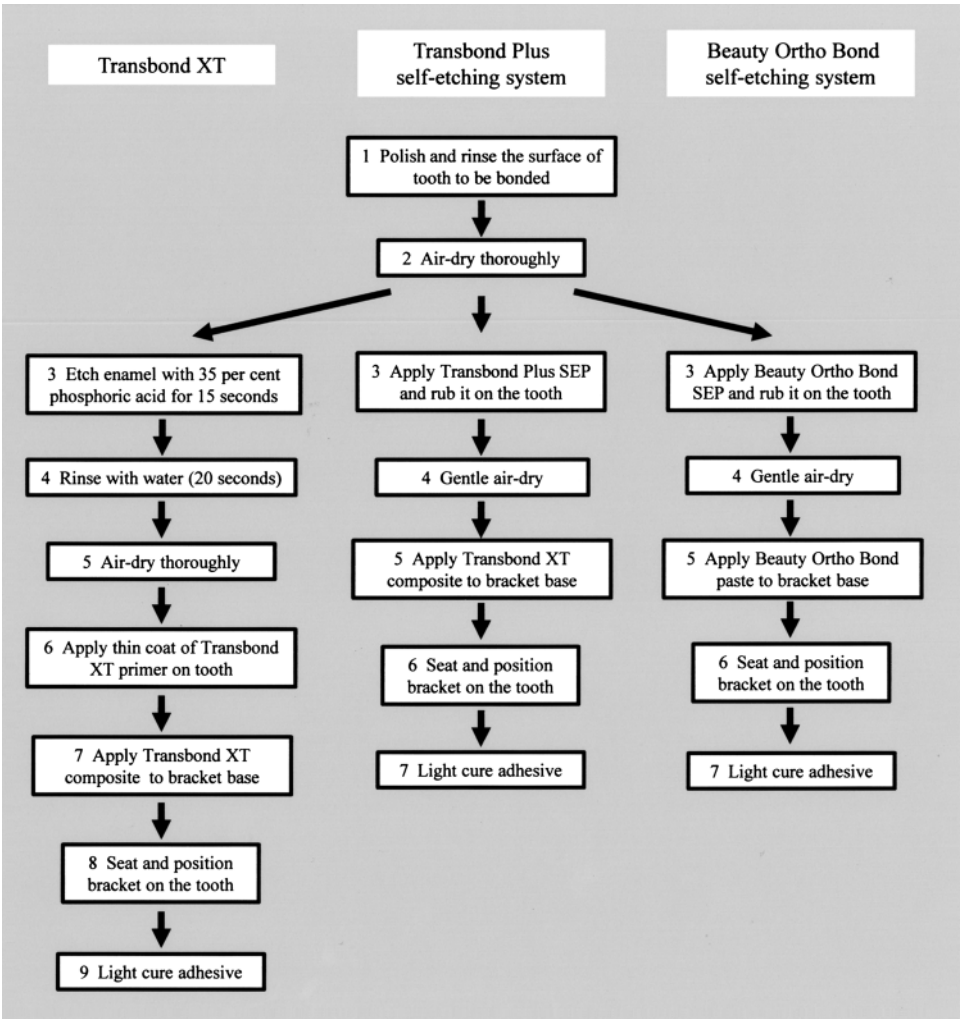

Figure 1 Flow chart of bracket bonding.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (version 14.0J for Windows, SPSS Inc., Chicago, Illinois, USA). The mean SBSs, along with the standard deviation (n = 14), for the groups of bonding materials were compared by two-way analysis of variance (ANOVA). The two factors for ANOVA were bonding material (Transbond XT etch and rinse, Transbond Plus SEP, Beauty Ortho Bond SEP) and storage method (24 hours, 2 years, thermocycling). Chi-square analysis was used to test the significance of differences in the distribution of ARI scores. The level of statistical significance was set at $P < 0.05$.

Results

The SBS results are shown in Figure 2. Two-way ANOVA showed that bonding material (Transbond XT etch and rinse adhesive system, Transbond Plus SEP adhesive system, Beauty Ortho Bond SEP adhesive system; $P = 0.000$) was a statistically significant factor. The storage method (24 hours, 2 years, thermocycling; $P = 0.408$) was not a statistically significant factor. Specimens bonded with Beauty Ortho Bond produced a significantly lower mean SBS (7.4 MPa) than those bonded with Transbond XT etch and rinse (9.8 MPa) or Transbond Plus SEP (9.1 MPa). There was no significant difference in the mean SBS among the three different ageing methods (T1, T2, and T3) for any of the bonding materials.

Chi-square analysis comparing the ARI scores for the three adhesives revealed a significant difference in the distribution of frequencies among the ARI categories for the three adhesive groups at each storage interval (Table 2). Transbond XT etch and rinse and Transbond Plus SEP had a greater frequency of ARI = 1 and 2, except for the Transbond Plus SEP with thermocycling (T3). On the other hand, the Beauty Ortho Bond SEP had a greater frequency of ARI = 2 and 3 for all three storage methods. Both of

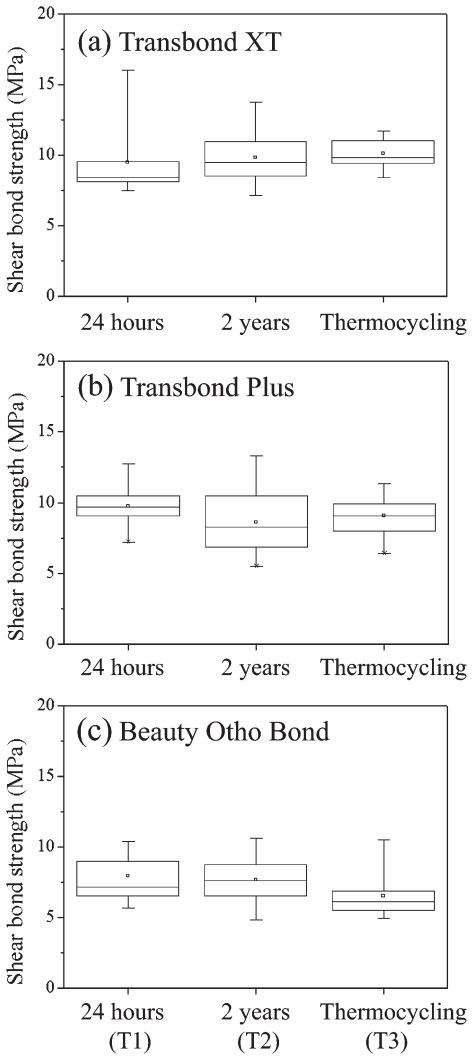

Figure 2 Shear bond strength (MPa) for (a) Transbond XT, (b) Transbond Plus, and (c) Beauty Ortho Bond. Horizontal bars in boxes are medians; 50 per cent of all values are within boxes. The horizontal bars represent the complete range of values. Small boxes within the boxes are average.

ageing methods (T2 and T3) for Transbond XT and Beauty Ortho Bond SEP showed less adhesive remaining on the teeth than the 24 hour storage group (T1).

Figure 3 shows the representative interfaces between the adhesive resin and the intact enamel after SBS testing for T1, T2, and T3, respectively. For Transbond XT, comparatively thick hybrid layers (Nakabayashi and Pashley, 1998), consisting of primer penetrating the surface enamel, were observed with tags ranging from 2 to 10 μm (Figure 3a). On the other hand, a distinct border was seen between the adhesive resin and the enamel for both self-etching adhesive systems (Transbond Plus and Beauty Ortho Bond) and the hybrid layers were less than 2 μm (Figure 3b and 3c). Tight contact between the adhesive resin and the enamel was observed for all specimens after 24 hours of storage (T1) and for the Transbond XT and the Transbond Plus SEP after 2 years of storage (T2) (Figure 3d and 3e) and thermocycling (T3) (Figure 3g and 3h). However, some of the Beauty Ortho Bond specimens showed leakage between the resin adhesive and enamel after 2 years of storage and thermocycling (Figure 3f and 3i).

Table 2 Frequency distribution of adhesive remnant index (ARI) scores.

		ARI scores					Mean
		1	2	3	4	5	
Transbond XT	24 Hours (T1)	10	3	1	—	—	1.4
	2 Years (T2)	4	9	—	1	—	1.9
	Thermocycling (T3)	—	11	3	—	—	2.2
Transbond Plus	24 Hours (T1)	4	6	4	—	—	2.0
	2 Years (T2)	6	6	2	—	—	1.7
	Thermocycling (T3)	1	8	3	1	—	2.4
Beauty Ortho Bond	24 Hours (T1)	4	8	2	—	—	1.9
	2 Years (T2)	1	3	8	2	—	2.8
	Thermocycling (T3)	—	5	6	3	—	2.1

ARI scores: 1, indicates all of the composite, with an impression of the bracket base, remains on the tooth surface; 2, more than 90% of the composite remains on the tooth surface; 3, more than 10% but less than 90% of the composite remains on the tooth surface; 4, less than 10% of composite remains on the tooth surface; 5, no composite remains on the tooth surface.

Discussion

The direct bonding of orthodontic appliances to enamel with acid etching, originally introduced by Newman (1965), has significantly improved the effectiveness of clinical orthodontics. Although acid etching of enamel may remove approximately 10–20 μm of enamel (Shinchi *et al.*, 2000; Powers and Messersmith, 2001), most clinicians accept acid etching of the enamel surface as a routine technique which may cause some iatrogenic effects such as the risk of enamel fracture, surface stains from increased surface porosity, discolouration by resin tags retained in the enamel, and loss of the outer enamel surface (Powers and Messersmith, 2001). Over the past decade, progress has been made in bonding enamel with resin-modified glass ionomers (Cehreli *et al.*, 2005) and SEPs (Cehreli *et al.*, 2005; Bishara *et al.*, 2006), and their lower etching ability might minimize the potential for iatrogenic damage to enamel.

The durability of SEP and resin-modified glass ionomers in clinical use must be evaluated. The most commonly used artificial ageing technique, especially in restorative dentistry, is long-term water storage. Thermocycling is another widely used ageing technique. Bishara *et al.* (2007) studied the effect of ISO standard thermocycling (500 × 5°C/55°C) on SBS for a resin-modified glass ionomer (Fuji Ortho LC) and a SEP adhesive system (Transbond Plus); their mean SBSs after thermocycling were at clinically acceptable levels (6.4 and 6.1 MPa, respectively). However, the number of cycles used in the ISO standard is probably too low to simulate the ageing

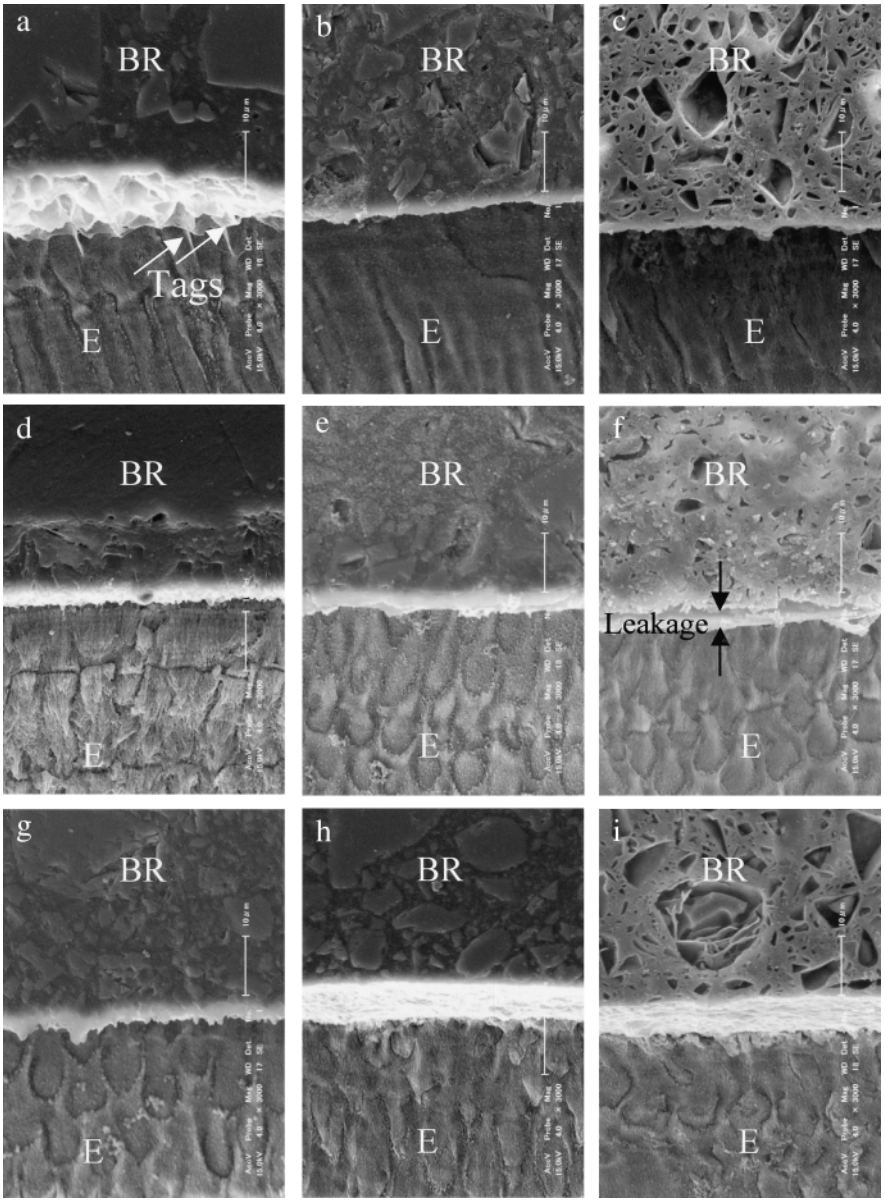

Figure 3 Scanning electron photomicrographs of the adhesive interface between the adhesive resin and the enamel after the shear bond strength (SBS) testing. The specimens were stored in artificial saliva at 37°C for 24 hours (a, Transbond XT; b, Transbond Plus; c, Beauty Ortho Bond), artificial saliva at 37°C for 2 years (d, Transbond XT; e, Transbond Plus; f, Beauty Ortho Bond), or thermocycled between 5 and 55°C for 6000 cycles after 24 hours of storage at 37°C in distilled water (g, Transbond XT; h, Transbond Plus; i, Beauty Ortho Bond) before SBS testing. BR, bonding resin; E, enamel. Magnification ×3000, bar = 10 μm.

effect during long-term orthodontic treatment. Faltermeier *et al.* (2007) compared SBS after thermocycling (6000 × 5°C/55°C) among one-, two- (self-etching systems), and three-component (etch and rinse system) adhesive systems and concluded that there was no significant difference in SBS between the two- and three-component adhesive systems, while the one-component adhesive had a lower bond strength. Most studies have used various types of thermocycling as an artificial ageing technique to understand the durability of bracket bonding but have not used long-term water storage. A decrease in bonding effectiveness is believed to be caused by degradation of the interface components by hydrolysis (Munck *et al.*, 2005). In addition, water can also infiltrate and weaken the mechanical properties of the polymer matrix (Ferracane *et al.*, 1998; Ito *et al.*, 2005). Although it is unclear whether the effect of thermocycling on bond strength is equal to that of long-term storage, limited information is available on the relative effects of thermocycling and long-term storage on bracket bonding. The present study measured SBS after 2 years, which almost corresponds to the duration of orthodontic

treatment with fixed appliances and compared the results to bond strength after thermocycling (6000 × 5°C/55°C). There was no significant difference in the mean SBS for any of the bonding materials among the three different storage methods (T1, 24 hours storage; T2, 2 years storage; T3, thermocycling between 5 and 55°C for 6000 cycles). These results confirm that thermocycling is an appropriate method for understanding the durability of orthodontic bracket-bonding materials. All the bonding materials used in the present study with both ageing methods had clinically acceptable levels of SBS.

The ARI scores in the present study showed that Transbond XT and Beauty Ortho Bond SEP adhesive systems had less adhesive remaining on the teeth with T2 and T3 than for the 24 hour storage group (T1). In SEM observation, some of specimens bonded with Beauty Ortho Bond showed leakage between the resin adhesive and enamel after artificial ageing. This may have been due to degradation of the interface components by hydrolysis since mild etching was carried out with a primer with a higher pH. As considerable chair time is needed to remove residual adhesive from the enamel surface, if brackets fail at the enamel–adhesive interface, there would be less residual adhesive, and this might be advantageous if the specimens still had an adequate bond strength.

Conclusions

Under the present study conditions, the following conclusions can be drawn:

1. Thermocycling is an appropriate method for understanding the durability of orthodontic bracket-bonding materials.
2. Both SEP adhesive systems, Transbond Plus and Beauty Ortho Bond, produced adequate SBS even after 2 years of storage and thermocycling between 5 and 55°C for 6000 cycles.

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References

Arhun N, Arman A, Sesen C, Karabulut E, Korkmaz Y, Gokalp S 2006 Shear bond strength of orthodontic brackets with 3 self-etch adhesives. American Journal of Orthodontics and Dentofacial Orthopedics 129: 547–550

Årtun J, Bergland S 1984 Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. American Journal of Orthodontics 85: 333–340

Bishara S E, Ajlouni R, Laffoon J F, Warren J J 2006 Comparison of shear bond strength of two self-etch primer/adhesive systems. Angle Orthodontist 76: 123–126

Bishara S E, Ostby A W, Laffoon J F, Warren J 2007 Shear bond strength comparison of two adhesive systems following thermocycling. Angle Orthodontist 77: 337–341

Buonocore M G 1955 A simple method of increasing the adhesion of acrylic filling materials to enamel surface. Journal of Dental Research 34: 849–850

Cehreli Z C, Kecik D, Kocadereli L 2005 Effect of self-etching primer and adhesive formations on the shear bond strength of orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 127: 573–579

Eliades T, Eliades G 2001 Orthodontic adhesive resins. In: Brantley W A, Eliades T (eds). Orthodontic materials: scientific and clinical aspects Thieme, Stuttgart, pp. 201–219

Eliades T, Kakaboura A, Eliades G, Bradley T G 2001 Comparison of enamel colour changes associated with orthodontic bonding using two different adhesives. European Journal of Orthodontist 23: 85–90

Faltermeier A, Behr M, Müssig D 2007 A comparative evaluation of bracket bonding with 1-, 2-, and 3-component adhesive systems. American Journal of Orthodontics and Dentofacial Orthopedics 132: 144.e1-5

Ferracane J L, Berge H X, Condon J R 1998 *In vitro* aging of dental composites in water–effect of degree of conversion, filler volume, and filler/matrix coupling. Journal of Biomedical Materials Research 42: 465–472

Gale M S, Darvell B W 1999 Thermal cycling procedures for laboratory testing of dental restorations. Journal of Dentistry 27: 89–99

Iijima M, Ito S, Yuasa T, Muguruma T, Saito T, Mizoguchi I 2008 Bond strength comparison and scanning microscopic evaluation of three orthodontic bonding systems. Dental Materials Journal 27: 392–399

Ito S *et al.* 2005 Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 26: 6449–6459

Munck J D *et al.* 2005 A critical review of the durability of adhesion of tooth tissue: methods and results. Journal of Dentistry 84: 118–132

Nakabayashi N, Pashley D H 1998 Hybridization of dental hard tissue. Quintessence Publishing Co., Tokyo, pp. 6–7

Newman G V 1965 Epoxy adhesives for orthodontic attachments: progress report. American Journal of Orthodontics 51: 901–912

Northrup R G, Berzins D W, Bradley T G, Schuckit W 2007 Shear bond strength comparison between two orthodontic adhesives and self-ligating and conventional brackets. Angle Orthodontist 77: 701–706

Oesterle L J, Shellhart W C 2008 Effect of aging on the shear bond strength of orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 133: 716–720

Pashley D H, Tay F R 2001 Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dental Materials 17: 430–444

Powers J M, Messersmith M L 2001 Enamel etching and bond strength. In: Brantley W A, Eliades T (eds). Orthodontic materials: scientific and clinical aspects Thieme, Stuttgart, pp. 105–122

Scougall Vilchis R J, Yamamoto S, Kitai N, Hotta M 2007 Shear bond strength of a new fluoride-releasing adhesive. Dental Materials Journal 26: 45–51

Shinchi M J, Soma K, Nakabayashi N 2000 The effect of phosphoric acid concentration on resin tag length and bond strength of a photo-cured resin to acid-etched enamel. Dental Materials 16: 324–329

Trites B, Foley T F, Banting D 2004 Bond strength comparison of 2 self-etching primers over a 30 month storage period. American Journal of Orthodontics and Dentofacial Orthopedics 126: 709–716

Zeppieri I L, Chung C-H, Mante F K 2003 Effect of saliva on shear bond strength of an orthodontic adhesive used with moisture-insensitive and self-etching primers. American Journal of Orthodontics and Dentofacial Orthopedics 124: 414–419

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The *in vitro* effect of repeated bonding on the shear bond strength with different enamel conditioning procedures

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SUMMARY The aims of this study were to evaluate the effect on shear bond strength (SBS), adhesive remnant, and enamel surface of repeated bonding of new brackets on the same tooth using different methods of enamel conditioning.

One hundred and thirty-five bovine incisors were used. Brackets were bonded to enamel using one of the following conditioning procedures: (1) 37 per cent phosphoric acid, (2) 37 per cent phosphoric acid (prior to first bond but not for further bonds), (3) Transbond Plus Self Etching Primer® (TSEP), and (4) non-rinse-conditioner (NRC). Brackets were sequentially bonded and debonded three times following the same conditioning procedure with the exception of group 2 where 37 per cent phosphoric acid was not reapplied prior to the second and third bonding sequences. SBS and adhesive remnant were evaluated for each debond. Scanning electron microscopy observations were made for each conditioning sequence. Statistical analysis was undertaken using ANOVA, Mann–Whitney, and Kruskal–Wallis tests.

Bond strength and adhesive remnant values were similar across the four groups for the first and second bonding sequences. At the third sequence, SBS was significantly less ($P < 0.008$) for group 2 (5.71 ± 1.56 MPa) than for group 1 (9.42 ± 2.75 MPa) and the adhesive remnant was significantly lower ($P < 0.008$) for group 2 (6.93% ± 3.34) than for the other groups (group 1: 16.95 ± 4.99 per cent, group 3: 14.40 ± 5.11 per cent, and group 4: 14.60 ± 5.33 per cent). When comparing the SBS and adhesive remnant of the three bonding/debonding sequences within each group, both the SBS and adhesive remnant for group 2 (SBS: 5.71 ± 1.56 MPa and adhesive remnant: 6.93 ± 3.34%) at the third sequence were significantly less ($P < 0.017$) than at the first (SBS: 10.44 ± 3.55 MPa and adhesive remnant: 13.81 ± 5.59%) and second (SBS: 9.23 ± 2.69 MPa and adhesive remnant: 15.32 ± 6.85%) sequences. Enamel changes were similar across all groups.

TSEP and NRC produced bonds that were similar to acid etching. When acid etching is used, it is possible to avoid etching for a second bond but not for following bonds.

Introduction

One of the most frequent problems facing the orthodontic clinician is bracket bond failure (Murfitt *et al.*, 2006; Banks and Thiruvenkatachari, 2007; Elekdag-Turk *et al.*, 2008), usually due to the patient applying excessive masticatory force, an inappropriate bonding technique or contamination of the tooth area during bonding (Donker *et al.*, 2001; Thiyagarajah *et al.*, 2006). Sometimes, the practitioner debonds a bracket in order to reposition it more appropriately to achieve a better outcome.

Whatever the cause of bond failure, repeated bonding on the same tooth involves both the removal of the bond material remaining on the tooth (Hong and Lew, 1995) and the repetition of acid etching. Both procedures are accompanied by some loss of the fluoride-rich surface enamel (Campbell, 1995; Tüfekçi *et al.*, 2004; Kim *et al.*, 2007).

Bonding brackets directly onto tooth enamel became possible with the introduction of the acid-etching technique developed by Buonocuore (1955). Since then, refinements of this technique have aimed to reduce both chair time and possible enamel loss. Weaker concentrations of phosphoric acid have been used and application time has been reduced (Kinch *et al.*, 1988), and some authors have proposed the use of other acids for etching: maleic (Urabe *et al.*, 1999; Bishara *et al.*, 2001) or nitric (Gardner and Hobson, 2001). At present, the most widely accepted etching technique is the application of phosphoric acid at 37 per cent for 30 seconds, although some authors argue that a 15 second application is sufficient (Wang and Yeh, 1994).

Recently, new alternatives to established acid-etch techniques have been introduced. Among these are non-rinse conditioners (NRCs), which condition the enamel without the need for washing, and self-etching primers (SEPs), which etch and prime in a single procedure. Various studies corroborate the efficacy of NRCs (Vicente *et al.*, 2005a) and SEPs (Vicente *et al.*, 2005b; Bishara and Ostby, 2006, 2007; Attar *et al.*, 2007; Davari *et al.*, 2007; Kitayama *et al.*, 2007) for bracket bonding, but research involving the use of these materials for successive rebonding is scarce. In fact, the use of NRCs for successive rebonds has not been evaluated, and only few studies exist on

the use of SEPs (Hirani and Sherriff, 2006; Montasser *et al.*, 2008a,b) for repeat bonding. Furthermore, there are no studies to date that evaluate the effect of avoiding acid etching for successive rebonds when enamel has been conditioned with phosphoric acid prior to the initial bond.

The aims of this study were to evaluate the effect (1) on shear bond strength (SBS), (2) on the percentage of the area occupied by adhesive remaining on the teeth after debonding, and (3) on the structural changes to the enamel surface after carrying out successive rebonds with new brackets on the same tooth using four enamel conditioning methods. The null hypothesis tested is that after carrying out successive rebonds with new brackets on the same tooth using four enamel conditioning methods, there are no significant differences in SBS, on the percentage of area occupied by adhesive remaining on teeth after debonding, and on the structural changes to the enamel surface.

Material and methods

Teeth

One hundred and thirty-five bovine upper central incisors visually intact and with no cracks on the enamel surface were used. The teeth were washed in water to remove any traces of blood and then placed in a 0.1 per cent thymol solution. They were then stored for a period of less than 1 month in distilled water, which was changed daily to avoid deterioration.

For SBS testing, 80 teeth were used; they were set in a 4 cm long copper cylinder with an internal diameter of 3 cm, with their roots in Type IV plaster. Fifty-five teeth were used for scanning electron microscopy (SEM) observations.

Brackets

Three hundred upper central incisor brackets (Victory Series®, 3M Unitek Dental Products, Monrovia, California, USA) were used, of which 240 were used for SBS testing and 60 for SEM observations. The base area of each bracket was calculated (mean = 10.25 mm^2) using image analysis equipment and MIP 4 software (Microm Image Processing Software, Digital Systems, Barcelona, Spain).

Bonding procedure

Eighty teeth were divided into the equal four groups and brackets were bonded on the buccal surfaces according to the manufacturer's instructions. For all groups, the buccal surfaces were polished with a rubber cup and polishing paste (Détartrine®, Septodont, Saint-Maur, France).

Group 1 (n = 20): the buccal surfaces were etched with 37 per cent *o*-phosphoric acid gel (Total Etch, Ivoclar, Vivadent, Schaan, Liechtenstein) for 30 seconds. The enamel was then washed with water and dried with compressed oil-free air. A layer of Transbond XT® primer (3M Unitek Dental Products) was applied to the tooth. Transbond XT® paste was applied to the base of the bracket and pressed firmly onto the tooth. Excess adhesive was removed from around the base of the bracket and the adhesive was light-cured, positioning the light guide of an Ortholux XT® lamp (3M Unitek Dental Products) on each interproximal side for 10 seconds.

Group 2 (n = 20): the brackets were bonded as in group 1.

Group 3 (n = 20): the enamel was treated with Transbond Plus Self-Etching Primer® (TSEP), 3M Unitek Dental Products, which was gently rubbed onto the enamel for 5 seconds with the disposable applicator supplied by the manufacturer. A moisture-free air source was used to deliver a gentle burst of air to the primer. The bracket was bonded with Transbond XT paste as in group 1.

Group 4 (n = 20): the enamel was treated with a NRC (Dentsplay de Trey, Konstanz, Germany). NRC was gently brushed onto the enamel leaving it undisturbed for 20 seconds. A moisture-free air source was then used to deliver a gentle burst of air to the enamel. The bracket was then bonded with Transbond XT (primer and paste) as in group 1.

Storage of test specimens

The specimens were immersed in distilled water at a temperature of 37°C for 24 hours.

Bond strength test

SBS was measured with a universal testing machine (Autograph AGS-1KND, Shimadzu, Kyoto, Japan) with a 1 KN load cell connected to a metal rod with one end angled at 30 degrees. The crosshead speed was 1 mm/minute.

The teeth were set at the base of the machine so that the sharp end of the rod incised in the area between the base and the wings of the bracket, exerting a force parallel to the tooth surface in an inciso-apical direction.

The force required to debond each bracket was registered in Newtons (N) and converted into megapascals (MPa) as a ratio of Newtons to surface area of the bracket (MPa = N/mm^2).

Repeated bonding

After debonding, the remaining bond material was removed from the enamel surface with a finishing carbide bur (Komet FG H22 GK016, Besigheim, Germany). Bonding/debonding procedures were repeated three times for each tooth. A new bracket was used for each successive bond procedure. Brackets were bonded following the same procedures as described for each group with the exception of group 2 in which for the second and third bonds, phosphoric acid was not used and Transbond XT® primer was applied directly to the enamel.

Evaluation of residual adhesive

The percentage of the surface of the bracket base covered by adhesive after debonding was determined using image analysis equipment (Sony dxc 151-ap video camera, connected to an Olympus SZ11 microscope) and MIP 4 software.

The percentage of the area occupied by adhesive remaining on the tooth after debonding was obtained by subtracting the area of adhesive covering the bracket base from 100 per cent.

Statistical analysis

SBS values and the data for the percentage of the area occupied by adhesive remaining on the teeth after debonding were compared for the four bonding procedures at each bonding/debonding sequence. Comparisons were also performed to determine whether significant differences existed in SBS and the percentage of area of adhesive remaining on teeth between the three bonding/debonding sequences within each bonding procedure.

Kolmogorov–Smirnov's normality and Levene's homogeneity of variance tests were applied to the data for bond strength and percentage of area of adhesive remaining on the teeth after debonding. When the data fulfilled the criteria for normality and homogeneity of variance, the existence of significant differences was determined by means of variance analysis (ANOVA) for one factor and Scheffé test for multiple comparisons ($P < 0.05$). When the data were not normally distributed or failed to fulfil the criteria for variance homogeneity, it was analysed using the Kruskal–Wallis test ($P < 0.05$), finding those groups that were significantly different with the Mann–Whitney test for two independent samples. In order to avoid an accumulation of errors due to multiple comparisons, the significance level was modified dividing this ($P < 0.05$) by the number of comparisons made (Bonferroni correction). $P < 0.017$ was considered significant when three comparisons were made and $P < 0.008$ for six comparisons.

SEM observation

The conditioned and reconditioned enamel surfaces of 55 teeth were observed after the brackets had been debonded following the procedures described above. The same procedures were used for bond strength testing, except that brackets were debonded using debonding pliers (Leone P 1920-00 CE, Florence, Italy).

Teeth treated with TSEP were rinsed with acetone for 10 seconds to remove the SEP (Kanemura *et al.*, 1999). Afterwards, the teeth were cleaned in distilled water with ultrasonic agitation for 30 minutes and gently air-dried. They were then fixed to SEM stubs, sputter coated with gold, and examined under a Jeol-6100 (Tokyo, Japan) scanning eletron microscope operating at 15kV. Representative images for each different surface treatment were captured and stored digitally.

Results

No significant differences were observed between the four procedures at the first ($P = 0.06$) or second ($P = 0.05$) bonding sequences. However, at the third sequence, the SBS in group 2 was significantly less than in group 1 ($P = 0.000$) (Table 1). For the percentage of tooth occupied by bond material remnant after debonding, the data showed no significant differences between the four bond procedures at the first ($P = 0.15$) or second ($P = 0.28$) sequence, while at the third sequence, the percentage of tooth area occupied was significantly less in group 2 than in the other groups (group 1 $P = 0.000$, group 3 $P = 0.000$, and group 4 $P = 0.000$) (Table 2).

When comparing the SBS of the three bonding/debonding sequences within each bonding procedure, only group 2 ($P = 0.00$) showed significant differences, bond strength at the third sequence being significantly less than at the first ($P = 0.000$) and second ($P = 0.000$) sequence. No significant difference was found between the first and second sequences ($P = 0.273$) for this group. For the other groups evaluated, there were no significant differences between the three bonding/debonding sequences (group 1 $P = 0.46$, group 3 $P = 0.94$, and group 4 $P = 0.77$) (Table 1). Data for the percentage of area occupied by adhesive did not show significant differences between the three bonding/debonding sequences for groups 1 ($P = 0.25$), 3 ($P = 0.06$), and 4 ($P = 0.84$). However, in group 2 at the third sequence, the area occupied by adhesive was significantly less than at the first ($P = 0.000$) and second ($P = 0.000$) sequences. No significant

Table 1 Mean shear bond strength (MPa) and standard deviation (SD) for group 1: 37 per cent phosphoric acid; group 2: 37 per cent phosphoric acid only etched for the first bond, not for further bonds; group 3: Transbond self-etching primer; and group 4: non-rinse conditioner, after repeated bonding and debonding ($n = 20$).

Debonding sequence	Group			
	1	2	3	4
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1	10.50 ± 3.73	10.44 ± 3.55A	8.19 ± 3.19	8.07 ± 2.73
2	10.13 ± 3.61	9.23 ± 2.69A	7.60 ± 1.12	7.97 ± 2.20
3	9.42 ± 2.75a	5.71 ± 1.56Bb	7.85 ± 3.12	7.14 ± 2.62

Different upper case letters within the same column indicate significant differences ($P < 0.017$). For each row, different lower case letters indicate significant differences ($P < 0.008$). Within the same column or the same row, the values unmarked by upper or lower case letters did not show significant differences with any other ($P > 0.05$).

differences were found between the first and second sequences ($P = 0.429$) (Table 2).

When enamel surfaces were examined under SEM after the first conditioning, the presence of porosities in the enamel could be seen in all groups, although the etching pattern for the NRC resulted in improved enamel conservation. After the second and third sequences, composite remnants were observed on the enamel surfaces in all groups (Figure 1).

Discussion

Bovine incisors were used in this study because of their microstructural similarity to human tooth enamel (Oesterle *et al.*, 1998) and also because of the easy availability of samples with intact vestibular surface enamel.

Various authors (Buchman, 1980; Egan *et al.*, 1996; Grabouski *et al.*, 1998; Chung *et al.*, 2000; Tavares *et al.*, 2006) have evaluated SBS obtained after rebonding reused brackets on teeth that had not undergone previous bonding. However, there are only a limited number of studies that have evaluated the rebonding of new brackets on the same tooth surface (Bishara *et al.*, 2000; Montasser *et al.*, 2008a,b).

No significant differences in bond strength between the different enamel conditioning procedures for the first bond/debond sequence were found in the present study. In agreement with Vicente *et al.* (2006), the NRC resulted in bond strength values similar to those obtained with phosphoric acid. For the SEPs, the results agree with other studies in which the bond strength values obtained with these materials were similar to those with the traditional technique (Hirani and Sherriff, 2006; Holzmeier *et al.*, 2008; Iijima *et al.*, 2008; Montasser *et al.*, 2008a,b). However, some authors have reported bond strength values, which were significantly less with SEPs than with phosphoric acid (Bishara *et al.*, 2001; Aljubouri *et al.*, 2003).

No significant differences were observed in bond strength between the four conditioning methods at the second bond/debond sequence. The fact that the bond strength in group 1 was similar to that obtained in group 2 in which brackets were rebonded without a second etching is interesting clinically, given that avoiding acid etching will have the advantage of reducing chair time as well as enamel loss. Surface enamel loss during acid etching is estimated at between 10 and 30 μm (Wickwire and Rentz, 1977). However, at the third debond, there was a significant difference in bond strength between the no prior acid-etch group (group 2) and the phosphoric acid-etch group (group 1), suggesting that when brackets are bonded for a third time on the same tooth, it would seem advisable to apply phosphoric acid again in order to achieve an acceptable bond strength. NRC and TSEP achieved similar bond strengths at the third bond/debond sequence to the acid-etch procedure.

Table 2 Mean and standard deviation (SD) of the percentage of tooth area occupied by adhesive for group 1: 37 per cent phosphoric acid; group 2: 37 per cent phosphoric acid only etched for the first bond, not for further bonds; group 3: Transbond self-etching primer; and group 4: non-rinse conditioner, after repeated bonding and debonding ($n = 20$).

Debonding sequence	Group			
	1	2	3	4
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1	14.34 ± 2.46	13.81 ± 5.59Aa	16.21 ± 7.80	13.71 ± 5.28
2	17.98 ± 8.45	15.32 ± 6.85Aa	17.00 ± 5.82	14.09 ± 4.01
3	16.95 ± 4.99a	6.93 ± 3.34Bb	14.40 ± 5.11a	14.60 ± 5.33a

Different upper case letters within the same column indicate significant differences ($P < 0.017$). For each row, different lower case letters indicate significant differences ($P < 0.008$). Within the same column or the same row, the values unmarked by upper or lower case letters did not show significant differences with any other ($P > 0.05$).

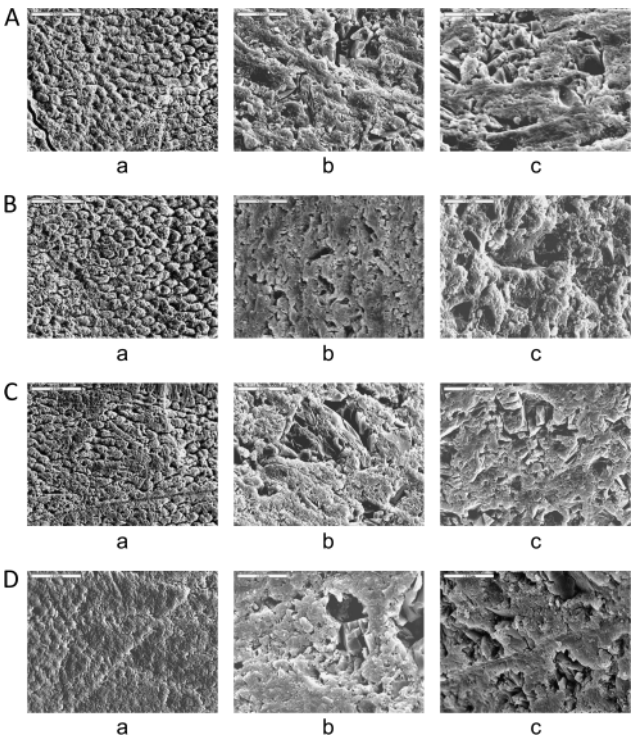

Figure 1 Scanning electron microphotographs of (A) group 1 (37 per cent phosphoric acid), (B) group 2 (37 per cent phosphoric acid only etched for the first bonding sequence, not for further bonds), (C) group 3 (Transbond self-etching primer), and (D) group 4 (non-rinse conditioner) for the first (a), second (b), and third (c) bond/debond sequences (×1500, bar = 20 μm).

When bond strength values for each conditioning procedure were compared at the three bond/debond sequences, a general reduction in SBS at each repetition

was seen. However, no significant differences were observed for the NRC, the SEP, or the group in which acid etching was applied prior to each bond.

The present results do not agree with Bishara *et al.* (2000), who, after etching with phosphoric acid for all bond/debond sequences, concluded that rebonded teeth have significantly lower and inconsistent SBS. This may be due to the different methodologies used and the fact that in the earlier study bond strength testing took place 30 minutes after bonding and the machine crosshead speed was 5 mm/minute.

For the self-etching adhesive, the present results were similar to those of Montasser *et al.* (2008a,b) who found that with repeated bonding/debonding, bond strength did not differ significantly.

In group 2, which did not undergo acid etching at the second and third bond/debond sequences, similar bond strengths were achieved as in the first sequence (that had been etched with phosphoric acid) and the second sequence. However, bond strength at the third sequence was significantly less than that at the first and second sequences. This result highlights the convenience of acid etching when bonding for a third time.

When the area occupied by adhesive remaining on the teeth after debonding was measured, no significant differences were found between the different conditioning procedures at either the first or second bond/debond sequence. However, at the third sequence, there was significantly less adhesive left on teeth in group 2, which had not been acid etched prior to bonding compared with the other groups (1, 3, and 4). Within each conditioning procedure, the adhesive remaining was similar for all three sequences, with the exception of the group that had not been acid etched at the second and third sequences, in which significantly less adhesive remnant was observed at the third sequence than at the first and second. These results may be explained by the fact that not etching does not create new porosity in the enamel, so reducing the microretention of the adhesive.

For the SEM observation, after the first sequence, the etching effects produced by the different procedures were similar, although the NRC showed a less aggressive etching pattern. However, at the second and third sequence, composite remnants were observed in all groups despite the fact that the enamel surfaces had been cleaned with a finishing carbide bur until all visible remnants had been eliminated. These observations agree with those of Bishara *et al.* (2003), who used the acid-etch technique for all bond/rebond sequences. The presence of these remnants may explain the gradual reduction in bond strength for each successive sequence within each group, given that these remnants contribute to a reduction in the roughness of the enamel and to the appearance of porosity when the tooth surface is repeatedly conditioned (Bishara *et al.*, 2003).

In group 2, in which acid etching was omitted at the second and third sequence, a greater reduction in surface roughness was noted at the third sequence than at the first and second, which, together with the cohesion characteristics of the material itself after polymerization, may explain the significant decrease in bond strength.

It must be taken into account that *in vitro* studies have their limitations. However, they are necessary and useful for initial evaluation of adhesive systems. However, *in vivo* research must be carried out to confirm *in vitro* results.

Conclusions

Both NRC and TSEP achieve a bond comparable with the acid-etch technique when new brackets are bonded repeatedly up to three times on the same tooth. When the acid-etch technique is used to condition enamel, etching need not be repeated when a bracket is bonded to a tooth for a second time but should be repeated for subsequent bonding.

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References

Aljubouri Y D, Millett D T, Gilmour W H 2003 Laboratory evaluation of a self-etching primer for orthodontic bonding. European Journal of Orthodontics 25: 411–415

Attar N, Taner T U, Tülümen E, Korkmaz Y 2007 Shear bond strength of orthodontic brackets bonded using conventional vs one and two-step self-etching/adhesive systems. The Angle Orthodontist 77: 518–523

Banks P, Thiruvenkatachari B 2007 Long-term clinical evaluation of bracket failure with a self-etching primer: a randomized controlled trial. Journal of Orthodontics 34: 243–251

Bishara S E, Ostby A W 2006 Early shear bond strength of a one-step self-adhesive on orthodontic brackets. The Angle Orthodontist 76: 689–693

Bishara S E, Ostby A W 2007 A self-conditioner for resin-modified glass ionomers in bonding orthodontic brackets. The Angle Orthodontist 77: 711–715

Bishara S E, VonWald L, Laffoon J F, Warren J J 2000 The effect of repeated bonding on the shear bond strength of a composite resin orthodontic adhesive. The Angle Orthodontist 70: 435–441

Bishara S E, VonWald L, Laffoon J F, Warren J J 2001 Effect of a self-etch primer/adhesive on the shear bond strength of orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 119: 621–624

Bishara S E, Ajlouni R, Oonsombat C 2003 Evaluation of a new curing light on the shear bond strength of orthodontic brackets. The Angle Orthodontist 73: 431–435

Buchman D J 1980 Effects of recycling on metallic direct-bond orthodontic brackets. American Journal of Orthodontics 77: 654–668

Buonocuore M G 1955 A simple method of increasing the adhesion of acrylic fillings materials to enamel surfaces. Journal of Dental Research 34: 849–853

Campbell P M 1995 Enamel surfaces after orthodontic bracket debonding. The Angle Orthodontist 65: 103–110

Chung C H, Fadem B W, Levitt H L, Mante F K 2000 Effects of two adhesion boosters on the shear bond strength of new and rebonded orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 118: 295–299

Davari A R, Yassaei S, Daneshkazemi A R, Yosefi M H 2007 Effect of different types of enamel conditioners on the bond strength of orthodontic brackets. Journal of Contemporary Dental Practice 8: 36–43

Donker H J, Melsen B, Frandsen E V 2001 Bacterial degradation of composite bonding materials. Clinical Orthodontics and Research 4: 112–118

Egan F R, Alexander S A, Cartwright G E 1996 Bond strength of rebonded orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics 109: 64–70

Elekdag-Turk S, Isci D, Turk T, Cakmak F 2008 Six-month bracket failure rate evaluation of a self-etching primer. European Journal of Orthodontics 30: 211–216

Gardner A, Hobson R 2001 Variations in acid-etch patterns with different acid and etch times. American Journal of Orthodontics and Dentofacial Orthopedics 120: 64–67

Grabouski J K, Staley R N, Jakobsen J R 1998 The effect of microetching on the bond strength of metal brackets when bonded to previously bonded teeth: an *in vitro* study. American Journal of Orthodontics and Dentofacial Orthopedics 113: 452–460

Hirani S, Sherriff M 2006 Bonding characteristics of a self-etching primer and precoated brackets: an *in vitro* study. European Journal of Orthodontics 28: 400–404

Holzmeier M, Schaubmayr M, Dasch W, Hirschfelder U 2008 A new generation of self-etching adhesives: comparison with traditional acid-etch technique. Journal of Orofacial Orthopedics 69: 78–93

Hong Y H, Lew K K 1995 Quantitative and qualitative assessment of enamel surfaces following five composite removal methods after bracket debonding. European Journal of Orthodontics 17: 121–128

Iijima M, Ito S, Yuasa T, Muguruma T, Saito T, Mizoguchi I 2008 Bond strength comparison and scanning electron microscopic evaluation of three orthodontic bonding systems. Dental Materials Journal 27: 392–399

Kanemura N, Sano H, Tagami J 1999 Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. Journal of Dentistry 27: 523–530

Kim S S, Park W K, Son W S, Ahn H S, Ro J H, Kim Y D 2007 Enamel surface evaluation after removal of orthodontic composite remnants by intraoral sandblasting: a 3-dimensional surface profilometry study. American Journal of Orthodontics and Dentofacial Orthopedics 132: 71–76

Kinch A P, Taylor H, Warltier R, Oliver R G, Newcombe R G 1988 A clinical trial comparing the failure rates of directly bonded brackets using etch times of 15 or 60 seconds. American Journal of Orthodontics and Dentofacial Orthopedics 94: 476–483

Kitayama S, Nikaido T, Ikeda M, Foxton R M, Tagami J 2007 Enamel bonding of self-etch and phosphoric acid-etch orthodontic adhesive systems. Dental Materials Journal 26: 135–143

Montasser M A, Drummond J L, Evans C A 2008a Rebonding of orthodontic brackets. Part I, a laboratory and clinical study. The Angle Orthodontist 78: 531–536

Montasser M A, Drummond J L, Roth J R, Al-Turki L, Evans C A 2008b Rebonding of orthodontics brackets. Part II, an XPS and SEM study. The Angle Orthodontist 78: 537–544

Murfitt P G, Quick A N, Swain M V, Herbison G P 2006 A randomised clinical trial to investigate bond failure rates using a self-etching primer. European Journal of Orthodontics 28: 444–449

Oesterle L J, Shellhart W C, Belanger G K 1998 The use of bovine enamel in bonding studies. American Journal of Orthodontics and Dentofacial Orthopedics 113: 514–519

Tavares S W, Consani S, Nouer D F, Magnani M B, Nouer P R, Martins L M 2006 Shear bond strength of new and recycled brackets to enamel. Brazilian Dental Journal 17: 44–48

Thiyagarajah S, Spary D J, Rock W P 2006 A clinical comparison of bracket bond failures in association with direct and indirect bonding. Journal of Orthodontics 33: 198–204

Tüfekçi E, Merrill T E, Pintado M R, Beyer J P, Brantley W A 2004 Enamel loss associated with orthodontic adhesive removal on teeth with white spot lesions: an *in vitro* study. American Journal of Orthodontics and Dentofacial Orthopedics 125: 733–739

Urabe H, Rossouw P E, Titley K C, Ymin C 1999 Combinations of etchants, composite resins and bracket systems: an important choice in orthodontic bonding procedures. The Angle Orthodontist 69: 267–274

Vicente A, Bravo L A, Romero M 2005a Influence of a nonrinse conditioner on the bond strength of brackets bonded with a resin adhesive system. The Angle Orthodontist 75: 400–405

Vicente A, Bravo L A, Romero M, Ortiz A J, Canteras M 2005b Shear bond strength of orthodontic brackets bonded with self-etching primers. American Journal of Dentistry 18: 256–260

Vicente A, Bravo L A, Romero M 2006 Self-etching primer and a non-rinse conditioner versus phosphoric acid: alternative methods for bonding brackets. European Journal of Orthodontics 28: 173–178

Wang W N, Yeh C L 1994 Effect of H_3PO_4 concentration on bond strength. The Angle Orthodontist 64: 377–382

Wickwire N A, Rentz D 1977 Enamel pretreatment: a critical variable in direct bonding systems. American Journal of Orthodontics 71: 542–553

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Unerupted incisors—characteristic features and associated anomalies

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SUMMARY The aims of this study were to investigate the association of unerupted incisors with other dental anomalies and to indicate the aetiological and clinical relevance of such associations. Forty-one patients with unerupted incisors were examined. The group comprised 30 males and 11 females, ranging in age from 7 to 39 years. The patients were assessed for nine dental anomalies: hyperdontia, hypodontia, microdontia, enamel hypoplasia, infraocclusion of the primary molars and ectopia of the canines, premolars, first permanent molars, and second permanent molars. The patients were matched with respect to age and gender to 41 consecutively selected control subjects with similar selection criteria but no history of problems with incisor eruption. The prevalence rates of the dental anomalies in association with failure of eruption of incisors were compared to the reference rates in the control group by means of Pearson chi-square tests.

The results of this study revealed that unerupted incisors were more frequent in males than in females. A statistically significant association ($P = 0.006$) was found between unerupted incisors and other inherited dental anomalies, namely ectopic teeth, hyperdontia, and enamel hypoplasia.

Unerupted incisors may be considered part of a spectrum of inheritable dental anomalies.

Introduction

Many articles in the dental literature describe the diagnosis and treatment of unerupted anterior teeth. The maxillary central incisor is, after the maxillary canine, the most common labially impacted unerupted tooth. Labial impaction of incisors accounts for 1–2 per cent of patients attending for orthodontic treatment (Crean *et al.*, 2000).

Maxillary incisors are the third most commonly impacted teeth in Caucasians, following the third molars and maxillary canines. They are more prevalent in Mongoloid races, suggesting that both hereditary and environmental factors may be implicated (Davis, 1987).

Heredity plays an important role in the appearance of dental anomalies. Supernumerary teeth are more likely to be present in affected relatives, although inheritance does not follow a simple Mendelian pattern. In 90 per cent of patients with hyperdontia, there exists a definite genetic influence to the incidence of supernumerary teeth (Stafne, 1932).

There also seems to be an association between tooth size and number, as hypodontia and microdontia often occur together and affect females more frequently than males (Baum and Cohen, 1971). Megadontia and supernumerary teeth have also been linked, with males being more commonly affected than females (Davis, 1987).

There is a spectrum of possible associations among various tooth anomalies, namely hypodontia, peg-shaped incisors, infraocclusion of primary molars, ectopic eruption of maxillary first permanent molars, and intraosseous displacement of maxillary canines (Bjerklin *et al.*, 1992; Baccetti, 1998).

Baccetti (1993), in a sample of 169 patients with inherited syndromes with tooth disturbances, found a 76.02 per cent prevalence of associated tooth anomalies. This suggests the possibility of a genetic relationship between the number, size, shape, and structural characteristics of the teeth.

The diagnosis, management, and treatment of unerupted incisors have previously been described (Munns, 1981; Jones and Husain, 1996). There are also papers on the association of various tooth anomalies (Hoffmeister, 1977; Baccetti, 1998), yet very little has been published on the association of unerupted incisors with other dental anomalies (Kobayashi *et al.*, 1999; Chaushu *et al.*, 2003). Therefore, the aim of this study was to investigate the association of unerupted incisors with other dental anomalies.

Materials and methods

An existing database of patients with unerupted incisors (Betts and Camilleri, 1999) was used as a starting point for data collection. Further contemporary records of patients with unerupted incisors were obtained from the Dental Outpatients Department at St. Luke's Hospital, the School Dental Clinic at Floriana Health Centre and from private dental clinics.

The inclusion criteria for a unilateral unerupted incisor were an unerupted incisor, which was not palpable, and a contralateral incisor, which had been erupted for more than 6 months.

The inclusion criteria for bilateral unerupted incisors were unerupted upper central incisors if the upper lateral incisors had already erupted, unerupted lower central incisors if the lower lateral incisors had already erupted, unerupted upper lateral incisors if the upper first premolars had already erupted, and unerupted lower lateral incisors if the lower canines had already erupted.

Patients excluded from the study were those with a history of dentoalveolar trauma, cleft lip and/or palate, or craniofacial malformations.

The study group comprised 41 patients, 30 males and 11 females, ranging in age from 7 to 39 years. The mean age was 15.8 years with a standard deviation of ±7.7. The patients were matched with respect to age and gender to 41 consecutively selected control subjects with similar selection criteria but no history of problems with incisor eruption. The patients in the control group were those who attended for a dental check-up at the Dental Outpatients Department at St. Luke's Hospital, the School Dental Clinic at Floriana Health Centre and from private dental clinics.

All the patients in the study and control groups attended for a dental examination and were assessed visually by one examiner (AB), using a mouth mirror and under a good operating light. Dental histories and panoramic radiographs were available for all patients.

The number of erupted/unerupted incisors and the presence or absence of inheritable dental anomalies were recorded on a data collection form. The inheritable dental anomalies were further categorized into the following eight types:

1. Infraocclusion: when the distance between the affected teeth and the occlusal plane was more than 1mm.
2. Enamel hypoplasia: when at least one permanent incisor showed an enamel lesion that was not related to dental caries or trauma. This was diagnosed from clinical examination and intraoral photographs.
3. Presence of supernumerary teeth (hyperdontia): diagnosed from existing radiographs and at the clinical examination.
4. Hypodontia and microdontia (including peg-shaped lateral incisors): diagnosed by measurement of the affected teeth and from existing radiographs.
5. Ectopic second premolars: diagnosed at the clinical examination and from existing radiographs.
6. Ectopic eruption of the first permanent molars: when the first permanent molar is initially blocked from complete eruption by the adjacent primary second molar. This was diagnosed at the clinical examination and from existing radiographs.
7. Ectopic eruption of the second molars: when the second molar erupts but becomes trapped under the distal bulge of the first molar. This was diagnosed at the clinical examination and from existing radiographs.
8. Ectopic position of canines: diagnosed at the clinical examination and from existing radiographs.

Sixty-four patients were contacted to attend for a dental examination. Forty-three attended and all gave their consent to participate. Twenty-one patients failed to attend and were excluded from the study. Two patients who did not have panoramic radiographs available were also excluded. Ethical approval was granted by the University of Malta Research Committee.

Statistical analysis

The results were processed using the Statistical Package for Social Sciences Version 12.0 for Windows® (SPSS Inc., Chicago, Illinois, USA). Numerical data were summarized. The prevalence rates of dental anomalies in association with failure of eruption of the incisors were compared with the reference prevalence rates in the control group by means of Pearson chi-square tests.

Where the subject was too young to determine whether an anomaly was present, the data were entered as not available and processed as missing values.

Results

The data for the study group are given in Table 1. The prevalence of unerupted incisors was higher in males than in females, with a male:female ratio of 2.7:1. Eighty-three per cent of patients had one unerupted incisor and 17 per cent two unerupted incisors.

Ninety-five per cent of patients had an unerupted maxillary central incisor, 2 per cent an unerupted maxillary lateral incisor, and 2 per cent an unerupted mandibular lateral incisor. No patients had unerupted mandibular central incisors.

Inheritable dental anomalies

Of the study group of 41 patients with unerupted incisors, 31 (75.6 per cent) had an inheritable dental anomaly. Out of the control group of 41 patients, 1 (2.4 per cent) had an inheritable dental anomaly.

The prevalence of dental anomalies was found to be higher in patients with unerupted incisors and this was highly statistically significant ($P = 0.006$). Pearson correlation analysis also showed that the number of inheritable dental anomalies increased with the number of unerupted incisors ($P < 0.01$).

Hypodontia and microdontia

Pearson chi-square test did not show the presence of hypodontia and microdontia to be statistically significant within the study group ($P = 0.078$). Three patients (7.3 per cent) had missing or small teeth in the study group. No patients were affected in the control group.

Infraocclusion of primary molars

The presence of infraoccluded primary molars was not statistically significant ($P = 0.314$) in the study group.

Enamel hypoplasia

Of the study group of 41 patients with unerupted incisors, five (12.2 per cent) had enamel hypoplasia on one or more of the incisor teeth. In the control group, no patients had enamel hypoplasia.

Table 1 Data of the patients with unerupted incisors showing the number of teeth affected by each anomaly.

No.	Age at examination	Gender	Number of teeth						
			Unerupted incisors	Missing teeth	Microdontic teeth	Infraoccluded primary molars	Ectopic teeth	Supernumerary teeth	Enamel hypoplasia
1	13	M	2		2		2	1	4
2	14	F	1					1	
3	20	M	1						
4	34	M	1					1	
5	10	M	2				n/a	1	
6	11	F	1				4		2
7	12	M	1					1	
8	11	M	1					1	
9	18	F	1				2		
10	16	M	2					3	
11	12	M	1					1	
12	22	M	1					4	1
13	14	M	1						
14	12	M	1			2	2		
15	33	F	1						
16	8	M	1				n/a		
17	15	M	2	1				1	
18	10	M	1				n/a		
19	21	F	1					1	
20	11	M	1				1	1	
21	13	M	1					1	
22	22	F	1						
23	16	M	1				1	1	
24	16	M	2					1	
25	21	M	1						
26	21	F	1						
27	40	M	2						
28	26	M	1						2
29	13	M	1						
30	32	F	1					2	
31	10	F	1				n/a	1	
32	10	M	1				n/a	1	
33	24	M	1					2	
34	16	M	1					1	
35	12	F	1					1	2
36	12	M	2					1	
37	12	M	1					2	
38	25	M	1						
39	13	M	1				1		
40	9	F	1				n/a	1	
41	11	M	1	1				1	

n/a, data not available.

The prevalence of enamel hypoplasia was found to be higher in patients with unerupted incisors and this was statistically significant ($P = 0.021$). The number of teeth with enamel hypoplasia increased with the number of unerupted incisors ($P < 0.01$).

Ectopic teeth

The ectopic teeth under investigation in this analysis were the canines and premolars. In the study group, seven patients (20.0 per cent) had ectopic teeth. While in the control group, one patient (2.9 per cent) had an ectopic tooth.

The prevalence of ectopic teeth was higher in patients with unerupted incisors and this was statistically significant ($P = 0.024$). Pearson correlation analysis showed that there was no statistically significant correlation between the number of ectopic teeth and the number of unerupted incisors ($P > 0.05$).

Hyperdontia

Twenty-six patients (63.4 per cent) in the study group had hyperdontia, with one exhibiting both hypodontia and hyperdontia. In the control group, no patients had supernumerary teeth.

The prevalence of hyperdontia was found to be higher in patients with unerupted incisors and this was highly statistically significant ($P < 0.001$). The number of supernumerary teeth increased with the number of unerupted

incisors and this correlation was statistically significant ($P < 0.01$). The prevalence of supernumerary teeth was higher in males than in females, with a male:female ratio of 2.7:1. The difference in gender distribution between the two groups was statistically significant ($P = 0.019$).

Comparison of inheritable dental anomalies between subgroups with and without supernumerary teeth

The data were analysed, using Pearson chi-square tests, in order to compare the prevalence of other inheritable dental anomalies between patients with and without supernumerary teeth.

The study group was divided into two subgroups:

1. Patients with unerupted incisors and supernumerary teeth;
2. Patients with unerupted incisors but no supernumerary teeth.

Twenty-six patients (63.4 per cent) in the study group had supernumerary teeth, while 15 (36.6 per cent) had no supernumerary teeth. These subgroups were compared with each other and with the control group. When supernumerary teeth were excluded, there was no significant difference in the number of other dental anomalies between the two groups. Both subgroups were also found to have a significantly higher prevalence of other inheritable dental anomalies when compared with the control group.

Comparison of the gender distribution of patients with unerupted incisors, with and without supernumerary teeth

In patients with unerupted incisors and supernumerary teeth, there was a significant gender bias towards males [19 males and 7 females ($P = 0.019$)]. In patients with unerupted incisors without supernumerary teeth, there was no significant difference between males and females.

Discussion

This investigation has shown that unerupted incisors occur together with other inheritable tooth abnormalities. There is evidence of a significant association between unerupted incisors and enamel hypoplasia, hyperdontia, and other ectopic teeth. This is in concordance with other studies where significant reciprocal associations were found among five anomalies (aplasia of second premolars, peg-shaped lateral incisors, infraocclusion of primary molars, enamel hypoplasia, and ectopic eruption of the maxillary canines; Hoffmeister, 1977; Baccetti, 1998).

A dental disturbance that appears to be significantly associated with unerupted incisors is enamel hypoplasia. In this study, enamel hypoplasia was found in 12.2 per cent of the dentitions of the study group. This correlates well with the theories proposed by Baccetti (1998), who showed that generalized enamel hypoplasia is part of an inheritable developmental disturbance due to a general disturbance of tooth development structures.

A significant association has also been found between unerupted incisors and ectopic teeth. Ectopic teeth form part of a spectrum of inheritable dental anomalies (Bjerklin *et al.*, 1992; Peck *et al.*, 1994; Baccetti, 1998). In this study, no correlation was found between the number of ectopic teeth and the number of unerupted incisors nor was an association found between unerupted incisors and ectopic canines or ectopic premolars. However, it is reasonable to suppose that as the eruption process is common to all teeth, ectopic teeth have a common genetic origin. As the expression of inheritable dental anomalies is highly variable, eruption defects may present with different phenotypes for the same genotype (Townsend *et al.*, 2005).

Anomalous lateral incisors and hypodontia are often associated with ectopic canines (Becker *et al.*, 1981). In this study, hypodontia and microdontia were analysed together. However, there seems to be no significant association between hypodontia, microdontia, and unerupted incisors. Infraoccluded primary molars also form part of the spectrum of inherited dental anomalies. No association was found between unerupted incisors and submerged primary molars. However, 43.9 per cent of the patients in this study were over 15 years of age. Therefore, it is expected that most of the patients would have already lost any submerged primary molars and so this finding is not reliable.

Supernumerary teeth were found more commonly in males, with an overall male:female ratio of 2.7:1. Seventy-three per cent of the supernumerary tooth subgroup was, in fact, male. This is in agreement with the findings of Brook (1974).

The prevalence of hyperdontia in patients with unerupted incisors was 63 per cent. This is not a surprising finding as supernumerary teeth have been associated with unerupted teeth (Howard, 1967). There was also an association between the number of supernumerary teeth and the number of unerupted incisors. This occurred irrespective of whether the supernumeraries were adjacent to the unerupted teeth or not. The fact that 36 per cent of the group had no supernumeraries would imply that supernumerary teeth are not the primary cause of unerupted incisors. Delayed eruption of incisors occurs in a relatively small proportion of supernumerary cases (Nik-Hussein, 1990). The findings of the present study revealed a higher prevalence of unerupted incisors in males than in females. Analysis of gender in the subgroup without supernumerary teeth showed no significant difference between the genders, while that of the subgroup with supernumerary teeth was significant. It is possible that the supernumerary trait, more common in males, carries a greater tendency to failure of eruption. Supernumerary teeth are more prevalent in males (Davis, 1987) and missing teeth are more prevalent in females (Baum and Cohen, 1971). This raises the possibility of gender influencing expression of the gene or genes determining

the number of teeth, though each is not mutually exclusive. However, both traits seem to be associated with an increased prevalence of other inheritable dental anomalies.

When supernumerary teeth were excluded from the analysis of the main group, unerupted incisors were still found to be significantly associated with inheritable dental anomalies. Furthermore, the percentage of anomalies (excluding supernumerary teeth) in the supernumerary subgroup was similar to that in the non-supernumerary subgroup and both groups had a significantly higher prevalence than the controls. Thus, both unerupted incisors and supernumerary teeth seem to be associated with the presence of other inheritable dental anomalies. This is in contrast to an earlier study where no association between supernumerary teeth and other inherited dental anomalies was found (Baccetti, 1998).

Unerupted incisors are relatively uncommon. The small size of the groups resulted in reduced power and was a major limitation of the study. This lack of power may lead to major incorrect rejection of the null hypothesis (Type II error) and may well explain the lack of association with some anomalies. The existing records of patients with unerupted incisors are continually being updated, with a view to repeating the study with a larger sample size. This would give more robust evidence regarding any anomalies associated with unerupted incisors. Further research would also investigate the incidence of dental anomalies in family members of probands with unerupted incisors, to further explore the genetic basis of inheritable dental anomalies.

Conclusions

The results of this study show that males present with more unerupted incisors than females, with a prevalence of 2.7:1.

Unerupted incisors occur together with other inheritable tooth abnormalities. There is evidence of a significant association between unerupted incisors and enamel hypoplasia, hyperdontia, and ectopic teeth.

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References

Baccetti T 1993 An analysis of the prevalence of isolated dental anomalies and of those associated with hereditary syndromes: a model for evaluating the genetic control of the dentition characteristics. Minerva Stomatologica 42: 281–294

Baccetti T 1998 A controlled study of associated dental anomalies. Angle Orthodontist 68: 267–274

Baum B J, Cohen M M 1971 Agenesis and tooth size in the permanent dentition. Angle Orthodontist 41: 100–102

Becker A, Smith P, Behar R 1981 The incidence of anomalous maxillary lateral incisors in relation to palatally-displaced cuspids. Angle Orthodontist 51: 24–29

Betts A, Camilleri G E 1999 A review of 47 cases of unerupted maxillary incisors. International Journal of Paediatric Dentistry 9: 285–292

Bjerklin K, Kurol J, Valentin J 1992 Ectopic eruption of maxillary first permanent molars and association with other tooth and developmental disturbances. European Journal of Orthodontics 14: 369–375

Brook A H 1974 Dental anomalies of number, form and size: their prevalence in British schoolchildren. Journal of the International Association of Dentistry for Children 5: 37–53

Chaushu S, Zilberman Y, Becker A 2003 Maxillary incisor impaction and its relationship to canine displacement. American Journal of Orthodontics and Dentofacial Orthopedics 124: 144–150

Crean S J, Banu B, Coonar H 2000 Modified apically repositioned flap in the treatment of unerupted maxillary central incisors. Dental Update 27: 137–139

Davis P J 1987 Hypodontia and hyperdontia of permanent teeth in Hong Kong schoolchildren. Community Dentistry and Oral Epidemiology 15: 218–220

Hoffmeister H 1977 Microsymptoms as an indication for familial hypodontia, hyperdontia and tooth displacement. Deutsche Zahnärztliche Zeitschrift 32: 551–561

Howard R D 1967 The unerupted incisor. A study of the postoperative eruptive history of incisors delayed in their eruption by supernumerary teeth. Dental Practitioner and Dental Record 17: 332–341

Jones J W, Husain J 1996 Management of the unerupted incisor. Dental Update 23: 36–39

Kobayashi H, Taguchi Y, Noda T 1999 Eruption disturbances of maxillary permanent central incisors associated with anomalous adjacent permanent lateral incisors. International Journal of Paediatric Dentistry 9: 277–284

Munns D 1981 Unerupted incisors. British Journal of Orthodontics 8: 39–42

Nik-Hussein N N 1990 Supernumerary teeth in the premaxillary region: its effects on the eruption and occlusion of the permanent incisors. Australian Orthodontic Journal 11: 247–250

Peck S, Peck L, Kataja M 1994 The palatally displaced canine as a dental anomaly of genetic origin. Angle Orthodontist 64: 249–256

Stafne E C 1932 Supernumerary teeth. Dental Cosmos 74: 653–659

Townsend G C, Richards L, Hughes T, Pinkerton S, Schwerdt W 2005 Epigenetic influences may explain dental differences in monozygotic twin pairs. Australian Dental Journal 50: 95–100

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Prevalence of hypodontia in orthodontic patients in Brasilia, Brazil

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SUMMARY The purpose of this retrospective study was to determine the prevalence of hypodontia and associated dental anomalies in patients undergoing orthodontic treatment in Brasília, Brazil, over a 2 year period (1998–2000). The records of 1049 orthodontic patients between 10 and 15.7 years of age (507 males and 542 females) from 16 orthodontic clinics were analysed. Descriptive statistics were performed for the study variables. A chi-square test was used to determine the difference in the prevalence of hypodontia between genders.

The prevalence of hypodontia was 6.3 per cent (39.4 per cent males and 60.6 per cent females) with no statistically significant difference between the genders. One case of oligodontia was observed. The maxillary lateral incisor was the most frequently missing tooth, followed by the mandibular second premolar. All cases of hypodontia, except one, were associated with at least one other dental anomaly. These associated dental anomalies were retained primary teeth (30.3 per cent), ectopic canine eruption (25.8 per cent), taurodontism (21.2 per cent), and peg-shaped maxillary lateral incisors (16.7 per cent).

Introduction

Tooth agenesis, which is defined as the congenital absence of one or more primary or permanent teeth, is one of the most frequent human dental anomalies (Symons *et al.*, 1993; Cameron and Sampson, 1996; Vastardis, 2000). Tooth agenesis can be classified as hypodontia, oligodontia, or anodontia. The term hypodontia is used to describe agenesis of one to six teeth (excluding the third molars), oligodontia the absence of more than six teeth (excluding the third molars), and anodontia representing complete absence of teeth (Arte, 2001). The term 'severe hypodontia' is often used as an alternative to oligodontia to describe those cases with more than four teeth missing, excluding the third molars (Vastardis, 2000; Wong *et al.*, 2005; Endo *et al.*, 2006; Worsaae *et al.*, 2007). Hypodontia can be an isolated condition or part of a syndrome (Van der Weide *et al.*, 1994).

Population studies have revealed that the prevalence of hypodontia differs with regard to the permanent and primary dentitions, tooth type, and racial groups. The prevalence of hypodontia varies from 0.03 to 10.1 per cent in various populations (Mattheeuws *et al.*, 2004). In the primary dentition, the prevalence is between 0.5 and 0.9 per cent, while oligodontia is rare, with an estimated prevalence of 0.25 per cent (Vastardis, 2000). In the permanent dentition, the most commonly missing teeth are the third molars, followed by either the lower second premolars or upper lateral incisors (Lavelle *et al.*, 1970; Müller *et al.*, 1970). The following differences in prevalence between racial groups have been reported: 1.5–3 per cent in Caucasians, 6–9.2 per cent in Orientals, and 7.7 per cent in Afro-Americans (Vastardis, 2000). Hypodontia prevalence also differs among studies of orthodontic patient (Thongudomporn and Freer, 1998; Meza, 2003; Fekonja, 2005; Endo *et al.*, 2006; Altug-Atac and Erdem, 2007).

A higher prevalence of hypodontia in females has also been suggested (Magnusson, 1977; Bäckman and Wahlin, 2001). However, other studies have not found any statistically significant differences between genders (Grahnén, 1956; Haavikko, 1971; Rølling, 1980).

Other dental anomalies have been reported in patients with hypodontia, such as peg-shaped incisors (Townsend *et al.*, 1995), taurodontism (Lai and Seow, 1989), enamel developmental defects (Symons *et al.*, 1993; Arte *et al.*, 2001), and lateral incisor–canine transposition (Peck *et al.*, 1998; Shapira and Kuftinec, 2001).

Some studies have reported the prevalence of hypodontia in orthodontic patients (Thongudomporn and Freer, 1998; Meza, 2003; Fekonja, 2005; Endo *et al.*, 2006; Altug-Atac and Erdem, 2007). Retrospective studies rely on good record keeping and orthodontic patients often have more complete records. Some Brazilian studies have investigated the prevalence of hypodontia (Castilho *et al.*, 1990; Ciamponi and Frassei, 1999), but there are no reports concerning orthodontic patients. Thus, the aim of the present study was to determine the prevalence of hypodontia and its association with other dental anomalies in orthodontic patients.

Materials and methods

The present study was approved by the Research Ethics Committee of the Faculty of Health Sciences, University of Brasília, Brazil.

The records of patients, between 10 and 15.7 years of age, who initiated orthodontic treatment at 16 orthodontic practices in the Federal District, Brazil, were used in the study. The records included panoramic radiographs and study models of all patients at the beginning of treatment, between 1998 and 2000.

Hypodontia was recorded when a tooth was absent on the panoramic radiograph, excluding a history of loss due to trauma, caries, periodontal disease, or orthodontic extraction. All permanent teeth were investigated, excluding third molars. Hypodontia was diagnosed if one to six teeth were absent. For those records where hypodontia was noted, the presence of any syndrome or systemic disease was registered, and the presence of other dental anomalies was also verified and registered. The dental anomalies investigated were retained primary teeth, ectopic canine eruption, lateral incisor–canine transposition, peg-shaped maxillary lateral incisors, supernumerary teeth, and taurodontism.

Study models were used to determine the presence of ectopic canine eruption, lateral incisor–canine transposition, and peg-shaped maxillary lateral incisors. A maxillary lateral incisor was considered peg-shaped when the mesiodistal incisor was shorter than the cervical width of the tooth crown (Bäckman and Wahlin, 2001).

The presence of retained primary teeth, supernumerary teeth, and taurodontism of the first mandibular molars was assessed on panoramic radiographs. Taurodontism was determined according to the criteria described by Seow and Lai (1989). The tooth was considered as taurodontic when the crown body–root ratio was equal or greater than 1 : 1. The molars were classified as hypo-, meso-, or hypertaurodont. To establish crown–root ratios, the radiographs were digitized at a resolution of 100 dpi, and the measurements were performed by one examiner (RRG) using Image Pro-Express software, version 4.5 (Media Cybernetics Inc., Bethesda, Maryland, USA).

Descriptive statistics were performed for the study variables. Data collected were analysed for frequency, gender, tooth type, and the association with other dental anomalies. The difference in the prevalence of hypodontia between genders was tested with the chi-square test, at a level of significance of 0.05, using SAS version 8.1 for Windows (SAS Institute Inc., Cary, North Carolina, USA).

To assess measurement error to determine taurodontism, Dahlberg's formula ($EM = \sqrt{\frac{\sum d^2}{2n}}$) was applied to 20 per cent of the analysed molars which were measured twice with an interval of 15 days. The error of the method was 0.07.

Results

A total of 1049 records were analysed, 507 males (48.3 per cent) and 542 females (51.7 per cent). No systemic diseases or syndromes were registered. Hypodontia was diagnosed in 66 subjects, i.e. 6.3 per cent of the studied sample [26 males (5.1 per cent) and 40 females (7.3 per cent)]. No significant statistical gender difference was observed (χ^2 = 2.25; df = 1; $P > 0.05$). The mean age of the patients at treatment initiation was 13.16 years (range 10–15.7 years). Only one case of oligodontia was verified, with 18 missing teeth. The patient was an 11-year-old female.

Maxillary hypodontia was seen in 59.2 per cent of patients and in the mandible in 40.8 per cent, with an overall ratio of 1.45 : 1. The maxillary lateral incisors were the most frequently missing teeth, followed by the mandibular second premolars. The distribution of the missing teeth is shown in Figure 1. A total of 108 permanent teeth were found to be congenitally absent, with the number of missing teeth in each case ranging from one to five, with an average of 1.63 missing teeth per patient. One missing tooth was found in 56.6 per cent of the studied cases, two missing teeth in 33.3 per cent, and three to five absent teeth were observed in 12.1 per cent.

All individuals, except one, had at least one dental anomaly coexisting with hypodontia. The distribution of the dental anomalies associated with hypodontia is shown in Table 1. A retained primary tooth was the most commonly observed anomaly (n = 20; 30.3 per cent), while the mandibular second primary molar was the most frequently retained tooth. Ectopic eruption was found in 25.8 per cent (n = 17) of individuals, with the maxillary canine being the most frequently affected tooth. The presence of lateral incisor–canine transposition was not verified.

Peg-shaped maxillary lateral incisors were observed in 11 individuals (16.7 per cent). All peg-shaped maxillary lateral incisors were unilateral and their occurrence was observed with agenesis of the contralateral maxillary lateral incisor. The association of supernumerary teeth and hypodontia was not verified.

Taurodontism of the first mandibular molars was found in 14 individuals (21.2 per cent): 10 had unilateral taurodontism (five on the right side and five on the left side). The remaining four cases were bilateral. All teeth were classified as

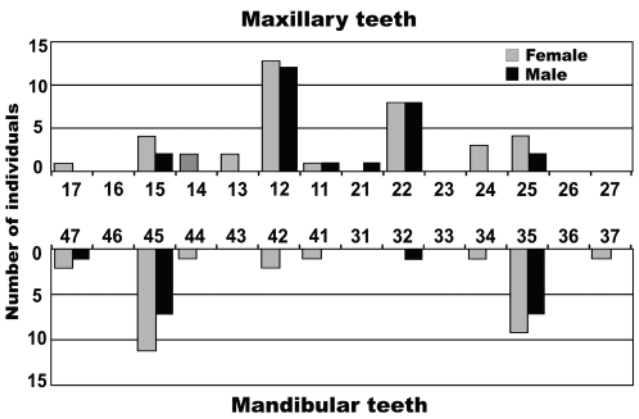

Figure 1 Distribution of missing teeth by genders. Tooth notation according to the Federation Dentaire International.

Table 1 Distribution and frequency (%) by gender of the dental anomalies associated with hypodontia in the 1049 orthodontic patients studied.

Dental anomaly	Subjects with hypodontia (n = 66)		
	Male (%)	Female (%)	Total (%)
Retained primary teeth	7 (10.6)	13 (19.7)	20 (30.3)
Ectopic eruption	7 (10.6)	10 (15.2)	17 (25.8)
Taurodontism	6 (9.1)	8 (12.0)	14 (21.1)
Peg-shaped upper lateral incisor	5 (7.6)	6 (9.1)	11 (16.7)
Incisor–canine transposition	0 (0.0)	0 (0.0)	0 (0.0)
Supernumerary	0 (0.0)	0 (0.0)	0 (0.0)

hypotaurodontic, except one, which was classified as a mesotaurodontic first molar.

Discussion

The sample in this study represented 0.6 per cent of the Federal District population in the age range studied (www.codeplan.df.gov.br). Since local public health policies do not include orthodontic treatment, the sample studied represents those requesting orthodontic treatment. Orthodontic patients do not necessarily reflect the number of individuals in the population with hypodontia; this will be dependent on the availability of orthodontic treatment and its uptake in this particular population.

The results of the present study showed a prevalence of 6.3 per cent of hypodontia in orthodontic patients in the Federal District of Brazil. This prevalence is lower than that reported in other similar studies. The prevalence of hypodontia observed in Australian orthodontic patients was 8.1 per cent (Thongudomporn and Freer, 1998), whereas in Japanese orthodontic patients the prevalence was 8.5 per cent (Endo *et al.*, 2006). On the other hand, the prevalence in Mexican orthodontic patients was 2.7 per cent (Meza, 2003), lower than the prevalence observed in the present study. There are important differences among countries in the organization of orthodontics for children and as such there will be differences in the availability and uptake of orthodontics. The wide range of prevalence values (1.6–9.6 per cent) observed in population studies has indicated geographic differences (Haavikko, 1971; Seow and Lai, 1989; Townsend *et al.*, 1995; Kotsomitis and Freer, 1997; Arte and Pirinen, 2003). Nevertheless, these reports are mainly for European, Australian, and North American populations, indicating the need for studies in other geographic regions in order to verify these differences.

Females presented a higher prevalence of hypodontia, but no significant statistical difference was observed, which is in accordance with the majority of reports (Grahnén, 1956; Haavikko, 1971; Lai and Seow, 1989; Thongudomporn and Freer, 1998; Fekonja, 2005; Endo *et al.*, 2006). A few studies have found significant difference between genders (Bäckman and Wahlin, 2001; Brook, 1984).

In the present study, of the individuals identified with hypodontia, 87.9 per cent had one or two missing teeth. Other studies have reported a higher frequency of one or two missing teeth (Haavikko, 1971; Rølling, 1980; Davis, 1987; Fekonja, 2005).

The maxillary lateral incisor was found to be the most frequently missing tooth in the current study. There is some variation in the literature concerning the description of the most frequently missing tooth, excluding third molars. The mandibular second premolar is normally the most frequently missing tooth reported (Rølling, 1980; Thongudomporn and Freer, 1998; Bäckman and Wahlin, 2001; Polder *et al.*, 2004; Mattheeuws *et al.*, 2004; Endo *et al.*, 2006). However, other studies have also shown the permanent upper lateral incisor to be the most affected tooth (Müller *et al.*, 1970; Ciamponi and Frassei, 1999; Meza, 2003; Fekonja, 2005). Müller *et al.* (1970) observed that in a North American population, the maxillary lateral incisor was the most frequently missing tooth in individuals with agenesis of only one or two teeth, while in those with more than two absent teeth, the second premolar was most commonly missing tooth. These variations probably reflect the fact that although populations in various countries have been studied (Castilho *et al.*, 1990; Thongudomporn and Freer, 1998; Ciamponi and Frassei, 1999; Bäckman and Wahlin, 2001; Meza, 2003; Mattheeuws *et al.*, 2004; Polder *et al.*, 2004; Endo *et al.*, 2006), some research has focused on patients undergoing orthodontic treatment (Thongudomporn and Freer, 1998; Meza, 2003; Fekonja, 2005; Endo *et al.*, 2006) and on the overall population (Castilho *et al.*, 1990; Ciamponi and Frassei, 1999; Polder *et al.*, 2004; Mattheeuws *et al.*, 2004). In addition, differences between populations of patients seeking orthodontic treatment may possibly reflect different psycho-social aspects between regions. It is thus probable that in countries where smile aesthetics are highly valued, lateral incisor hypodontia may motivate parents and patients to seek orthodontic treatment.

Previous studies have demonstrated that hypodontia may be related to other dental anomalies such as microdontia, peg-shaped incisors, taurodontism, enamel defects, and lateral incisor–canine transposition (Lai and Seow, 1989; Symons *et al.*, 1993; Townsend *et al.*, 1995; Peck *et al.*, 1998; Arte *et al.*, 2001; Shapira and Kuftinec, 2001). The results suggest that ectopic canines, peg-shaped lateral incisors, and taurodontism are associated with hypodontia.

The percentage of peg-shaped maxillary lateral incisors observed in the individuals studied was higher than previously reported (Grahnén, 1956; Meskin and Gorlin, 1963; Lai and Seow, 1989). It has been suggested that hypodontia and peg-shaped lateral incisors are different forms of genotypic manifestation of the same gene (Alvesalo and Portin, 1969; Jorgenson, 1980; Brook, 1984). These data suggest that if a patient with hypodontia undergoes

orthodontic treatment, careful attention must be given to the analysis of the possible existence of Bolton's discrepancies in the maxillary anterior region (Carreiro *et al.*, 2005).

In the present study, taurodontism was observed in 21.2 per cent of individuals with hypodontia, confirming previous reports (Lai and Seow, 1989; Arte *et al.*, 2001). Lai and Seow (1989) observed that the occurrence of taurodontism was higher in individuals with hypodontia (34.3 per cent) than in a control group (7.1 per cent). These findings may also suggest that there is an association between hypodontia and taurodontism. Complementary studies must be conducted to better understand the impact of these morphological alterations on orthodontic treatment. Alterations in crown–root ratio are probably followed by other subtle morphological differences that hamper the successful conclusion of orthodontic treatment (Casko *et al.*, 1998). Moreover, the tendency to root resorption is greater in dentitions in which hypodontia and taurodontism occur (Kjær, 1995).

The most common dental anomaly associated with hypodontia was found to be retained primary teeth. This was expected since the absence of permanent teeth is related to persistence of the primary predecessor (Bjerklin and Bennett, 2000; Ith-Hansen and Kjær, 2000).

Tooth morphogenesis is a complex process that involves epithelial–ectomesenchymal interactions. Numerous transcription factors, growth factors, and their receptors, as well as extracellular matrix components, have been associated with early tooth development (Thesleff and Nieminen, 1996; Jernvall and Thesleff, 2000; Miletich and Sharpe, 2003). The genetic basis of tooth development is supported by the identification of mutations in genes that participate in dental development (Vastardis *et al.*, 1996; Mostowska *et al.*, 2003). Mutations in transcription factors *MSX 1* (Mostowska *et al.*, 2006b) and PAX 9 (Stockton *et al.*, 2000; Nieminen *et al.*, 2001) have been identified in families with autosomal dominant oligodontia. More recently, a mutation in the *AXIN2* gene was identified in families with oligodontia and colorectal cancer suggesting that tooth agenesis might be an indicator of colorectal cancer susceptibility (Lammi *et al.*, 2004). Although mutations in these genes have not yet been associated with hypodontia and its genetic origin remains unknown, polymorphisms in the 5′ flanking region of the *PAX9* gene (Peres *et al.*, 2005) and *AXIN2* (Mostowska *et al.*, 2006a) have been associated with hypodontia in humans.

Some studies have demonstrated other dental anomalies in association with hypodontia (Lai and Seow, 1989; Symons *et al.*, 1993; Townsend *et al.*, 1995; Peck *et al.*, 1993, 1996, 1998, 2002; Baccetti, 1998; Arte *et al.*, 2001; Shapira and Kuftinec, 2001), suggesting that these associated anomalies are controlled by similar genetic mechanisms (Svinhufvud *et al.*, 1988; Baccetti, 1998; Peck *et al.*, 1998; 2002; Camilleri, 2005). Nevertheless, at present, there is no available data concerning the molecular aetiology of other dental anomalies in association with hypodontia.

Conclusions

The prevalence of hypodontia in Brazilian orthodontic patients was 6.3 per cent, with no statistically significant difference between genders. The maxillary lateral incisor was the most frequently missing tooth, followed by the mandibular second premolar. The associated dental anomalies were retained primary teeth (30.3 per cent), ectopic canine eruption (25.8 per cent), taurodontism (21.2 per cent), and peg-shaped maxillary lateral incisors (16.7 per cent).

Further studies are necessary to verify the aetiology of dental anomalies associated with hypodontia. A detailed description of these dental anomalies, when characterizing families with tooth agenesis, is essential to better correlate phenotype with genotype.

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References

Altug-Atac A T, Erdem D 2007 Prevalence and distribution of dental anomalies in orthodontic patients. American Journal of Orthodontics and Dentofacial Orthopedics 131: 510–514

Alvesalo L, Portin P 1969 The inheritance pattern of missing, peg-shaped, and strongly mesio-distally reduced upper lateral incisors. Acta Odontologica Scandinavica 27: 563–575

Arte S 2001 Phenotypic and genotypic features of familial hypodontia. Thesis, University of Helsinki

Arte S, Pirinen S 2003 Hypodontia. Orphanet encyclopedia. http://orpha.net/data/patho/G8/uk-hypodontia.pdf (18 February 2009, date last accessed)

Arte S, Nieminen P, Apajalahti S, Haavikko K, Thesleff I, Pirinen S 2001 Characteristics of incisor-premolar hypodontia in families. Journal of Dental Research 80: 1445–1450

Baccetti T 1998 A controlled study of associated dental anomalies. Angle Orthodontist 68: 267–274

Bäckman B, Wahlin Y B 2001 Variations in number and morphology of permanent teeth in 7-year-old Swedish children. International Journal of Paediatric Dentistry 11: 11–17

Bjerklin K, Bennett J 2000 The long-term survival of lower second primary molars in subjects with agenesis of the premolars. European Journal of Orthodontics 22: 245–255

Brook A H 1984 A unifying aetiological explanation for anomalies of human tooth number and size. Archives of Oral Biology 29: 373–378

Cameron J, Sampson W J 1996 Hypodontia of the permanent dentition. Case reports. Australian Dental Journal 41: 1–5

Camilleri S 2005 Maxillary canine anomalies and tooth agenesis. European Journal of Orthodontics 27: 450–456

Carreiro L S, Santos-Pinto A, Raveli D B, Martins L P 2005 Bolton tooth size discrepancy in normal occlusion and in different types of malocclusions and its relationship to arch form and tooth positioning. Revista Dental Press de Ortodontia e Ortopedia Facial 10: 97–117

Casko J S *et al.* 1998 Objective grading system for dental casts and panoramic radiographs. American Journal of Orthodontics and Dentofacial Orthopedics 114: 589–599

Castilho J C M, Nicodemo R A, Bazzarella C B, Moraes L C 1990 Prevalência de anodontia entre estudantes do 2º grau da cidade de São José dos Campos—correlação dessa anomalia entre terceiros molares e outros órgãos dentários. Revista de Odontologia da UNESP 19: 269–276

Ciamponi A L, Frassei V A S 1999 Anodontias parciais congênitas de dentes permanentes: estudo da prevalência em crianças residentes na cidade de São Paulo. Revista da Pós-Graduação Faculdade de Odontologia da Universidade de São Paulo 6: 213–217

Davis P J 1987 Hypodontia and hyperdontia of permanent teeth in Hong Kong schoolchildren. Community Dentistry and Oral Epidemiology 15: 218–220

Endo T, Ozoe R, Kubota M, Akiyama M, Shimooka S 2006 A survey of hypodontia in Japanese orthodontic patients. American Journal of Orthodontics and Dentofacial Orthopedics 129: 29–35

Fekonja A 2005 Hypodontia in orthodontically treated children. European Journal of Orthodontics 27: 457–460

Grahnén H 1956 Hypodontia in the permanent dentition. Odontologisk Revy Supplement 7: 1–100

Haavikko K 1971 Hypodontia of permanent teeth. An orthopantomographic study. Suomen Hammaslaakariseuran Toimituksia 67: 219–225

Ith-Hansen K, Kjær I 2000 Persistence of deciduous molars in subjects with agenesis of the second premolars. European Journal of Orthodontics 22: 239–243

Jernvall J, Thesleff I 2000 Reiterative signaling and patterning during mammalian tooth morphogenesis. Mechanisms of Development 92: 19–29

Jorgenson R J 1980 Clinician's view of hypodontia. Journal of the American Dental Association 101: 283–286

Kjær I 1995 Morphological characteristics of dentitions developing excessive root resorption during orthodontic treatment. European Journal of Orthodontics 16: 25–34

Kotsomitis N, Freer T J 1997 Inherited dental anomalies and abnormalities. Journal of Dentistry for Children 64: 405–408

Lai P Y, Seow W K 1989 A controlled study of the association of various dental anomalies with hypodontia of permanent teeth. Pediatric Dentistry 11: 291–296

Lammi L *et al.* 2004 Mutations in *AXIN2* cause tooth agenesis and predispose to colorectal cancer. American Journal of Human Genetics 74: 1043–1050

Lavelle C L B, Ashton E H, Flinn R M 1970 Cusp pattern, tooth size and third molar agenesis in the human mandibular dentition. Archives of Oral Biology 15: 227–237

Magnusson T E 1977 Prevalence of hypodontia and malformations of permanent teeth in Iceland. Community Dentistry and Oral Epidemiology 5: 173–178

Mattheeuws N, Dermaut L, Martens G 2004 Has hypodontia increased in Caucasians during the 20th century? A meta-analysis. European Journal of Orthodontics 26: 99–103

Meskin L H, Gorlin R J 1963 Agenesis and peg-shaped permanent maxillary lateral incisors. Journal of Dental Research 42: 1476–1479

Meza R S 2003 Radiographic assessment of congenitally missing teeth in orthodontic patients. International Journal of Paediatric Dentistry 13: 112–116

Miletich I, Sharpe P T 2003 Normal and abnormal dental development. Human Molecular Genetics 12: R69–R73

Mostowska A, Biedziak B, Jagodzinski P P 2006a Axis inhibition protein 2 (AXIN2) polymorphisms may be a risk factor for selective tooth agenesis. Journal of Human Genetics 51: 262–266

Mostowska A, Biedziak B, Trzeciak W H 2006b A novel c.581CT transition localized in a highly conserved homeobox sequence of MSX1 is it responsible for oligodontia. Journal of Applied Genetics 47: 159–164

Mostowska A, Kobielak A, Trzeciak W H 2003 Molecular basis of non-syndromic tooth agenesis: mutations of MSX1 and PAX9 reflect their role in patterning human dentition. European Journal of Oral Sciences 111: 365–370

Müller T P, Hill I N, Peterson A C, Blayney J R 1970 A survey of congenitally missing permanent teeth. American Journal of Orthodontics 81: 101–107

Nieminen P *et al.* 2001 Identification of a nonsense mutation in PAX9 gene in molar oligodontia. European Journal of Human Genetic 9: 743–746

Peck L, Peck S, Attia Y 1993 Maxillary canine-first premolar transposition, associated dental anomalies and genetic bases. Angle Orthodontist 63: 99–109

Peck S, Peck L, Kataja M 1996 Prevalence of tooth agenesis and peg-shaped maxillary lateral incisor associated with palatally displaced canine (PDC) anomaly. American Journal of Orthodontics and Dentofacial Orthopedics 110: 441–443

Peck S, Peck L, Kataja M 1998 Mandibular lateral incisor–canine transposition, concomitant dental anomalies, and genetic control. Angle Orthodontist 68: 455–466

Peck S, Peck L, Kataja M 2002 Concomitant occurrence of canine malposition and tooth agenesis: evidence of orofacial genetic fields. American Journal of Orthodontics and Dentofacial Orthopedics 122: 657–660

Peres R C R, Scarel-Caminaga R M, Santo A R E, Line S R P 2005 Association between PAX-9 promoter polymorphisms and hypodontia in humans. Archives of Oral Biology 50: 861–871

Polder B J, Van 't Hof M A, Van der Linden F P G M, Kuijpers-Jagtman A M 2004 A meta-analysis of the prevalence of dental agenesis of permanent teeth. Community Dentistry and Oral Epidemiology 32: 217–226

Rølling S 1980 Hypodontia of permanent teeth in Danish schoolchildren. Scandinavian Journal of Dental Research 88: 365–369

Seow W K, Lai P Y 1989 Association of taurodontism with hypodontia: a controlled study. Pediatric Dentistry 11: 214–218

Shapira Y, Kuftinec M M 2001 Maxillary tooth transpositions: characteristic features and accompanying dental anomalies. American Journal of Orthodontics and Dentofacial Orthopedics 119: 127–134

Stockton D W, Das P, Goldenberg M, D'Souza R N, Patel P I 2000 Mutation of PAX9 is associated with oligodontia. Nature Genetics 24: 18–19

Svinhufvud E, Myllärniemi S, Norio R 1988 Dominant inheritance of tooth malpositions and their association to hypodontia. Clinical Genetics 34: 373–381

Symons A L, Stritzel F, Stamation J 1993 Anomalies associated with hypodontia of the permanent lateral incisor and second premolar. Journal of Clinical Pediatric Dentistry 17: 109–111

Thesleff I, Nieminen P 1996 Tooth morphogenesis and cell differentiation. Current Opinion in Cell Biology 8: 844–850

Thongudomporn U, Freer T J 1998 Prevalence of dental anomalies in orthodontic patients. Australian Dental Journal 43: 395–398

Townsend G, Rogers J, Richards L, Brown T 1995 Agenesis of permanent maxillary lateral incisors in South Australian twins. Australian Dental Journal 40: 186–192

Van der Weide Y S, Beemer F A, Faber J A J, Bosman F 1994 Symptomatology of patients with oligodontia. Journal of Oral Rehabilitation 21: 247–261

Vastardis H 2000 The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. American Journal of Orthodontics and Dentofacial Orthopedics 117: 650–655

Vastardis H, Karimbux N, Guthua S, Seidman J G, Seidman C E 1996 A human *MSX1* homeodomain missense mutation causes selective tooth agenesis. Nature Genetics 13: 417–421

Wong A T Y, McGrath C, McMillan A S 2005 Oral health of southern Chinese children and adolescents with severe hypodontia. International Journal of Paediatric Dentistry 15: 256–263

Worsaae N, Jensen B N, Holm B, Holsko J 2007 Treatment of severe hypodontia—oligodontia—an interdisciplinary concept. International Journal of Oral Maxillofacial Surgery 36: 473–780

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Intermaxillary tooth size discrepancies among different malocclusion groups

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SUMMARY The aims of this study were to identify possible gender-related differences in tooth size ratios, to determine whether there is a prevalence for intermaxillary tooth size discrepancies in any malocclusion group, and to detect the percentage of tooth size discrepancies outside 1 or 2 standard deviations (SDs) from Bolton's mean. The material comprised the models of 500 subjects (284 females and 216 male aged between 12 and 28 years). Five groups were formed: normal occlusion, Class I, Class II division 1, Class II division 2, and Class III, which had an equal number of subjects. Tooth size measurements were undertaken using an electronic measuring device. Overall, anterior, and posterior ratios were computed as described by Bolton. For statistical evaluation, analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests were used.

A significant gender difference was found only for posterior ratio in all groups ($P<0.01$). There was no significant difference among the malocclusion groups in anterior ratio, but the differences for overall and posterior ratios were significant ($P<0.05$ and $P<0.001$, respectively). A large number of subjects had discrepancies greater than 2 SD from Bolton's mean. In addition, the means and SDs in this investigation were found to be larger than those of Bolton. Intermaxillary tooth size ratios may vary in different malocclusion types and may, to some degree, contribute to the severity of a malocclusion.

Introduction

One of the main tasks of an orthodontist is to obtain a functionally balanced occlusion between the upper and lower dental arches. For an ideal occlusion, the mesiodistal crown diameters of the teeth in both arches should correspond. Bolton (1958) investigated the relationship between the mesiodistal crown diameters of the upper and lower teeth and developed an analysis. This analysis is made directly on study casts, and rotations or other malpositions are not taken into account. In the calculation of a possible tooth size discrepancy, the sum of the diameters of the mandibular teeth is divided by that of the maxillary teeth and the result multiplied by 100. For evaluation of the two sets of 12 opposing teeth, the term 'overall ratio' is used and for the two sets of six anterior teeth, the term 'anterior ratio'. Bolton (1958) stated that for a good interdigitation and occlusion, overall ratio should be 91.3 ± 1.91 and anterior ratio 77.2 ± 1.65. Clinical application of the analysis has been described by Bolton (1962).

Bolton's analysis has been investigated in different racial groups and populations (Lavelle, 1972; Freeman *et al.*, 1996; Nie and Lin, 1999; Santoro *et al.*, 2000; Smith *et al.*, 2000; Ta *et al.*, 2001; Uysal and Sarı, 2005; Uysal *et al.*, 2005; Paredes *et al.*, 2006; Endo *et al.*, 2007; Othman and Harradine, 2007; Al-Omari *et al.*, 2008). These investigations were generally carried out on subjects with good or excellent occlusion. A limited number of studies in malocclusion groups have been undertaken, but their results were contradictory (Sperry *et al.*, 1977; Crosby and Alexander, 1989; Nie and Lin, 1999; Ta *et al.*, 2001; Araujo and Souki, 2003; Laino *et al.*, 2003; Uysal *et al.*, 2005). In addition, gender differences were considered only in the studies of Lavelle (1972), Arya *et al.* (1974), Smith *et al.* (2000), and Uysal and Sarı (2005).

Stifter (1958) replicated Bolton's study on Class I occlusion subjects and reported similar results. Lavelle (1972) showed that there was sexual dimorphism in tooth dimensions and in the ratio of upper to lower arch tooth size. Arya *et al.* (1974) observed tooth size differences between genders, in agreement with Moorrees *et al.* (1957), Lysell and Myrberg (1982), Smith *et al.* (2000), and Uysal and Sarı (2005).

Sperry *et al.* (1977) analyzed Bolton's ratios for Class I, II, and III subjects and found a mandibular tooth size excess in the overall ratio of the Class III patients, while Crosby and Alexander (1989) found no difference in tooth size in different malocclusion groups (Class I, Class II division 1, Class II division 2, and Class II surgery). Nie and Lin (1999) carried out a similar study on normal occlusion and malocclusion groups (Class I with bimaxillary protrusion, Class II division 1, Class II division 2, Class III, and Class III surgery) but found no sexual dimorphism for these ratios in any of the groups, and no significant difference between the subcategories of the malocclusion groups. However, they observed significant differences among the ratios of the Class I, II, and III groups. The subjects with a Class III malocclusion had larger ratio values than the other groups.

Uysal *et al.* (2005) found no significant sexual dimorphism except in the normal occlusion group in overall ratio; there

were no statistically significant differences among the malocclusion groups for anterior and overall ratios.

The aims of the present study were (1) to identify possible gender-related differences in tooth size ratios, (2) to determine whether there is a difference in intermaxillary tooth size discrepancies among the malocclusion groups classified by dental and skeletal variables, and (3) to determine the percentage of tooth size discrepancies outside 1 or 2 standard deviations (SDs) from Bolton's mean.

Subjects and methods

The sample comprised the study models of 100 subjects with normal occlusion and 400 patients with varying malocclusions. The distribution of the subjects according to gender and malocclusion is shown in Table 1. The models were randomly selected from the archives of the Department of Orthodontics, Faculty of Dentistry, Atatürk University, Erzurum, Turkey. The normal occlusion group included those with ideal occlusion and well-balanced faces. All subjects were between 12 and 28 years of age and of Turkish origin with Turkish grandparents. Occlusal categories, classified by Angle classification, coincided with the skeletal categories. Skeletal diagnosis was made on the basis of ANB angle; in skeletal Class I, ANB angle was from 0 to 5 degrees, for skeletal Class II more than 5 degrees, and for skeletal Class III less than 0 degrees (Laino *et al.*, 2003; Uysal *et al.*, 2005). The subjects were divided into five equal groups: normal occlusion, Class I, Class II division 1, Class II division 2, and Class III.

Table 1 The number of subjects in each gender and malocclusion group.

Malocclusion groups	Female	Male	Total
Normal occlusion	39	61	100
Class I	65	35	100
Class II division 1	61	39	100
Class II division 2	63	37	100
Class III	58	42	100
Total	284	216	500

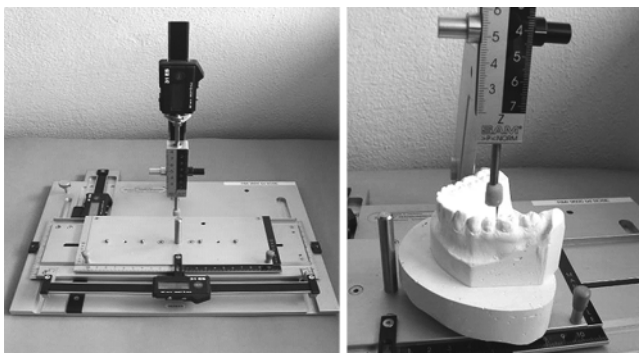

Figure 1 The electronic measuring device.

The following study model selection criteria were used:

1. Good quality models of the normal occlusion and pre-treatment models of the malocclusion groups.
2. All permanent teeth erupted except second and third molars.
3. No mesiodistal or occlusal tooth abrasion.
4. No residual crown or crown–bridge restoration.
5. Absence of tooth anomalies regarding form, structure, and development.

A RMI 550 three-dimensional measuring device (SAM Präzisionstechnick GmbH, München, Germany) was used to measure the casts to the nearest 0.01 mm (Figure 1). The mesiodistal crown diameters of all teeth were measured according to the method described by Moorrees *et al.* (1957), i.e. from the mesial contact point to the distal contact point at the greatest interproximal distance. The individual tooth diameters were summed to derive the anterior (canine to canine), posterior (first molar to first premolar), and overall (first molar to first molar) arch segments. The segments were used to define the following ratios:

1. Overall ratio: overall mandibular arch segment divided by the overall maxillary arch segment.
2. Anterior ratio: anterior mandibular arch segment divided by the anterior maxillary arch segment.
3. Posterior ratio: posterior mandibular arch segment divided by the posterior maxillary arch segment.

Overall, anterior, and posterior ratios were computed for all subjects whose values were outside 1 or 2 SDs from the mean value.

To determine the errors associated with the measurements, 30 study casts were randomly selected. Their measurements were repeated 4 weeks after the first measurement by the same examiner (EU). The first and second measurements were compared as described by Houston (1983). Coefficients of reliability were computed as 0.981, 0.990, and 0.965 for overall, anterior, and posterior ratios, respectively. The results showed that the measurements could be repeated with high accuracy.

In order to determine whether there was sexual dimorphism in the incidence of intermaxillary tooth size discrepancy and to compare intermaxillary tooth size discrepancies among the groups, analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were applied. Statistical analyses were carried out using the Statistical Package for Social Sciences (Version 11.5, SPSS Inc., Chicago, Illinois, USA).

Results

The means and SDs of the tooth size ratios for each gender and occlusion group are summarized in Table 2. Table 3 shows the results of ANOVA. A statistically significant

gender difference was found only for posterior ratio ($P<0.01$). As there was no sexual dimorphism in overall and anterior ratios, males and females were combined for each malocclusion groups for these ratios. Comparisons between the male and female subjects indicated larger posterior ratio values for males in all groups except Class III.

ANOVA demonstrated that there were statistically significant differences among the malocclusion groups for overall and posterior ratios ($P<0.05$ and $P<0.001$, respectively; Table 3). The results of Tukey's HSD analysis, which was used for comparisons of tooth size ratios of different malocclusion groups, are presented in Table 4. For overall ratio, the difference between the Class II division 1 and Class III groups was statistically significant ($P<0.01$). For the posterior ratio, the differences between the normal occlusion and Class I groups and between the normal occlusion and Class III groups were statistically significant at the 0.05 and 0.001 levels, respectively. Other differences among the groups were not statistically significant. The highest ratio values were in the Class III group, i.e. the subjects with a Class III malocclusion had larger mandibular teeth.

The frequencies of tooth size discrepancy 1, 2, and more than 2 SDs from Bolton's mean for overall and anterior ratios of all groups are presented in Table 5. For overall ratio in the total sample, 61.6 per cent were inside 1 SD, 28.4 per cent inside 2 SD, and 11 per cent outside 2 SD. The percentage distributions for anterior ratio were 41.4, 30.4, and 28.2 per cent, respectively.

Table 2 Tooth size ratios [mean (X) and standard deviation (SD)] for the groups.

	Female		Male		Pooled	
	X	SD	X	SD	X	SD
Normal occlusion						
Overall ratio	91.63	2.04	92.39	1.84	92.10	1.95
Anterior ratio	79.17	2.65	79.35	2.47	79.28	2.53
Posterior ratio	103.49	2.54	104.98	2.63		
Class I malocclusion						
Overall ratio	92.24	2.32	92.33	1.88	92.27	2.16
Anterior ratio	78.58	3.01	78.66	2.41	78.61	2.80
Posterior ratio	105.53	2.81	105.85	2.92		
Class II division 1 malocclusion						
Overall ratio	91.64	2.07	92.22	2.05	91.86	2.07
Anterior ratio	78.38	2.46	78.30	2.17	78.35	2.34
Posterior ratio	104.56	3.09	105.99	3.07		
Class II division 2 malocclusion						
Overall ratio	92.16	2.26	92.42	2.15	92.26	2.22
Anterior ratio	79.11	2.69	78.76	2.67	78.98	2.67
Posterior ratio	104.88	3.08	105.82	3.05		
Class III malocclusion						
Overall ratio	92.92	1.83	92.81	2.05	92.87	1.92
Anterior ratio	79.24	2.83	79.39	3.13	79.30	2.94
Posterior ratio	106.11	2.57	105.87	2.12		

Table 3 The results of analysis of variance.

Parameters	Factors	df	*F*	*P*
Overall ratio	Groups	4	3.020	0.018
	Gender	1	2.761	0.097
	Interaction	4	0.701	0.592
Anterior ratio	Groups	4	2.307	0.570
	Gender	1	0.000	0.985
	Interaction	4	0.161	0.958
Posterior ratio	Groups	4	5.316	0.000
	Gender	1	9.221	0.003
	Interaction	4	1.661	0.158

df, degree of freedom.

Discussion

It has been commonly accepted that the mesiodistal crown diameters of the upper and lower teeth should match each other for a balanced occlusion. Significant higher overall ratios can be explained by relatively larger mandibular or smaller maxillary arch segments, and thus there might be an association between malocclusion and tooth size. In other words, tooth size discrepancies between maxillary and mandibular teeth may be an important factor in the cause of malocclusions (Othman and Harradine, 2006).

In order to predict the occlusal relationships at the end of orthodontic treatment, a number of studies have been carried out. Many investigators have attempted to quantify interarch tooth size discrepancies, but none are as useful or as well accepted as Bolton's analysis (White, 1982; Crosby and Alexander, 1989; Shellhart *et al.*, 1995; Freeman *et al.*, 1996; Smith *et al.*, 2000). According to Sheridan (2000), the vast majority of orthodontists (91 per cent) use Bolton's tooth size analysis. This analysis has been investigated in different racial and malocclusion groups (Lavelle, 1972; Arya *et al.*, 1974; Crosby and Alexander, 1989; Freeman *et al.*, 1996; Nie and Lin, 1999; Santoro *et al.*, 2000; Smith *et al.*, 2000; Ta *et al.*, 2001; Araujo and Souki, 2003; Uysal and Sarı, 2005; Uysal *et al.*, 2005; Paredes *et al.*, 2006; Endo *et al.*, 2007; Othman and Harradine, 2007; Al-Omari *et al.*, 2008). The present study was carried out on Turkish subjects. Both skeletal classification, according to ANB, and Angle's dental classification were used for determination of the groups, and all malocclusions in the sagittal direction were included.

ANB is affected by several factors in the craniofacial structures (Oktay, 1991; Hurmerinta *et al.*, 1997), and thus floating norms have been introduced for ANB angle (Järvinen, 1986). In order to overcome the limitations of this angle, the selection criteria in the present study included Class II patients with ANB angle greater than 5 degrees and Class III patients with an ANB less than 0 degrees similar to the studies of Laino *et al.* (2003) and Uysal *et al.* (2005).

Table 4 Differences between the groups for overall, anterior, and posterior ratios and their levels of significance determined by Tukey's honestly significant difference analysis.

Comparisons	Overall ratio		Anterior ratio		Posterior ratio	
	Mean difference	*P*	Mean difference	*P*	Mean difference	*P*
Normal occlusion–Class I	−0.17	0.976	0.67	0.390	−1.25	**0.016**
Normal occlusion–Class II division 1	0.23	0.932	0.94	0.101	−0.72	0.370
Normal occlusion–Class II division 2	−0.16	0.981	0.30	0.932	−0.83	0.226
Normal occlusion–Class III	−0.78	0.063	−0.19	1.000	−1.61	**0.001**
Class I–Class II division 1	0.41	0.634	0.26	0.958	0.53	0.677
Class I–Class II division 2	0.01	1.000	−0.37	0.865	0.41	0.836
Class I–Class III	−0.60	0.242	−0.69	0.361	−0.36	0.892
Class II division 1–Class II division 2	−0.40	0.657	−0.63	0.454	−0.11	0.999
Class II division 1–Class III	−1.00	**0.006**	−0.95	0.089	−0.89	0.168
Class II division 2–Class III	−0.61	0.225	−0.32	0.916	−0.78	0.291

Bold font indicates statistically significant differences.

Table 5 The percentage distribution of anterior and overall tooth size discrepancies outside 1 or 2 standard deviations (SDs) from Bolton's means.

	Anterior ratio						
	Outside 2 SD (%)	2 SD (%)	1 SD (%)	Mean (%)	1 SD (%)	2 SD (%)	Outside 2 SD (%)
	<73.9	73.9–75.4	75.5–77.1	77.2	77.3–78.8	78.9–80.5	>80.5
Normal occlusion	0	5	12	3	26	26	28
Class I malocclusion	3	6	22	2	16	27	24
Class II division 1 malocclusion	4	5	23	1	26	23	18
Class II division 2 malocclusion	3	6	17	1	18	28	27
Class III malocclusion	4	4	14	2	24	22	30
	Overall ratio						
	Outside 2 SD (%)	2 SD (%)	1 SD (%)	Mean (%)	1 SD (%)	2 SD (%)	Outside 2 SD (%)
	<87.5	87.5–89.3	89.4–91.2	91.3	91.4–93.2	93.3–95.1	>95.1
Normal occlusion	2	6	25	3	39	18	7
Class I malocclusion	1	5	27	3	33	22	9
Class II division 1 malocclusion	1	11	29	1	36	15	7
Class II division 2 malocclusion	1	11	21	3	30	25	9
Class III malocclusion	0	3	18	1	39	26	13

Needle-pointed orthodontic dividers are commonly used to determine the greatest mesiodistal diameter of the teeth. Digital callipers are also used to measure the teeth to the nearest 0.1 or 0.01 mm. In recent years, new techniques and devices have been developed in order to achieve more accurate and reliable tooth measurements (Yen, 1991; Schirmer and Wiltshire, 1997; Mok and Cooke, 1998; Nie and Lin, 1999; Tomasetti *et al.*, 2001; Othman and Harradine, 2006). All tooth measurements in this study were carried out using an electronic measuring device. This device has a needle-pointed measuring rod which can move in three dimensions of space, allowing the greatest mesiodistal diameters of the teeth to be easily measured, even if crowding is present.

Moorrees *et al.* (1957) showed gender differences in overall ratio. Lavelle (1972) reported relatively larger overall and anterior ratios in males compared with white, black, and mongoloid female populations. Smith *et al.* (2000) found larger overall and posterior ratios in black, Hispanic, and white males. Uysal and Sarı (2005) found statistically significant gender difference only in overall ratio. Crosby and Alexander (1989) did not differentiate between genders and did not mention whether there was

sexual dimorphism for tooth size ratios in their sample. Santoro *et al.* (2000), Ta *et al.* (2001), Basaran *et al.* (2006), Endo *et al.* (2007), and Al-Omari *et al.* (2008) on the other hand observed no sexual dimorphism in overall and anterior ratios. Nie and Lin (1999) found no difference between the genders for the three tooth size ratios. The results of the present study showed no sexual dimorphism in overall and anterior ratios but sexual dimorphism in the posterior ratio ($P<0.01$; Table 3). For this reason, males and females were combined only for comparison of overall and anterior ratios.

Different results have been reported in the literature regarding tooth size ratios in different malocclusion groups. Xia and Wu (1983) found no statistically significant difference in tooth size ratios between malocclusion and normal occlusion groups. Crosby and Alexander (1989), in a comparison of tooth size ratios among Class I, Class II division 1, Class II division 2, and Class II surgery groups, found no significant differences. The present study showed that only anterior ratio was not significantly different among the groups (Table 4). The possible reason for these different results may be ethnic or racial because tooth sizes show considerable variation in different racial and occlusal categories (Lavelle, 1972).

Sperry *et al.* (1977) observed, in a Class III group with mandibular prognathism, more mandibular tooth size excess for overall ratio than in the Class I and Class II groups. Similarly, Lavelle (1972) and Nie and Lin (1999) demonstrated that Class III subjects were characterized by smaller maxillary tooth dimensions and larger mandibular teeth. Araujo and Souki (2003) investigated the correlations between anterior tooth size discrepancies and Angle Class I, II, and III malocclusions. They concluded that Angle Class I and III individuals showed a significantly greater prevalence of tooth size discrepancies than those with a Class II malocclusion, and that the mean anterior tooth size discrepancy for Angle Class III subjects was significantly greater than for Class I and II subjects. Othman and Harradine (2006) reviewed the literature on Bolton's tooth size discrepancy and concluded that subjects with a Class III malocclusion probably had higher average ratios.

In the present study, there were significant differences among the normal occlusion and malocclusion groups for overall and posterior ratios ($P<0.05$ and $P<0.001$, respectively; Table 3). The results of Tukey's HSD analysis showed that the Class III group had the largest overall, anterior, and posterior ratios, but statistical significance was seen only between the Class II division 1 and Class III groups for overall ratio ($P<0.01$), between the normal occlusion and Class III groups ($P<0.001$), and between the normal occlusion and Class I groups ($P<0.05$) for posterior ratio (Table 4). There was a tendency for mandibular tooth size excess in the Class III malocclusion subjects and for maxillary tooth size excess in the Class II malocclusion patients. These results are compatible with the literature.

According to Bolton (1962), a ratio greater than 1 SD from the reported mean values indicates a need for diagnostic consideration. Crosby and Alexander (1989) and Freeman *et al.* (1996) defined a significant discrepancy as a value of more than 2 SDs from Bolton's mean. On the other hand, Othman and Harradine (2006) stated that Bolton's SDs were not a good guide to the prevalence of a clinically significant tooth size discrepancy.

In the present sample, the frequency of tooth size discrepancy outside 1 or 2 SDs from Bolton's mean values was used to determine the clinical significance of tooth size imbalance. The results for overall and anterior ratios were nearly the same. Most subjects in all groups had overall and anterior ratios within a 1 SD interval (Table 5). It should be noted, however, that the 11 per cent of the subjects had a discrepancy greater than 2 SDs from Bolton's means for overall ratio and 28.2 per cent for anterior ratio. Thus maxillary and mandibular anterior teeth had a greater incidence of tooth size deviations, i.e. the greatest variables in mesiodistal tooth width occurred in the anterior region. It should also be noted that there was a larger percentage of subjects with mandibular tooth size excess since almost all those with ratios outside 2 SDs had larger ratio values than Bolton's means. In other studies, percentage values of 9.5 (Al-Omari *et al.*, 2008), 11 (Santoro *et al.*, 2000), 13.4 (Freeman *et al.*, 1996), and 15.3 (Uysal *et al.*, 2005) for overall ratio and of 21.3 (Uysal *et al.*, 2005), 22.9 (Crosby and Alexander, 1989), 23.7 (Al-Omari *et al.*, 2008), 28 (Santoro *et al.*, 2000), and 30.6 (Freeman *et al.*, 1996) for anterior ratio have been reported in different patient populations. The present results are compatible with those in the literature.

For both overall and anterior ratios in the present study, the means and SDs were larger than in Bolton's (1958) study. This finding is consistent with the results of Nie and Lin (1999), Smith *et al.* (2000), and Al-Omari *et al.* (2008). Crosby and Alexander (1989), Freeman *et al.* (1996), and Santoro *et al.* (2000) found that the means in their studies and those of Bolton's study were nearly identical although the ranges and SDs were significantly larger. The probable reason for these findings may be the types of population that constituted the samples.

Conclusions

On the basis of the results of this investigation, the following conclusions can be drawn:

1. Statistically significant gender differences were found only for posterior ratio. The gender differences in overall and anterior ratios were not significant.
2. There were no statistically significant differences among the groups for anterior ratio.
3. There were statistically significant differences among the groups for overall and posterior ratios. The significance originated from the Class III group.
4. A large number of subjects in each group had discrepancies greater than 2 SD from the Bolton mean.

The cause of these discrepancies was the larger mesiodistal diameter of the mandibular teeth.

5. The means and SDs for overall and anterior ratios in the present study were larger than those in Bolton's study.

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References

Al-Omari I K, Al-Bitar Z B, Hamdan A M 2008 Tooth size discrepancies among Jordanian schoolchildren. European Journal of Orthodontics 30: 527–531

Araujo E, Souki M 2003 Bolton anterior tooth size discrepancies among different malocclusion groups. Angle Orthodontist 73: 307–313

Arya B S, Savara B S, Thomas D, Clarkson Q 1974 Relation of sex and occlusion to mesiodistal tooth size. American Journal of Orthodontics 66: 479–486

Basaran G, Selek M, Hamamcı O, Akkus Z 2006 Intermaxillary Bolton tooth size discrepancies among different malocclusion groups. Angle Orthodontist 76: 26–30

Bolton W A 1958 Disharmony in tooth size and its relation to the analysis and treatment of malocclusion. Angle Orthodontist 28: 113–130

Bolton W A 1962 The clinical application of a tooth size analysis. American Journal of Orthodontics 48: 504–529

Crosby D R, Alexander C G 1989 The occurrence of tooth size discrepancies among different malocclusion groups. American Journal of Orthodontics and Dentofacial Orthopedics 95: 457–461

Endo T, Shundo I, Abe R, Ishida K, Yoshino S, Shimooka S 2007 Applicability of Bolton's tooth size ratios to a Japanese orthodontic population. Odontology 95: 57–60

Freeman J E, Maskeroni A J, Lorton L 1996 Frequency of Bolton tooth-size discrepancies among orthodontic patients. American Journal of Orthodontics and Dentofacial Orthopedics 110: 24–27

Houston W J B 1983 The analysis of errors in orthodontic measurements. American Journal of Orthodontics 83: 382–390

Hurmerinta K, Rahkamo A, Haavikko K 1997 Comparison between cephalometric classification methods for sagittal jaw relationships. European Journal of Oral Sciences 105: 221–227

Järvinen S 1986 Floating norms for the ANB angle as guidance for clinical considerations. American Journal of Orthodontics and Dentofacial Orthopedics 90: 383–387

Laino A, Quaremba G, Paduana S, Stanzione S 2003 Prevalence of tooth-size discrepancy among different malocclusion groups. Progress in Orthodontics 4: 37–44

Lavelle C L B 1972 Maxillary and mandibular tooth size in different racial groups and in different occlusal categories. American Journal of Orthodontics 61: 29–37

Lysell L, Myrberg N 1982 Mesiodistal tooth size in the deciduous and permanent dentitions. European Journal of Orthodontics 4: 113–122

Mok K H Y, Cooke M S 1998 Space analysis: a comparison between sonic digitization (DigiGraph Workstation) and the digital caliper. European Journal of Orthodontics 20: 653–661

Moorrees C F A, Thomsen S O, Jensen E, Yen P K J 1957 Mesiodistal crown diameters of the deciduous and permanent teeth in individuals. Journal of Dental Research 36: 39–47

Nie Q, Lin J 1999 Comparison of intermaxillary tooth size discrepancies among different malocclusion groups. American Journal of Orthodontics and Dentofacial Orthopedics 116: 539–544

Oktay H 1991 A comparison of ANB, Wits, AF-BF, and APDI measurements. American Journal of Orthodontics and Dentofacial Orthopedics 99: 122–128

Othman S A, Harradine N W T 2006 Tooth-size discrepancy and Bolton's ratios: a literature review. Journal of Orthodontics 33: 45–51

Othman S A, Harradine N W T 2007 Tooth size discrepancies in an orthodontic population. Angle Orthodontist 77: 668–674

Paredes V, Gandia J L, Cibrian R 2006 Do Bolton's ratios apply to a Spanish population? American Journal of Orthodontics and Dentofacial Orthopedics 129: 428–430

Santoro M, Ayoub M E, Pardi V A, Cangialosi T J 2000 Mesiodistal crown dimensions and tooth size discrepancy of the permanent dentition of Dominican Americans. Angle Orthodontist 70: 303–307

Schirmer U R, Wiltshire W A 1997 Manual and computer-aided space analysis: a comparative study. American Journal of Orthodontics and Dentofacial Orthopedics 112: 676–680

Shellhart W C, Lange W, Kluemper G T, Hicks P, Kaplan A 1995 Reliability of the Bolton tooth-size analysis when applied to crowded dentitions. Angle Orthodontist 65: 327–334

Sheridan J J 2000 The reader's corner. Journal of Clinical Orthodontics 34: 593–597

Smith S S, Buschang P H, Watanabe E 2000 Interarch size relationships of three populations: does Bolton's analyses apply? American Journal of Orthodontics and Dentofacial Orthopedics 117: 169–174

Sperry T P, Worms F W, Isaacson R J, Speidel T M 1977 Tooth-size discrepancy in mandibular prognathism. American Journal of Orthodontics 72: 183–190

Stifter J 1958 A study of Pont's, Howes', Rees', Neff's and Bolton's analyses on Class I adult dentitions. Angle Orthodontist 28: 215–225

Ta T A, Ling J Y K, Hägg U 2001 Tooth-size discrepancies among different occlusion groups of southern Chinese children. American Journal of Orthodontics and Dentofacial Orthopedics 120: 556–558

Tomasetti J J, Taloumis L J, Denny J M, Fisher J R 2001 A comparison of 3 computerized Bolton tooth size analyses with a commonly used method. Angle Orthodontist 71: 351–357

Uysal T, Sarı Z 2005 Intermaxillary tooth size discrepancy and mesiodistal crown dimensions for a Turkish population. American Journal of Orthodontics and Dentofacial Orthopedics 128: 226–230

Uysal T, Sarı Z, Başçiftçi F A, Memili B 2005 Intermaxillary tooth size discrepancy and malocclusion: is there a relation? Angle Orthodontist 75: 208–213

White L W 1982 The clinical use of occlusograms. Journal of Clinical Orthodontics 16: 92–103

Xia Z, Wu X Y 1983 The application of dentocclusal measurement in malocclusion. Stomatology 3: 126–127

Yen C H 1991 Computer-aided space analysis. Journal of Clinical Orthodontics 25: 236–238

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Overall and anterior Bolton ratio in Class I, II, and III orthodontic patients

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SUMMARY The aim of the investigation was to compare overall and anterior Bolton ratios in different malocclusion groups with Bolton's standards. The material comprised 600 pre-treatment study casts (262 males and 338 females, aged 12–25 years), selected from the models of 3088 patients who had applied for orthodontic treatment based on the following criteria: permanent dentition from the first right molar to the first left molar and no interproximal caries or restorations. There were 162 Class I, 144 Class II division 1, 155 Class II division 2, and 139 Class III patients. Statistical analysis of the data was undertaken using a Student's *t*-test.

Statistically significant differences were found for the mean overall ratio when compared with the original Bolton norm for the whole study group, as well as for patients with Class I and III malocclusions when the mean anterior ratio was compared with the original Bolton norm. Significant differences were observed in all malocclusion groups for both genders. Discrepancies exceeding 2 SD were found in 31.2 per cent of the studied population for the anterior ratio when compared with Bolton's norm. The highest mean values for anterior ratio were in males with Class I (79.1) and Class III (80.1) malocclusions.

Introduction

Bolton's analysis is one of the most popular methods for determining tooth size abnormality. It is useful in aiding diagnosis as well as in treatment planning. The overall Bolton ratio is the percentage obtained by summing the widths of the 12 mandibular teeth divided by the sum of the widths of the 12 maxillary teeth and should be 91.3 ± 0.26 per cent. Anterior ratio is the percentage obtained by summing the widths of the six mandibular anterior teeth divided by the sum of the widths of the six maxillary anterior teeth and should be 77.2 ± 0.22 per cent (Bolton, 1958, 1962). The original analysis was performed on 55 patients with excellent occlusion, including 44 orthodontically treated (non-extraction) and 11 untreated subjects.

Similar research was carried out by Lundström (1954), where the medium ratio for the incisors and canines was stated to be equal to 78.5 ± 0.13 and the medium ratio of all the 12 teeth equal to 92.3 ± 0.26. The same values for overall and anterior ratios were found for a group of 57 dental students and eight Navaho Indians with a Class I occlusion (Stifter, 1958).

Later studies of the Bolton ratio concerned mainly patients from different Caucasian populations of the United States (Neff, 1957; Sperry *et al.*, 1977; Doris *et al.*, 1981; Crosby and Alexander, 1989) as well as Europeans (Lundström, 1954; Lavelle, 1972; Ebeling *et al.*,1973; Arya *et al.*, 1974; Manke and Miethke, 1983; Redahan and Lagerström, 2003). All publications are listed in Table 1. Most of the studies were based on patients applying for orthodontic treatment with different malocclusions. The sample sizes varied between 55 and 710.

As Bolton used casts of subjects with an ideal occlusion, it is not possible to clinically determine the size of significant discrepancies. Bolton suggested that a discrepancy greater than 1 SD may create clinical problems. Most authors define a clinically significant ratio as 2 SD outside Bolton's mean (Crosby and Alexander, 1989; Freeman *et al.*, 1996; Santoro *et al.*, 2000). Proffit *et al.* (2007) stated that a tooth width discrepancy larger than 1.5 mm creates problems that should be considered in the treatment plan. Most authors assert that a tooth size discrepancy, compared with Bolton's norm, greater than 1.5 mm, or 2 SD, results in difficulties in tooth alignment in the finishing phase of treatment (Crosby and Alexander, 1989; Freeman *et al.*, 1996; Santoro *et al.*, 2000; Araujo and Souki, 2003; Bernabé *et al.*, 2004; Othman and Harradine, 2007). No evidence has been found for the clinical importance of a discrepancy exceeding 2 SD or 1.5 mm and both values seem to be suggestions.

Numerous authors claim that it is necessary to measure the teeth before initiating orthodontic treatment (Freeman *et al.*, 1996; Alkofide and Hashim, 2002; Othman and Harradine, 2007).

Only one Bolton study of the Polish population has been published (Bielawska, 1994). It comprised 51 patients with malocclusions, 22 with a Class II, 10 with a Class III, and 19 with a crossbite.

The aim of the present investigation therefore was to calculate the overall and anterior Bolton ratios in different malocclusion groups of Polish patients applying for orthodontic treatment and to compare them with Bolton's standards.

Table 1 Previous Bolton studies.

Author	Year of publication	Population	Occlusion	Sample size			Anterior ratio	Overall ratio
Lundström	1954	Swedish schoolchildren	No data	140			78.5	92.3
Bolton	1958 1962	American selected orthodontic	Ideal	55			77.2	91.3
Stifter	1958	American selected students and Navaho Indians	Normal	65			77.55	91.04
Lavelle	1972	British selected dental	Ideal untreated	40	Caucasoid	Male	76.8	91.7
						Female	77.5	90.8
				40	Negroid	Male	79.4	93.5
						Female	78.6	92.9
				40	Mongoloid	Male	78.7	92.6
						Female	78.2	92.1
Manke and Miethke	1983	German orthodontic	No data	100			78.28	No data
Crosby and Alexander	1989	American orthodontic	Class I and II	109			77.5	91.4
Lew and Keng	1991	Chinese selected orthodontic	Ideal	85			77.89	No data
Bielawska	1994	Polish orthodontic	Distal occlusion	22			No data	91.2 92.1
			Mesial occlusion	10				92.1
			Crossbite	19				
Freeman *et al.*	1996	American orthodontic	No data	157			77.8	91.4
Nie and Lin	1999	Chinese orthodontic	Class I, II, and III	300			81.52	93.27
Santoro *et al.*	2000	Dominican orthodontic	No data	54			78.1	91.3
Smith *et al.*	2000	American orthodontic	No data	180		White	79.6	92.3
						Black	79.3	93.4
						Hispanic	80.5	93.1
Ta *et al.*	2001	Chinese selected schoolchildren	Class I, II, and III	110			77.5	90.9
Alkofide and Hashim	2002	Saudi Arabian selected orthodontic	Class I, II, and III	240			78.86	92.61
Araujo and Souki	2003	Brazilian orthodontic	Class I, II, and III	300			78.18	No data
Redahan and Lagerström	2003	Swedish orthodontic	Different malocclusions	137			78.0	No data
Bernabé *et al.*	2004	Peruvian schoolchildren	Different malocclusions	200		Male	78.39	91.33
						Female	77.78	90.79
Baidas and Hashim	2005	Turkish orthodontic	No data	184			79.11	92.03
Al-Tamimi and Hashim	2005	Saudi Arabian orthodontic	Normal	65			77.4	91.4
Nourallah *et al.*	2005	Syrian orthodontic	Class I	55			78.99	92.26
Uysal *et al.*	2005	Turkish orthodontic	Class I, II, and III	710			78.26	89.88
Uysal and Sari	2005	Turkish orthodontic	Normal	150			78.26	89.88
Paredes *et al.*	2006b	Spanish orthodontic	No data	100			78.32	91.97
Akyalcin *et al.*	2006	Turkish orthodontic	Class I, II, and III	152			78.15	91.34
Fattahi *et al.*	2006	Iranian orthodontic	Class I, II, and III	200			79.01	91.68
Endo *et al.*	2007	Japanese orthodontic	Class I	60			78.39	91.6
Freire *et al.*	2007	Brazilian orthodontic	Normal	30			77.83	91.46
Al-Omari *et al.*	2008	Jordanian schoolchildren	No data	367			78.6	92.2

Materials and methods

The study material comprised 600 pre-treatment study casts, selected from models of 3088 patients, who during 2003–2006 applied to the university department of orthodontics and two private orthodontic practices in Szczecin and Kolobrzeg for orthodontic treatment. Each of the patients had a cephalometric radiograph. The diagnosis of a Class I occlusion was based on a Class I molar and canine relationship as well as an ANB angle between 0 and 4 degrees. The diagnosis of a Class II was based on the presence of Class II molar and canine relationship accompanied by an ANB angle greater than 4 degrees. Overjet was a criterion to differentiate between Class II division 1 and 2. Class III was diagnosed based on an inverse overjet, a Class III molar and canine relationship as well as an ANB angle less than 0 degrees.

The inclusion criteria were:

1. between 12 and 25 years of age
2. fully erupted permanent dentition from the first molar to the first molar in both arches
3. diagnostic records, including study casts, panoramic view, and a lateral cephalogram

4. the clinical diagnosis of Class I, II, or III malocclusion exemplified by the presence of its features regarding molar and canine relationship and overjet, as well as cephalometric analysis
5. absence of interproximal caries or restorations as well as prosthetic crowns or bridges

The study group included 162 Class I patients (73 males and 89 females), 144 Class II division 1 patients (60 males and 84 females), 155 Class II division 2 patients (67 males and 88 females), and 139 Class III (62 males and 77 females). All were of Polish nationality and Caucasian. The age distribution of the groups is shown in Table 2.

All measurements on the study models were undertaken by one author (BWS) with sliding callipers (Dentaurum, Pforzheim, Germany), accurate to the nearest 0.1 mm. The following were calculated for each pair of study casts.

S_{12mand}—sum of the widths of the 12 mandibular teeth (mm)
S_{12max}—sum of the widths of the 12 maxillary teeth (mm)
S_{6mand}—sum of the widths of the 6 mandibular teeth (mm)
S_{6max}—sum of the widths of the 6 maxillary teeth (mm)

Overall and anterior Bolton ratios, B_{or} and B_{ar}, were calculated according to the following equations: $B_{or} = S_{12mand}/S_{12max}$ and $B_{ar} = S_{6mand}/S_{6max}$, respectively. Since the reasons for tooth size discrepancies in individual patients were not diagnosed, each discrepancy was described as the relative mandibular or maxillary tooth size excess. The formulae for the relative tooth size excess, E, depends on the relationship between the obtained ratios and Bolton standards:

$$E = S_{12mand} - 91.3\%\ S_{12max} \quad \text{for } B_{or} > 91.3\%,$$

$$E = S_{12max} - S_{12mand}/91.3\% \quad \text{for } B_{or} < 91.3\%,$$

$$E = S_{6mand} - 77.2\%\ S_{6max} \quad \text{for } B_{ar} > 77.2\%,$$

$$E = S_{6max} - S_{6mand}/77.2\% \quad \text{for } B_{ar} < 77.2\%.$$

The relative tooth size excess was calculated for each patient.

Table 2 Age distribution between the groups investigated.

Malocclusion group	Sample size	Range (year.month)	Mean (year.month)
Class I male	73	12.25–25.30	14.67
Class I female	89	12.00–25.00	14.33
Class II division 1 male	60	12.42–25.17	14.25
Class II division 1 female	84	12.08–25.42	14.25
Class II division 2 male	67	12.25–25.17	15.33
Class II division 2 female	88	12.17–25.08	14.33
Class III male	62	12.50–25.58	16.33
Class III female	77	12.33–25.00	16.25

Statistical analysis

The Shapiro–Wilk test showed that the data distribution in all the malocclusion groups was normal. The overall and anterior ratios for a particular malocclusion group was compared with Bolton's standard using the Student's *t*-test.

In order to assess the error of the method, 30 study casts were randomly chosen from the total of 600 and remeasured 3 months later by the same investigator. The standard deviation (SD) of all 360 measurement differences was 0.22 mm, giving a coefficient of variation of 2.74 per cent. The error of the method was calculated using Dahlberg's equation. The average measurement error of tooth widths was 0.17 mm corresponding to a relative error of 2.11 per cent. All measurement errors were found to be less than 10 per cent, which was considered acceptable.

Bolton ratios obtained from both measurements were compared using three approaches. First, according to Dahlberg's equation, the errors of the overall and anterior ratios were equal to 0.74 and 0.96 or 0.80 and 1.21 per cent, respectively. In the second approach, the confidence intervals of Bolton ratios obtained from the two measurements were calculated and compared. The lower confidence bounds differed by 0.09 and 0.45 per cent for the overall and anterior ratios, respectively, and the upper confidence bounds by 0.16 and 0.05 per cent, respectively. This means, that the confidence intervals for both measurements overlapped to a high degree. Finally, conservative intraclass correlation coefficients (2;1) were calculated. The results were 0.886 and 0.872 for overall and anterior ratios, respectively. All these approaches confirmed an acceptable level of measurement error.

Results

The overall Bolton ratio for all the malocclusion groups is shown in Table 3. Statistically significant differences were found for the whole study group as well as for the subgroups of subjects with Class I and III malocclusions.

The results for anterior Bolton ratio in the individual malocclusion groups are presented in Table 4. Comparing anterior ratio in the individual malocclusion groups with Bolton's standards, statistically significant differences were observed for all groups and for both genders; the greatest difference was for males with Class III (80.1 ± 3.0) and Class I (79.1 ± 2.2) malocclusions.

The percentage of patients with a significant discrepancy in overall and anterior Bolton ratios in the various malocclusion groups is shown in Table 5. Comparing anterior ratio with Bolton's norm, discrepancies exceeding 2 SD were found in 31.2 per cent of the population studied. The highest percentage of subjects with an anterior Bolton discrepancy exceeding 2 SD (45.2 per cent) was found in males with a Class III malocclusion.

Table 3 Overall Bolton ratio in the individual malocclusion groups.

	Sample size	Minimum	Maximum	Mean	SD	Coefficient of variation (%)	*P* value
Class I male	73	87.9	97.8	92.3	1.86	2.0	***
Class I female	89	86.5	96.0	91.9	2.09	2.3	NS
Class II division 1 male	60	86.0	95.9	91.2	2.22	2.4	NS
Class II division 1 female	84	83.9	95.9	91.1	2.16	2.4	NS
Class II division 2 male	67	84.7	96.1	91.7	2.21	2.4	NS
Class II division 2 female	88	86.7	99.1	91.5	2.23	2.4	NS
Class III male	62	86.6	98.0	93.0	2.29	2.5	***
Class III female	77	86.4	97.7	92.0	2.46	2.7	**
Total male	262	84.7	98.0	92.1	2.22	2.4	***
Total Female	338	83.9	99.1	91.6	2.25	2.5	**
Total	600	83.9	99.1	91.8	2.24	2.4	***

NS, not significant. **$P<0.01$, ***$P<0.001$.

Table 4 Anterior Bolton ratio in the individual malocclusion groups.

	Sample size	Minimum	Maximum	Mean	SD	Coefficient of variation (%)	*P* value
Class I male	73	74.4	86.2	79.1	2.20	2.8	***
Class I female	89	68.5	86.0	78.4	2.98	3.8	***
Class II division 1 male	60	72.5	84.5	78.1	2.46	3.1	**
Class II division 1 female	84	72.2	84.4	78.8	2.63	3.3	***
Class II division 2 male	67	71.5	83.8	78.4	2.71	3.5	***
Class II division 2 female	88	72.5	87.3	78.4	2.80	3.6	***
Class III male	62	73.2	89.2	80.1	3.00	3.7	***
Class III female	77	70.9	88.5	78.9	2.89	3.7	***
Total male	262	71.5	89.2	78.9	2.70	3.4	***
Total Female	338	68.5	88.5	78.6	2.83	3.6	***
Total	600	68.5	89.2	78.8	2.77	3.5	***

**$P<0.01$, ***$P<0.001$.

Table 5 The frequency of Bolton tooth size discrepancies exceeding 2 SD.

	Sample size	Frequency of overall ratio discrepancy			Frequency of anterior ratio discrepancy		
		Total (%)	Relative maxillary excess (%)	Relative mandibular excess (%)	Total (%)	Relative maxillary excess (%)	Relative mandibular excess (%)
Class I male	73	4.1	0.0	4.1	28.8	0.0	28.8
Class I female	89	7.9	1.1	6.7	31.5	5.6	25.8
Class II division 1 male	60	10.0	6.7	3.3	20.0	6.7	13.3
Class II division 1 female	84	9.5	4.8	4.8	34.5	4.8	29.8
Class II division 2 male	67	7.5	1.5	6.0	34.3	3.0	31.3
Class II division 2 female	88	9.1	2.3	6.8	25.0	2.3	22.7
Class III male	62	22.6	1.6	21.0	45.2	1.6	43.5
Class III female	77	13.0	2.6	10.4	31.2	3.9	27.3
Total male	262	10.7	2.3	8.4	32.1	2.7	29.4
Total female	338	9.8	2.7	7.1	30.5	4.1	26.3
Total	600	10.2	2.5	7.7	31.2	3.5	27.7

Discussion

In the present study, the mean overall Bolton ratio was 91.8 per cent, which is significantly higher than Bolton's standard. Anterior and overall Bolton ratios were found to be higher in the malocclusion groups than in the untreated subjects with normal occlusion (Table 1). The mean anterior Bolton ratio was 78.8 per cent, which was higher when compared with Bolton's standard for all malocclusion groups and for both genders. The size of the maxillary anterior teeth, particularly the lateral incisors, may differ

within populations. A greater percentage of patients with anterior tooth size discrepancy than with discrepancies in overall ratio can be explained by the fact that the size of the anterior teeth has, mathematically, less effect on overall ratio (Othman and Harradine, 2006).

The finding that males with a Class III malocclusion have higher Bolton ratios is in agreement with previous reports (Lavelle, 1972; Nie and Lin, 1999). However, some authors found no differences in gender or type of malocclusion (Arya *et al.*, 1974; Sperry *et al.*, 1977; Crosby and Alexander, 1989; Nourallah *et al.*, 2005; Uysal *et al.*, 2005). The only study concerning the Polish population by Bielawska (1994), performed on 51 orthodontic patients with different malocclusions, also did not find any statistically significant differences. It is likely that in the most studies citied, the sample sizes of particular malocclusions were not sufficiently large to detect differences between the malocclusion groups. Moreover, some investigations did not include Class III subjects.

In the present study, the prevalence of a significant (exceeding 2 SD) discrepancy in overall ratio was 10.2 per cent. Both Bolton (1958, 1962) and Proffit *et al.* (2007) reported less than 5 per cent of cases with an overall Bolton discrepancy exceeding 2 SD, but their studies included populations with excellent occlusion, which may be considered representative of the general population, but not of patients beginning orthodontic treatment.

The frequency of a significant anterior Bolton discrepancy in the present investigation was 31.2 per cent, which supports earlier findings in other populations (Santoro *et al.*, 2000; Smith *et al.*, 2000; Alkofide and Hashim, 2002; Araujo and Souki, 2003; Bernabé *et al.*, 2004; Fattahi *et al.*, 2006; Paredes *et al.*, 2006a,b; Endo *et al.*, 2007).

It has been suggested that it is necessary to determine specific standards, especially for anterior Bolton ratio, for different populations (Ta *et al.*, 2001; Uysal and Sari, 2005; Endo *et al.*, 2007) as well as for different malocclusions (Ta *et al.*, 2001) since the relationships established by Bolton for American whites are not always appropriate. The standard, however, should be the range of the proportion of tooth size that allows alignment of the teeth in perfect occlusion. Thus, it seems to be impractical to produce 'specific particular standards' for the numerous different malocclusions since these should be considered as discrepancies.

Bolton's standards have not been adequately verified on large groups of individuals of different ethnicity with ideal Class I occlusions, perfect alignment, and no crowding. Uysal and Sari (2005) calculated Bolton ratios in a group of 150 subjects with a normal occlusion, but with minor crowding. The obtained mean values and SDs seem to differ from Bolton's means, but the authors did not verify the compatibility with Bolton's norms, so the difference may be statistically insignificant. Nie and Lin (1999) calculated the Bolton ratio for 60 subjects with normal occlusion and found the received mean values to be higher than those of Bolton, but statistical comparisons were made only between normal occlusion and malocclusion groups and the inclusion criteria for the subjects with a normal occlusion were not reported. Lew and Keng (1991), who undertook measurements on 85 study casts with perfect occlusions (32 untreated and 53 post-orthodontic), reported anterior ratios almost identical to Bolton's mean. This finding is also in concordance with the study of Ta *et al.* (2001) who stated that Bolton standard applies to Chinese children with a Class I occlusion.

Based on the fact that Bolton's standards apply to patients with ideal occlusion (Lavelle, 1972; Lew and Keng, 1991), it seems that the standards, e.g. values characterizing individuals with a perfect Class I occlusion, could be similar for different populations, but various ethnic groups may be characterized by a different prevalence of Bolton discrepancy.

The fact that Bolton used casts with ideal occlusion has made it impossible to determine the size of any discrepancy that would make an ideal occlusion unachievable. An attempt to verify the clinical importance of tooth size discrepancy was undertaken by Heusdens *et al.* (2000) who created different amounts of discrepancy in an experimental set-up. By simultaneously altering the curve of Spee, an acceptable occlusion according to the Peer Assessment Rating (PAR) Index was achieved even with 6 mm of anterior stripping in the upper dental arch (PAR Index 2.35). Tooth size discrepancy was not studied as an isolated factor. Thus, the clinical importance of tooth size discrepancy, especially relative to anterior mandibular tooth size excess, has not been adequately verified. It should be remembered that patients treated with fixed appliances expect at the termination of treatment an occlusion, which is ideal, not acceptable according to some index. This may be difficult in Class III malocclusion subjects with an anterior Bolton discrepancy due to a relative mandibular tooth size excess, if not diagnosed before the initiation of treatment.

Conclusions

1. Bolton ratios in patients with malocclusions differ from Bolton's standards.
2. An anterior Bolton discrepancy exceeding 2 SD occurs in 31.2 per cent of Polish orthodontic patients.
3. It is necessary to calculate Bolton's ratios in all orthodontic patients, especially in males with a Class III malocclusion.

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References

Akyalcin S, Dogan S, Dincer B, Erdinc A M E, Oncag G 2006 Bolton tooth size discrepancies in skeletal Class I individuals presenting with different dental Angle classifications. Angle Orthodontist 76: 637–643

Alkofide E, Hashim H A 2002 Intermaxillary tooth size discrepancies among different malocclusion Classes: a comparative study. Journal of Clinical Pediatric Dentistry 26: 383–387

Al-Omari I K, Al.-Bitar Z B, Hamdan A M 2008 Tooth size discrepancies among Jordanian schoolchildren. European Journal of Orthodontics 30: 527–531

Al-Tamimi T, Hashim H A 2005 Bolton tooth-size ratio revisited. World Journal of Orthodontics 6: 289–295

Araujo E, Souki M 2003 Bolton anterior tooth size discrepancies among different malocclusion groups. Angle Orthodontist 73: 307–313

Arya B S, Savara B S, Thomas D, Clarkson Q 1974 Relation of sex and occlusion to mesiodistal tooth-size. American Journal of Orthodontics 66: 479–486

Baidas L, Hashim H 2005 An anterior tooth size comparison in unilateral and bilateral congenitally absent maxillary lateral incisors. Journal of Contemporary Dental Practice 6: 56–63

Bernabé E, Major P W, Flores-Mir C 2004 Tooth-width ratio discrepancies in a sample of Peruvian adolescents. American Journal of Orthodontics and Dentofacial Orthopedics 125: 361–365

Bielawska H 1994 Wskaźnik Boltona a niektóre wady zgryzu. Czasopismo Stomatologiczne 47: 360–362

Bolton W A 1958 Disharmony in tooth size and its relation to the analysis and treatment of malocclusion. Angle Orthodontist 28: 112–130

Bolton W A 1962 The clinical application of a tooth-size analysis. American Journal of Orthodontics 48: 504–529

Crosby D R, Alexander C G 1989 The occurrence of tooth size discrepancies among different malocclusion groups. American Journal of Orthodontics and Dentofacial Orthopedics 95: 457–461

Doris J M, Bernard B W, Kuftinec M M 1981 A biometric study of tooth size and dental crowding. American Journal of Orthodontics 79: 326–336

Ebeling C F, Ingervall B, Hedegård B, Lewin T 1973 Secular changes in tooth size in Swedish men. Acta Odontologica Scandinavica 31: 140–147

Endo T, Shundo I, Abe R 2007 Applicability of Bolton's tooth size ratios to a Japanese orthodontic population. Odontology 95: 57–60

Fattahi H R, Pakshir H R, Hedayati Z 2006 Comparison of tooth size discrepancies among different malocclusion groups. European Journal of Orthodontics 28: 491–495

Freeman J E, Maskeroni A J, Lorton L 1996 Frequency of Bolton tooth-size discrepancies among orthodontic patients. American Journal of Orthodontics and Dentofacial Orthopedics 110: 24–27

Freire S M, Nishio C, Mendes A M, Quintão C C, Almeida M A 2007 Relationship between dental size and normal occlusion in Brazilian patients. Brazilian Dental Journal 18: 253–257

Heusdens M, Dermaut L, Verbeeck R 2000 The effect of tooth size discrepancy on occlusion: an experimental study. American Journal of Orthodontics and Dentofacial Orthopedics 117: 184–191

Lavelle C L 1972 Maxillary and mandibular tooth size in different racial groups and in different occlusal categories. American Journal of Orthodontics 61: 29–37

Lew K K, Keng S B 1991 Anterior crown dimensions and relationship in an ethnic Chinese population with normal occlusions. Australian Orthodontic Journal 12: 105–109

Lundström A 1954 Intermaxillary tooth width ratio and tooth alignment and occlusion. Acta Odontologica Scandinavica 12: 265–292

Manke M, Miethke R R 1983 Die Größe des anterioren Bolton-Index und die Häufigkeit von Bolton-Diskrepanzen im Frontzahnsegment bei unbehandelten kieferorthopädischen Patienten. Fortschritte der Kieferorthopädie 44: 59–65

Neff C W 1957 The size relationship between the maxillary and mandibular anterior segments of the dental arch. American Journal of Orthodontics 27: 138–147

Nie Q, Lin J 1999 Comparison of intermaxillary tooth size discrepancies among different malocclusion groups. American Journal of Orthodontics and Dentofacial Orthopedics 116: 539–544

Nourallah A W, Splieth C H, Schwahn C, Khurdaji M 2005 Standardizing interarch tooth-size harmony in a Syrian population. Angle Orthodontist 75: 996–999

Othman S A, Harradine N W T 2006 Tooth-size discrepancy and Bolton's ratios: a literature review. Journal of Orthodontics 33: 45–51

Othman S, Harradine N 2007 Tooth size discrepancies in an orthodontic population. Angle Orthodontist 77: 668–674

Paredes V, Gandia J L, Cibrian R 2006a Determination of Bolton tooth-size ratios by digitization, and comparison with the traditional method. European Journal of Orthodontics 28: 120–125

Paredes V, Gandia J L, Cibrian R 2006b Do Bolton's ratios apply to a Spanish population? American Journal of Orthodontics and Dentofacial Orthopedics 129: 428–430

Proffit W R, Fields H W, Sarver D M 2007 Contemporary orthodontics, 4th edn. Mosby, St Louis, pp. 199–201

Redahan S, Lagerström L 2003 Orthodontic treatment outcome: the relationship between anterior dental relations and anterior inter-arch tooth size discrepancy. Journal of Orthodontics 30: 237–277

Santoro M, Ayoub M E, Pardi V A, Cangialosi T J 2000 Mesiodistal crown dimensions and tooth size discrepancy of the permanent dentition of Dominican Americans. Angle Orthodontist 70: 303–307

Smith S S, Buschang P H, Watanabe E 2000 Interarch tooth size relationships of 3 populations: 'does Bolton's analysis apply?' American Journal of Orthodontics and Dentofacial Orthopedics 117: 169–174

Sperry T P, Worms F W, Isaacson R J, Speidel T M 1977 Tooth-size discrepancy in mandibular prognathism. American Journal of Orthodontics 72: 183–190

Stifter J 1958 A study Pont's, Howes', Rees', Neff's and Bolton's analyses on Class I adult dentitions. Angle Orthodontist 28: 215–225

Ta T A, Ling J Y K, Hägg U 2001 Tooth-size discrepancies among different occlusion groups of southern Chinese children. American Journal of Orthodontics and Dentofacial Orthopedics 120: 556–558

Uysal T, Sari Z 2005 Intermaxillary tooth size discrepancy and mesiodistal crown dimensions for a Turkish population. American Journal of Orthodontics and Dentofacial Orthopedics 128: 226–230

Uysal T, Sari Z, Basciftci F A, Memili B 2005 Intermaxillary tooth size discrepancy and malocclusion: is there a relation? Angle Orthodontist 75: 208–213

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Lower incisor intrusion with intraoral transosseous stainless steel wire anchorage in rabbits

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SUMMARY The purpose of this research was to investigate the potential use of intraoral transosseous stainless steel wires as anchorage for intrusion of the lower incisors using a rabbit model. Placement of intraoral transosseous stainless steel wires around incisors is similar to that of intraoral transosseous wiring of edentulous mandibular fractures. Ten male New Zealand rabbits, 9 ± 1.5 months of age, average weight 1.8 ± 0.3 kg, were used in this study. One lower incisor was intruded with a 50 g bilateral force using a coil spring for 10 weeks, while the other incisor served as the control. Clinical measurements of the distances between the occlusal edges of the incisors (EE) were performed weekly with a calliper. In addition to standard descriptive statistical calculations, a paired Student's *t*-test was used for comparison of the two groups.

All surgical sites healed uneventfully after insertion of the wires. Significant differences were found in the change of EE between the experimental and control sides from 4 weeks onwards. Intrusion of the incisor, 4 ± 0.58 mm, was seen on the test side, while EE on the control side remained unchanged. Within the limits of this animal study, it is concluded that the intraoral transosseous stainless steel wire anchorage system is a cost-effective method for intrusion of lower incisors when the use of other anchorage system is not possible.

Introduction

Problems caused by over-erupted molars and incisors are often encountered during orthodontic treatment. Intrusion of over-erupted teeth is a challenge with traditional methods such as intrusion archwire systems and subapical alveolar osteotomy in adult patients (Poulton, 1989; Simmons *et al.*, 1992; Pangrazio *et al.*, 2001; Huang *et al.*, 2006). The use of mini-implants for orthodontic anchorage has increased in popularity over the past decade. These temporary anchorage devices offer absolute anchorage for orthodontic retraction of incisors, mesialization of posterior segments, as well as intrusion (Kanomi,1997; Melsen and Costa, 2000; Kyung *et al.*, 2003a,b; Maino *et al.*, 2003; Kuroda *et al.*, 2004; Wu *et al.*, 2006).

Intrusion of the teeth applying only apically directed force to the buccal tooth attachment will tip the teeth buccally. The most preferable biomechanics for tooth movement is balanced bilateral loading. In order to achieve an optimal bilateral intrusive force system, two miniscrews or miniplates, one placed buccally and the other lingually, has been reported in both animal and human studies (Daimaruya *et al.*, 2001; Ohmae *et al.*, 2001; Park *et al.*, 2003; Erverdi *et al.*, 2004). However, when human mandibular teeth are intruded, it is difficult to place the miniscrews from the lingual aspect due to the limitations of the oral cavity.

Therefore, the purpose of this study was to investigate a new method, the intraoral transosseous stainless steel wire anchorage system, for obtaining a bilateral force system for absolute intrusion of mandibular teeth when other anchorage system such as miniscrew anchorage is not possible.

Materials and methods

The research protocol was approved by the Experimental Animal Committee of Zhejiang University.

Ten New Zealand male rabbits, average age 9 ± 1.5 months, average weight 1.8 ± 0.3 kg, were used in this study. The rabbits were kept in cages at a temperature of approximately 20°C and fed with a diet of Chinese cabbage and clear water. One lower incisor was intruded using intraoral transosseous stainless steel wire anchorage, while the other served as control. A special needle, modified from those used in lumbar anaesthesia, was used to guide the stainless steel wire. It consisted of a hollow tube with sharp tip and a removable core (Figure1), which prevented the soft tissues from blocking the tube at the beginning of the procedure (Figure 2).

All experimental procedures, including the surgical procedures and clinical intraoral examinations, were performed under general anaesthesia by intramuscular application of a combination of ketamine, meperidine hydrochloride, and dihydroetorphine at 0.5 ml/kg.

Surgical procedure

Placement of the transosseous stainless steel wire around the incisor was similar to that for intraoral transosseous wiring of edentulous mandibular fractures (Freihofer and Sailer, 1973). The puncture point, opposite to the lower incisor to be intruded, was located on the inferior border of the mandible. Under local anaesthesia at the puncture point,

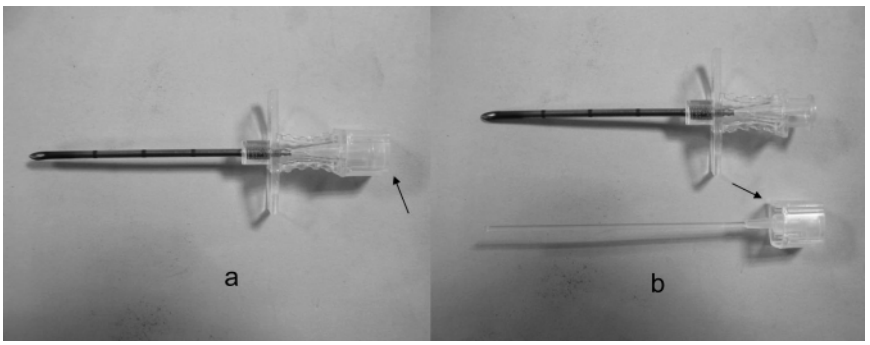

Figure 1 The needle used to guide the stainless steel wire (a and b) consists of a hollow tube with a sharp tip and a removable core (arrow).

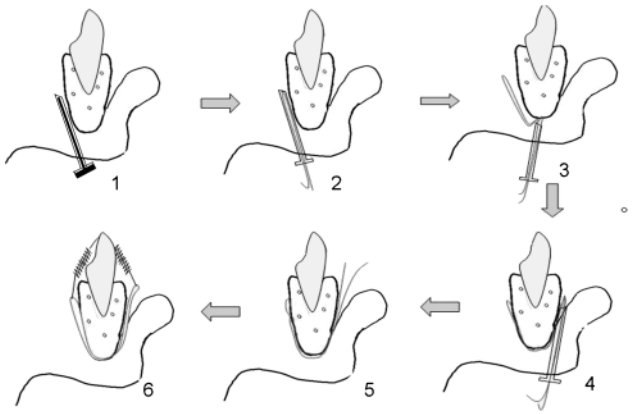

Figure 2 Schematic drawing showing the insertion of the transosseous stainless steel wire. Step 1: the needle with the removable core was inserted from the lingual aspect. Note, the inside core which prevents the soft tissues from blocking the tube. Step 2: after withdrawl of the core, a double-strand of 0.20 mm stainless steel wire was inserted through the needle. Step 3: the needle with the wire inside was then drawn back to the inferior border of the mandible. Step 4: the needle was reinserted from the buccal aspect. Step 5: the needle was withdrawn and the wire was left in the oral cavity. Step 6: the Ni–Ti spring was connected.

the needle was inserted along the lingual bone surface to the lingual side of the incisor until it reached the oral cavity. A double-strand of 0.20 mm stainless steel wire was inserted through the needle after the core was withdrawn (Figure 2). The needle, with steel wire inside, was then drawn back to the inferior border of the mandible and reinserted along the buccal jaw bone surface to the buccal side of the incisor until it reached the oral cavity. After withdrawal of the needle, the transosseous wire was left in the oral cavity. The free ends of the wire on both sides were twisted to produce hooks which connected the Ni–Ti spring (Westlake, Hangzhou, China). The coil spring, delivering a force of 50 g on both sides, was placed across the edge of the incisor to be intruded. The coil spring was bonded to the incisor at the incisor edge to ensure that the spring remained stable during orthodontic intrusion. Figure 2 shows the surgical procedures schematically. The springs were carefully calibrated biweekly for 10 weeks. The rabbits were given a soft diet to prevent breakage of the appliance as a result of the biting force. The appliances and teeth were brushed and cleaned with 2 per cent chlorhexidine digluconate weekly.

Measurement of intrusion

Measurements of lower incisor intrusion were based on the assumption that the upper incisors remained stable during the experiment. Clinical measurements were performed weekly with a digital callipers (KT5-230-62, Kenta Technologies, Singapore) in each rabbit under general anaesthesia. The distances between the occlusal edges of the antagonistic incisors, edge to edge (EE), were measured. At the end of experimental period, the rabbits were killed by intravenous injection of an overdose of ketamine. One mandible was dissected for radiographic examination of the transosseous stainless steel wire.

Statistical analysis

The data were normally distributed. The mean and standard deviation were calculated for the data. Time changes in EE were evaluated using analysis of variance and the difference between the test and control side for each period was determined by paired Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

Reproducibility of the measurements was assessed by statistically analysing the difference between 10 double measurements made at 30 minutes interval by one author (JCW). The method error was calculated using the formula: $Sx = \sqrt{(\sum D^2 / 2n)}$, where Sx is the error of the measurement, D is the difference between duplicate measurements, and n is the number of double measurements. The errors for EE were 0.18 mm.

Results

All puncture sites healed uneventfully after surgery. The rabbits did not eat anything until 12 hours after surgery or spring adjustment. At the end of the experiment, no loss of weight was found.

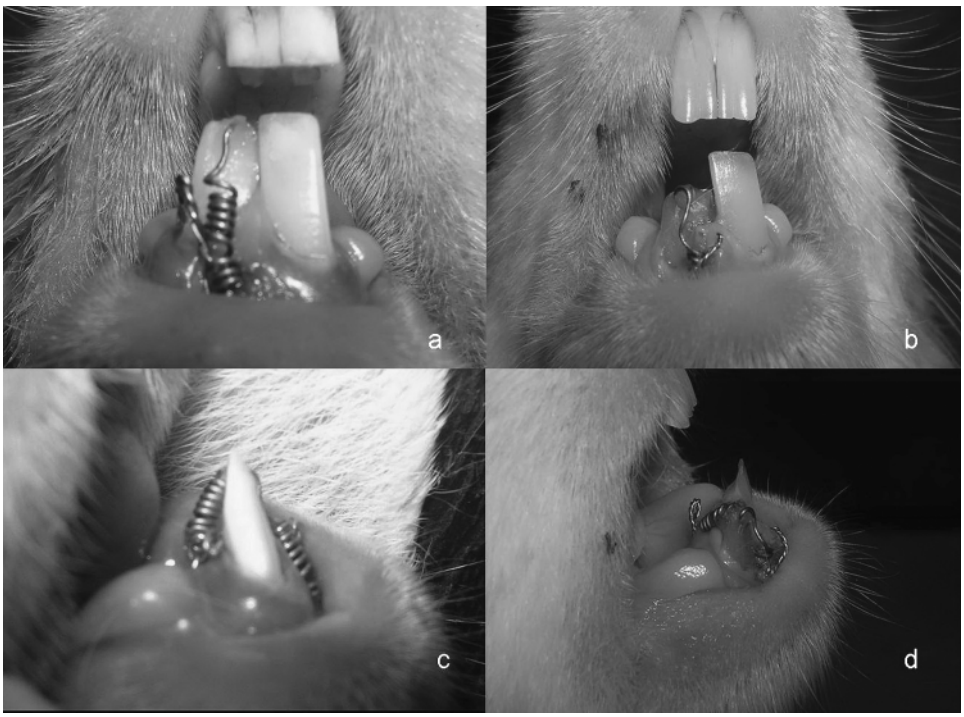

Figure 3 Intrusion of the lower incisor with the transosseous stainless steel wire anchorage system. Before intrusion (a and c) and after intrusion using a 50 g bilateral intruded force for 10 weeks (b and d). The coil spring was connecting to the bilateral hooks made of the twisted free ends of the double stranded transosseous stainless steel wire.

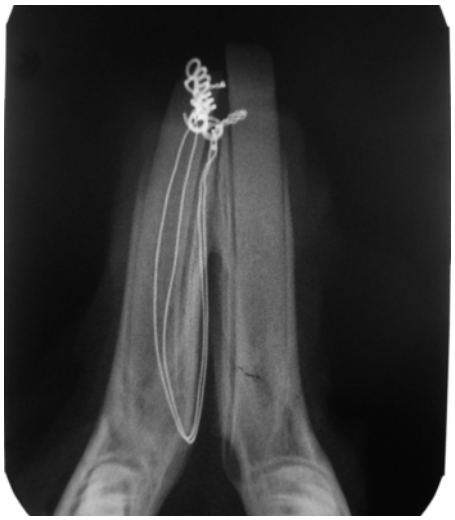

Figure 4 Radiograph of the transosseous stainless steel wire anchorage system, showing the relationship between the mandible and the wire. The radiograph is of a dissected free mandible including the intruded incisor after 10 weeks of orthodontic intrusion with a bilateral intrusive force of 50 g.

Intrusion of the experimental lower incisors was observed while no displacement was found for the control incisors at the end of treatment. The intruded incisors showed no buccal or lingual tipping (Figure 3). Deepening of gingival sulcus of the intruded teeth and bleeding on probing were frequently observed. Slight mucositis was found around the wire at both the buccal and lingual sides. There was no traumatic ulceration of the tongue or buccal mucosa. At the end of the experiment, the wire was easily removed by pulling one end, after 1 per cent iodine sterilization. Figure 4 shows a radiographic view of the transosseous stainless steel wire and the intruded lower incisor.

The values of EE in both groups at the different time points are shown in Table 1. Significant differences were found in the change of EE between the experimental and control sides from 4 weeks onwards. Intrusion of the incisor, 4 ± 0.58 mm, was seen on the experimental side, while the control side remained unchanged. Figure 5 shows root apex resorption of the intruded incisor.

Discussion

Simultaneous application of intrusive forces from the buccal and lingual aspects guarantees good control of the tooth (van Steenbergen *et al.*, 2005). Due to their advantages, miniscrews or miniplates have recently been introduced in orthodontics (Kanomi, 1997; Melsen and Costa, 2000; Kyung *et al.*, 2003 a,b; Maino *et al.*, 2003; Kuroda *et al.*, 2004; Wu *et al.*, 2006; Leung *et al.*, 2008). Intrusion of teeth using two miniscrews or miniplates, one buccally and the other lingually, has been reported in both animal and human studies (Daimaruya *et al.*, 2001; Ohmae *et al.*, 2001; Park *et al.*, 2003; Erverdi *et al.*, 2004). Although miniscrew anchorage has many advantages, there are still some limitations when intruding lower

Table 1 Time-course changes in the distance between the upper and antagonistic lower incisor edge in each rabbit.

	Week 0		Week 4		Week 8		Week 10	
Rabbit	Test	Control	Test	Control	Test	Control	Test	Control
1	−1	−1	0.2	−1.2	2.2	−1	2.9	−1
2	−1.3	−1.3	0.2	−1.1	2.2	−1.2	2.8	−1.2
3	−0.8	−0.7	0.5	−0.7	2	−0.8	3.5	−0.7
4	−0.8	−0.9	0.5	−0.8	2.1	−0.8	2.5	−1
5	−1	−0.8	0.3	−0.8	2.3	−1	2.9	−0.8
6	−0.9	−1	0.5	−1.1	1.8	−0.8	2.8	−1
7	−1.2	−1.2	0.2	−1.1	1.7	−1.2	2.7	−1.1
8	−1.2	−1	0.3	−1	1.8	−1.1	3	−1
9	−0.8	−0.7	0.8	−0.8	2.8	−0.8	3.6	−0.7
10	−0.8	−0.8	0.7	−0.7	2.5	−0.8	3.5	−0.8
Mean	−0.98	−0.94	0.42*$^{\Delta}$	−0.93	2.14*$^{\Delta}$	−0.95	3.02*$^{\Delta}$	−0.93
Standard deviation	0.19	0.2	0.21	0.19	0.34	0.17	0.37	0.17

*Significant difference between each experimental time point and week 0 in the test group $P < 0.05$.
$^{\Delta}$Significant difference between the test and control groups at each experimental time point $P < 0.05$.

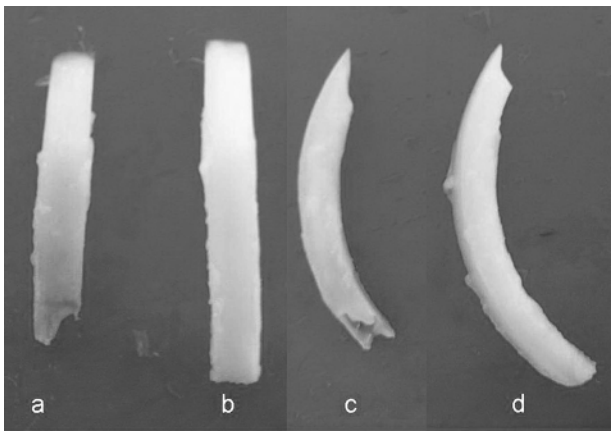

Figure 5 Extracted incisors after 10 weeks of intrusion. Intruded incisor (**a** and **c**) and control incisor (**b** and **d**). Frontal (a and b) and lateral (c and d) views. Root resorption was found at the apex of the intruded incisor.

human teeth. If lower teeth are to be intruded, it is difficult to place miniscrews from the lingual aspect. Furthermore, the discomfort, potential injury to the roots, and looseness of microimplants inserted in the interradicular region are a concern. Thus, the objective of this study was to investigate a new anchorage system, intraoral transosseous stainless steel wire, to obtain bilateral intrusive force for intrusion of lower teeth when bilateral miniscrew anchorage is not possible.

Before intraoral transosseous wire anchorage can be used in humans, it must be proven safe and efficient in an animal model. To investigate the potential anchorage of intraoral transosseous wires for intrusion of lower teeth, a rabbit model was used because it is difficult to intrude the lower incisors of rabbit with traditional anchorage systems, even with the recently advocated miniscrew anchorage. As the lower incisors of the rabbits are intruded, the upper incisors would over-erupt due to the lack of occlusal contact. As overeruption of the upper incisors is not obvious during short experimental periods, to simplify the measurement method, the distances between the upper and lower incisors (EE) were measured to calculate intrusion.

Zygomatic ligatures made of stainless steel wire have been proven to be an effective anchorage device for intrusion and retraction of the maxillary incisors in partially edentulous patients (Melsen *et al.*, 1998). The method of placing transosseous stainless steel wire around incisors is similar to that of intraoral transosseous wiring of the endentulous mandible for mandibular fractures. Generally, it does not result in infections at the interface of the stainless steel wires. In the present study based on a rabbit model, no infection was seen.

With bilateral miniscrew anchorage, Daimaruya *et al* (2001) intruded the lower molars 3.4 mm using an intrusive force from a buccal miniplate and lingual bone screw in dogs. Ohmae *et al* (2001) reported, in their beagle dog intrusion model, that the lower third premolars were intruded 4.5 mm, on average, after 12–18 weeks of bilateral orthodontic force application. In this rabbit model, the lower incisors were intruded approximately 4 mm after 10 weeks of intrusion using intraoral transosseous stainless steel wire anchorage. Two hooks, made from the ends of the inserted transosseous stainless steel wire, successfully served as bilateral anchors.

Previous studies suggest that external apical root resorption (EARR) occurs during treatment when forces at the apex exceed the resistance and reparative ability of the periapical tissue (Parker and Harris, 1998) and heavy forces increase the risk of EARR (Ohmae *et al.*, 2001). In this study, shortening of the intruded roots caused by severe root resorption after orthodontic treatment was observed. Severe root resorption indicates 'high stress' at the apex of

the incisor. In this study, a bilateral force of less than 50 g (20 g) might have been appropriate. The correct amount of force for intrusion of the lower incisors needs to be investigated.

With intraoral transosseous stainless steel wire anchorage, the incisors could be easily and rapidly intruded. However, insertion of the transosseous stainless steel wire is difficult. Good command of the topical anatomy and surgical skill are necessary. The discomfort and the consistent slight mucositis around the wire are of concern. Moreover, there are still questions that need to be answered such as the influence of the wire on the mandible. Further research should focus on these issues.

Conclusion

Easy and rapid intrusion of the lower incisors was achieved with the intraoral transosseous stainless steel wire anchorage system in rabbits. Within the limitations of this animal study, it is concluded that the intraoral transosseous stainless steel wire anchorage system is cost-effective for intruding the lower incisors when the use of other types of anchorage system such as miniscrews is not possible.

Further studies are necessary to determine the influence of the intraoral transosseous stainless steel wire anchorage system on the mandible.

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References

Daimaruya T, Nagasaka H, Umemori M, Sugawara J, Mitani H 2001 The influences of molar intrusion on the inferior alveolar neurovascular bundle and root using the skeletal anchorage system in dogs. Angle Orthodontist 71: 60–70

Erverdi N, Keles A, Nanda R 2004 The use of skeletal anchorage in open bite treatment: a cephalometric evaluation. Angle Orthodontist 74: 381–390

Freihofer Jr. H P, Sailer H 1973 Experiences with intraoral trans-osseous wiring of mandibular fractures. Journal of Maxillofacial Surgery 1: 248–252

Huang C S, de Villa G H, Liou E J, Chen Y R 2006 Mandibular remodeling after bilateral sagittal split osteotomy for prognathism of the mandible. Journal of Oral and Maxillofacial Surgery 64: 167–172

Kanomi R 1997 Mini-implant for orthodontic anchorage. Journal of Clinical Orthodontics 31: 763–767

Kuroda S, Katayama A, Takano T 2004 Severe anterior open-bite case treated using titanium screw anchorage. Angle Orthodontist 74: 558–567

Kyung H M, Park H S, Bae S M, Sung J H, Kim I B 2003a Development of orthodontic micro-implants for intraoral anchorage. Journal of Clinical Orthodontics 37: 321–328

Kyung S H, Choi J H, Park Y C 2003b Miniscrew anchorage used to protract lower second molars into first molar extraction sites. Journal of Clinical Orthodontics 37: 575–579

Leung M T, Rabie A B, Wong R W 2008 Stability of connected mini-implants and miniplates for skeletal anchorage in orthodontics. European Journal of Orthodontics 30: 483–489

Maino B G, Bednar J, Pagin P, Mura P 2003 The spider screw for skeletal anchorage. Journal of Clinical Orthodontics 37: 90–97

Melsen B, Costa A 2000 Immediate loading of implants used for orthodontic anchorage. Clinical Orthodontic Research 3: 23–28

Melsen B, Petersen J K, Costa A 1998 Zygoma ligatures: an alternative form of maxillary anchorage. Journal of Clinical Orthodontics 32: 154–158

Ohmae M, Saito S, Morohashi T 2001 A clinical and histological evaluation of titanium mini-implants as anchors for orthodontic intrusion in the beagle dog. American Journal of Orthodontics and Dentofacial Orthopedics 119: 489–497

Pangrazio V, Berger J L, Kaczynski R, Shunock M 2001 Stability of skeletal Class II correction with 2 surgical techniques: the sagittal split ramus osteotomy and the total mandibular subapical alveolar osteotomy. American Journal of Orthodontics and Dentofacial Orthopedics 120: 134–143

Park Y C, Lee S Y, Kim D H, Jee S H 2003 Intrusion of posterior teeth using mini-screw implants. American Journal of Orthodontics and Dentofacial Orthopedics 123: 690–694

Parker R J, Harris E F 1998 Directions of orthodontic tooth movements associated with external apical root resorption of the maxillary central incisor. American Journal of Orthodontics and Dentofacial Orthopedics 114: 677–683

Poulton D R 1989 Correction of extreme deep overbite with orthodontics and orthognathic surgery. American Journal of Orthodontics and Dentofacial Orthopedics 96: 275–280

Simmons K E, Turvey T A, Phillips C, Proffit W R 1992 Surgical-orthodontic correction of mandibular deficiency: five-year follow-up. International Journal of Adult Orthodontics and Orthognathic Surgery 7: 67–79

van Steenbergen E, Burstone C J, Prahl-Andersen B, Aartman I H 2005 The influence of force magnitude on intrusion of the maxillary segment. Angle Orthodontist 75: 723–729

Wu J C, Huang J N, Zhao S F, Xu X J, Xie Z J 2006 Radiographic and surgical template for placement of orthodontic microimplants in interradicular areas: a technical note. International Journal of Oral and Maxillofacial Implants 21: 629–634

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Expression levels of endothelin-1, endothelin-2, and endothelin-3 vary during the initial, lag, and late phase of orthodontic tooth movement in rats

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SUMMARY Endothelins (ET)-1, ET-2, and ET-3 are one group of cytokines likely to be released during orthodontic tooth movement (OTM). Therefore, the expression of ET levels was investigated to determine the importance and involvement of isopeptides during the several phases of OTM.

Thirty-two male Wistar rats (12–13 weeks old) were divided into four groups of eight: control, 14, 28, and 42 day groups. Tooth movement was induced by a closed-coil spring inserted between the upper left first molar and the upper incisors. The distance between the teeth was measured on days 0, 2, 7, 14, 21, 28, 35, and 42 using a digital calliper. The rate of tooth movement was calculated. The animals were sacrificed on days 14, 28, and 42 and gene expression levels of all three ET were determined using reverse transcription polymerase chain reaction. Statistical analysis was performed using two-way analysis of variance, Bonferroni's correction, and paired *t*-tests.

The distance between the teeth decreased in all appliance groups ($P < 0.001$). The rate of tooth movement was 0.20 ± 0.02, 0.03 ± 0.01, and 0.06 ± 0.02 mm/day between days 0–2, 3–21, and 22–42, respectively. On day 14, gene expression levels for ET-1 ($P < 0.05$) and ET-3 ($P < 0.001$) increased compared with day 0. On day 28, a downregulation of ET-3 was observed when compared with day 0 ($P < 0.001$). On day 42, ET-1 ($P < 0.001$) and ET-3 ($P < 0.01$) gene expression levels were strongly upregulated, while ET-2 gene expression level was downregulated ($P < 0.01$) when compared with day 0. ET-1 and ET-3, but not ET-2, are involved in all three phases of OTM, and ET-1 seems to be the predominant form in the late phase of OTM.

Introduction

Endothelins (ET) are a family of peptide cytokines that include ET-1, ET-2, and ET-3. ET-1 is the principal and most studied isoform in humans (Miyauchi and Masaki, 1999; Rich and McLaughlin, 2003). ET are involved in many physiological and pathophysiological processes and probably also in orthodontic tooth movement (OTM; Drevenšek *et al.*, 2006; Sprogar *et al.*, 2008).

Dependent on force characteristics, OTM comprises three phases: an initial phase, which lasts 1–2 days after force application; a lag phase, which lasts 20–30 days after force application and a late phase, which lasts as long as a force is applied to the teeth. During the initial phase, several changes cause the release of many different hormones, growth factors, cytokines, and other chemical messengers, which modulate OTM (Krishnan and Davidovitch, 2006). ET could be one group of these cytokines since it has been shown that ET-1 is released in the periodontal ligament (PDL) microvasculature after 3 hours continuous loading of a rat molar (Sims, 2001). During the lag phase, in which only minor tooth movement is present, necrotic tissue that has formed due to hypoxia in the initial phase is removed by cells, which functioning is influenced by ET, namely macrophages, foreign body giant cells, and osteoclasts (Ehrenreich *et al.*, 1990; Alam *et al.*, 1992). Therefore, ET could also be involved in the lag phase of OTM. During the late phase, rapid bone formation on the tension side, mediated by osteoblasts and bone resorption on the pressure side, mediated by osteoclasts, are the predominant processes (Reitan, 1967; Rygh, 1976; Krishnan and Davidovitch, 2006). ET-1 has been found in both major types of bone remodelling cells: in osteoblasts (Kitten and Andrews, 2001) as well as in osteoclasts (Sasaki and Hong, 1993). Furthermore, ET-1 increases osteoblast differentiation, proliferation and activity and, therefore, bone formation (Tatrai *et al.*, 1992; Nelson *et al.*, 1999, von Schroeder *et al.*, 2003; Yin *et al.*, 2003; Guise and Mohammad, 2004; Clines *et al.*, 2007). The findings on the influence of ET on bone resorption are inconsistent. Some researchers found that ET, mainly ET-1, increase bone resorption (Tatrai *et al.*, 1992; Sprogar *et al.*, 2008), while others concluded the opposite effect (Alam *et al.*, 1992).

While it has been shown that ET-1 is involved in the late phase of OTM in rats (Sprogar *et al.*, 2008), there is no evidence of the involvement of ET-2 or ET-3. Therefore, the aim of this study was to determine whether ET-2 and

ET-3 are involved in OTM and, furthermore, since ET-1 is the predominant isopeptide in other processes, whether it also predominates in all phases of OTM. Therefore, the expression levels of ET-1, ET-2, and ET-3 during the initial, lag, and late phases of OTM in rats were investigated.

Materials and methods

Animals and study protocol

The investigation was approved by the Veterinary Administration of the Republic of Slovenia (No. 323-02-234/2005/2) and complied with the guiding principles in the 'Care and Use of Animals'.

The study was performed on 32 male Wistar rats (330–350 g, 12–13 weeks old). The animals were housed in groups of four in polycarbonate cages under normal laboratory conditions at a constant temperature (24–25°C) and humidity with a 12 hour circadian cycle. They were fed with soaked standard laboratory rat chow diet (Krka, Novo mesto, Slovenia) and water *ad libitum*.

The animals were divided into four groups (n = 8): control, 14, 28, and 42 day groups. In the last three groups, a closed-coil spring (25cN, wire diameter 0.15 mm; GAC International, Bohemia, New York, USA) was placed between the upper left first molar and the maxillary incisors. Before placing the appliance, the animals were anaesthetized as described previously (Drevenšek *et al.*, 2006; Sprogar *et al.*, 2007). The closed-coil spring was attached to the upper left first molar with a stainless steel ligature wire (diameter 0.25 mm; Dentaurum, Ispringen, Germany) and to the upper incisors by a surgical steel wire (4-0, multifilament, W310, Ethicon; Johnson&Johnson, New Brunswick, New Jersey, USA). To improve fixation of the appliance, a 0.5 mm hole was made using a hard metal burr (HM 1, 204, 005, Meisinger, Neuss, Germany). The hole was drilled through the approximal tooth surfaces, perpendicular to the longitudinal axis of the incisors at the gingival level. The steel wire was inserted through the hole and bent on the approximal surface of the right incisor. Light curing bonding material (Tetric flow, Ivoclar Vivadent, Schaan, Lichtenstein) was used to protect the soft tissues from the sharp wire ends (Sprogar *et al.*, 2007). The superelasticity of the coil spring ensured a constant force (25 cN) during activation (Drevenšek *et al.*, 2006).

Measurements

In the control group and in the 42 day group, the distance between the most mesial point of the upper left first molar and the most palatal point of the ipsilateral incisor at the gingival level was measured. Measurements were undertaken on days 0, 2, 7, 14, 21, 28, 35, and 42 using a digital calliper (Wilson & Wolpert, Utrecht, The Netherlands; Sprogar *et al.* 2007). During this procedure, the animals were anaesthetized. All measurements were carried out twice by two investigators (AH and ŠS) independently within a period of a few minutes. Interexaminer reliability was tested using the intraclass correlation coefficient (ICC) and a paired t-test was used to assess the systematic bias. Tooth movement was calculated by subtracting the distance between teeth on each day (2, 7, 14, 21, 28, 35, and 42) from the distance between the teeth on day 0. The rate of tooth movement was calculated by subtracting the distance between the teeth on each day from the distance between the teeth on the previous day and dividing this difference by the number of days between these two measurements (2, 5, or 7 days).

Semi-quantitative reverse transcription polymerase chain reaction

All animals in the 14, 28, 42 day, and control group were killed by an intraperitoneal injection of anaesthetic and CO_2. Tissue samples of the alveolar bone containing all three left maxillary molars and their PDL were excised, frozen in liquid nitrogen, and mechanically powdered. The total RNA content was isolated from 100–150 mg of each of the powdered sample using TRIzol reagent (Invitrogen, Carlsbad, California, USA), according to the manufacturer's protocol. Prior to reverse transcription, RNA was treated with RNase-free DNase I (Fermentas, Vilnius, Lithuania). Approximately 500 ng of DNA-free RNA was reverse transcribed into cDNA in a 20 µl reaction using Improm II reverse transcriptase (Promega, Mannheim, Germany) and random hexamer primers (Promega), according to the manufacturer's instructions. Polymerase chain reaction (PRC) with Gotaq Green master mix (Promega) was performed using 1 µl of the reverse transcription reaction in a 25 µl PCR reaction with 15 nmol of the transcript specific primers (Invitrogen): 5′-GCTCCTGCTCCTCCTTGAT-3′ and 5′-CTCGCTCTATGTAAGTCATG-3′ for ET-1; 5′-ggccatccctgcatactcta-3′ and 5′-ctagggaaggggaaccagaG-3′ for ET-2; and 5′-GCACTTGCTTCACTTATAAG-3′ and 5′-CAGAAGCAAGAAGCATCAGT-3′ for ET-3. The internal normalization gene of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified using 3 nmol of primers 5′-TCCCTCAAGATTGTCAGCAA-3′ and 5′-AGATCCACAACGGATACATT-3′. Thermal cycling conditions comprised 32 cycles, each consisting of 30 seconds at 94°C, 1 minute at 55°C, and 30 seconds at 72°C. The number of cycles (32) was empirically determined as the optimal cycle number within the linear range of amplification by measuring the concentration of PCR products after 28, 30, 32, and 34 cycles. Ten microlitres of PCR products were resolved in 1.5 per cent agarose gels containing ethidium bromide. The band intensities were quantified using the TotalLab gel analysis program (Nonlinear Dynamics, Newcastle-upon-Tyne, UK) after image acquisition from ultra violet-illuminated gels by MiniBis (DNR BioImaging Systems, Jerusalem, Israel).

Statistics

Descriptive statistics (mean and standard error) were calculated for each parameter (tooth movement, rate of tooth movement, and gene expression levels) for all animals in all groups. Interexaminer reliability was tested with the ICC and a paired *t*-test was used to assess systematic bias. Within and between groups comparisons were made for gene expression levels using the analysis of variance and Bonferroni's correction in GraphPad Prism 4.00 (GraphPad Software, San Diego, California, USA). Values of $P < 0.05$ were considered statistically significant.

Results

Measurements

Systematic bias, which showed a value of $P > 0.88$ was determined using a paired *t*-test. The ICC was found to be 0.93 ± 0.02. Since reliability was within the standards, the mean value of the four measurements was used for further statistical analysis.

In the control group, the distance between the teeth increased from days 0 to 42 ($P < 0.001$). While in the 42 day group, it decreased from days 0 to 42 ($P < 0.001$). Changes in the distances significantly differed between the control group compared with the 42 day group on days 7, 14, 21, 28, 35, and 42 ($P < 0.001$). The rate of tooth movement was significantly faster in the initial phase compared with the lag and late phases ($P < 0.001$) and was also significantly faster in the late phase compared with the lag phase ($P < 0.05$; Figure 1).

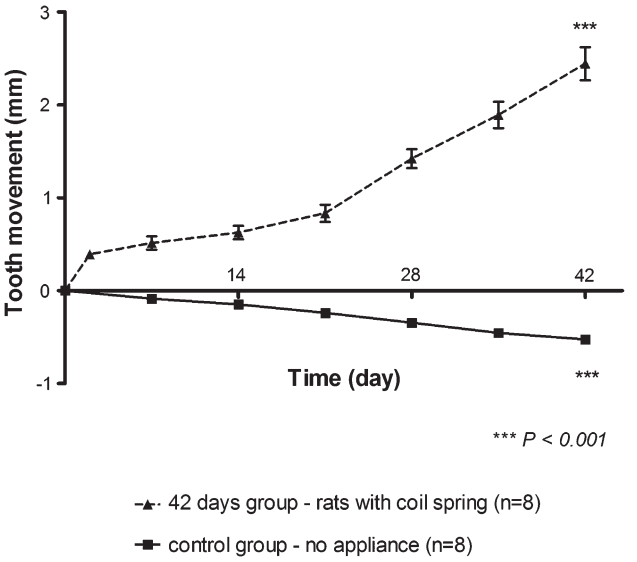

Figure 1 Tooth movement and rates of tooth movement ± mean and standard error in rats in the control (solid line) and the 42 day (dotted line) group in all three phases of orthodontic tooth movement. Filled triangles: 42 days group—rats with coil spring ($n = 8$). Filled squares: control group—no appliance ($n = 8$). Initial phase ≈ days 0–2 (0.20 ± 0.02 mm/day); lag phase ≈ days 3–21 (0.03 ± 0.01 mm/day); and late phase ≈ days 22–42 (0.08 ± 0.02 mm/day).

Gene expression levels of ET-1, ET-2, and ET-3 during OTM

Reverse transcription PCR analysis showed that the expression of ET-1, ET-2, and ET-3 genes varied considerably during the time course of force application (Figure 2). For all three transcripts tested, time course-dependent gene expression profiles were observed after normalization to the expression level of the housekeeping gene GAPDH. The basal levels of ET-1 and ET-3 transcripts in the control group (day 0) were almost the same, while ET-2 was significantly higher when compared with ET-1 and ET-3 ($P < 0.001$). A subsequent consensus increase during the following 14 days of force application was further observed for ET-1 and ET-3 but not for ET-2 gene expression level (Figure 2), bringing all three transcripts to almost the same level. A strong downregulation of ET-3 and a non-significant downregulation of ET-1 and ET-2 levels were observed on day 28 of force application. Therefore, on day 28, the ET-2 transcript level remained significantly higher compared with both ET-1 and ET-3 transcript levels ($P < 0.001$). Even more evident change in gene expression for all three ET was observed after 42 days of force application. On day 42, a strong upregulation of ET-1 and ET-3 genes was observed. The ET-1 transcript level rose sevenfold compared with the level on day 28, thus exceeding the expression level at any previous time point (Figure 2). The expression level of ET-1

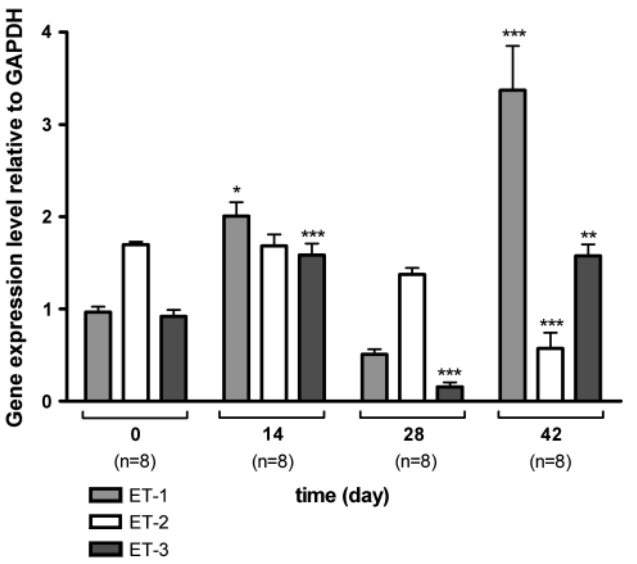

Figure 2 Gene expression levels of endothelins (ET)-1, ET-2, and ET-3 in alveolar bone and the periodontal ligament during orthodontic tooth movement on days 0, 14, 28, and 42. The relative expression levels of the indicated genes were performed after normalization against the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

on day 42 was also significantly higher compared with ET-2 and ET-3 transcript levels ($P < 0.001$ and $P < 0.01$, respectively). In contrast, the ET-2 transcript level was significantly downregulated and reached the lowest level compared with previous time points (Figure 2).

Discussion

OTM is modulated by many chemical messengers (Krishnan and Davidovitch, 2006), among which may be ET. Until now, only the involvement of ET-1 in bone modelling has been studied, using *in vitro* and *ex vivo* models. However, the involvement of ET-2 and ET-3 in these processes is not known nor is it clear whether ET-1 is the predominant isopeptide form of the ET family in all phases of OTM. Studies examining the role of ET at the molecular level using *in vivo* models could provide a deeper insight into the process of OTM, thereby identifying the important pharmacological targets for modulating this process. The present data showed that ET-1 and ET-3 could have a parallel role in OTM since their gene expression levels demonstrated a similar pattern. ET-2 appeared to have no significant role during OTM in this model (Figure 2).

The present animal model expressed all three phases of OTM (Figure 1). The initial phase lasted 48 hours and the lag phase approximately 20 days, followed by the late phase. This is in agreement with other studies (Shirazi *et al.*, 2002; von Böhl *et al.*, 2004; Krishnan and Davidovitch, 2006; Yoshimatsu *et al.*, 2006). The proposed model that shows the increases in gene expression levels of ET on day 14 represents the involvement of ET in the initial and later lag phase. Gene expression levels of ET on day 28 represent their involvement in the shift from the lag phase to the late phase, and on day 42 their involvement in the late phase of OTM, during which bone formation on the tension side and bone resorption on the pressure side enabled movement of teeth through alveolar bone.

ET-1 and ET-3, but not ET-2, are most likely involved in the events of the initial and lag phase of OTM since their gene expression levels were significantly upregulated on day 14. It appears that both ET-1 and ET-3 contribute equally to these events since their gene expression levels were similar (Figure 2). ET are released probably due to mechanical loading, ischaemia, and hypoxia (Kourembanas *et al.*, 1991; Schmitz-Spanke and Schipke 2000), which appear immediately after force application and last throughout most of the initial and lag phase of OTM. It has been shown that after acute loading of a rat molar, ET-1 immunofluorescence increased in the microvasculature of the PDL and alveolar bone socket surface (Sims, 2001). During the initial phase in the present study, the rate of tooth movement was high (Figure 1). This rapid movement is the result of the shift of the tooth in its PDL space and early bone resorption (Keeling *et al.* 1993, King *et al.* 1997; Noxon *et al.*, 2001), following which the lag phase starts. The events of the lag phase, where the rate of tooth movement is significantly decreased due to hyalinization (Figure 1), are intense cellular activity and the reestablishment of cell and fibre function (Rygh, 1973). These events are mediated by a variety of cells, among which are fibroblasts, macrophages, osteoclasts, and osteoblasts, which are all under the influence of ET (Ehrenreich *et al.* 1990; Zeballos *et al.*, 1991; Alam *et al.*, 1992; Kitten and Andrews, 2001). Gene expression levels of ET on day 28 showed the involvement of these peptides in the last days of necrotic tissue degradation and the start of the bone modelling process. Since the gene expression levels of ET-1 and ET-2 did not differ from those on day 0 and as the significant downregulation of the gene expression level of ET-3 seems to be a compensatory mechanism activated due to excessive peptide levels from the earlier phases, it seems that none of the ET isopeptides seems to contribute to these events (Figure 2). The late phase is dominated by bone resorption on the pressure side and bone formation on the tension side of the loaded tooth. During that time, the rate of tooth movement increased compared with the lag phase (Figure 1). This is a result of an increase in osteoblast and osteoclast number and activity (Ren *et al.*, 2005, Krishnan and Davidovitch, 2006). These cells mediate bone remodelling and, therefore, enable teeth to move through alveolar bone. ET-1 and ET-3 peptides contribute to the bone modelling processes since their gene expression levels on day 42 were upregulated. Furthermore, it appears that ET-1 dominates that phase of OTM since its gene expression level was significantly higher compared with that of ET-3 (Figure 2). However, it appears that ET-2 has no role in that phase of OTM, due to the relatively small change and downregulation of its gene expression (Figure 2).

Many studies have already shown that ET-1 increases bone formation (Tatrai *et al.*, 1992, Yin *et al.*, 2003; Guise and Mohammad, 2004; Clines *et al.*, 2007). Lately, it has also been suggested that ET-1 increases osteoclastic bone resorption during OTM (Sprogar *et al.*, 2008). No data on the effects of ET-3 on bone modelling are currently available. However, considering the similar expression patterns between ET-1 and ET-3, which could be a result of the involvement of the same transcript factors, it is likely that ET-3 plays a similar role to ET-1 in bone remodelling, increasing both bone formation and resorption.

Conclusions

During the late phase of OTM, when bone remodelling comprises bone formation and bone resorption, ET-1 and ET-3 are the predominant ET isoforms. ET-1 and ET-3 are equally important during the initial and lag phase, but ET-1 predominates the late phase. The role of ET-2 seems to be irrelevant during OTM.

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References

Alam A S *et al.* 1992 Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: implications for the vascular control of bone resorption. Endocrinology 130: 3617–3624

Clines G A *et al.* 2007 Dickkopf homolog 1 mediates endothelin-1-stimulated new bone formation. Molecular Endocrinology 21: 486–498

Drevenšek M, Sprogar Š, Boras I, Drevenšek G 2006 Effects of endothelin antagonist tezosentan on orthodontic tooth movement in rats. American Journal of Orthodontics and Dentofacial Orthopedics 129: 555–558

Ehrenreich H *et al.* 1990 Endothelins, peptides with potent vasoactive properties, are produced by human macrophages. The Journal of Experimental Medicine 172: 1741–1748

Guise T A, Mohammad K S 2004 Endothelins in bone cancer metastases. Cancer Treatment and Research 118: 197–212

Keeling S D, King G J, McCoy E A, Valdez M 1993 Serum and alveolar bone phosphatase changes reflect bone turnover during orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 103: 320–326

King G J, Latta L, Rutenberg J, Ossi A, Keeling S D 1997 Alveolar bone turnover and tooth movement in male rats after removal of orthodontic appliances. American Journal of Orthodontics and Dentofacial Orthopedics 111: 266–275

Kitten A M, Andrews C J 2001 Endothelin-1 expression in long-term cultures of fetal rat calvarial osteoblasts: regulation by BMP-7. Journal of Cellular Physiology 187: 218–225

Kourembanas S, Marsden P A, McQuillan L P, Faller D V 1991 Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. The Journal of Clinical Investigation 88: 1054–1057

Krishnan V, Davidovitch Z 2006 Cellular, molecular, and tissue-level reactions to orthodontic force. American Journal of Orthodontics and Dentofacial Orthopedics 129: 469.e1–469.e32

Miyauchi T, Masaki T 1999 Pathophysiology of endothelin in the cardiovascular system. Annual Review of Physiology 61: 391–415

Nelson J B *et al.* 1999 New bone formation in an osteoblastic tumor model is increased by endothelin-1 overexpression and decreased by endothelin A receptor blockade. Urology 53: 1063–1069

Noxon S J, King G J, Gu G, Huang G 2001 Osteoclast clearance from periodontal tissues during orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 120: 466–476

Reitan K 1967 Clinical and histologic observations on tooth movement during and after orthodontic treatment. American Journal of Orthodontics 53: 721–745

Ren Y, Kuijpers-Jagtman A M, Maltha J C 2005 Immunohistochemical evaluation of osteoclast recruitment during experimental tooth movement in young and adult rats. Archives of Oral Biology 50: 1032–1039

Rich S, McLaughlin V V 2003 Endothelin receptor blockers in cardiovascular disease. Circulation 108: 2184–2190

Rygh P 1973 Ultrastructural changes in pressure zones of human periodontium incident to orthodontic tooth movement. Acta Odontologica Scandinavica 31: 109–122

Rygh P 1976 Ultrastructural changes in tension zones of rat molar periodontium incident to orthodontic tooth movement. American Journal of Orthodontics 70: 269–281

Sasaki T, Hong M H 1993 Localization of endothelin-1 in the osteoclast. Journal of Electron Microscopy 42: 193–196

Schmitz-Spanke S, Schipke J D 2000 Potential role of endothelin-1 and endothelin antagonists in cardiovascular diseases. Basic Research in Cardiology 95: 290–298

Shirazi M, Nilforoushan D, Alghasi H, Dehpour A R 2002 The role of nitric oxide in orthodontic tooth movement in rats. The Angle Orthodontist 72: 211–215

Sims M R 2001 Endothelin-1 expression in the microvasculature of normal and 3-hour continuously loaded rat molar periodontal ligament. European Journal of Orthodontics 23: 647–662

Sprogar Š, Vaupotic T, Cör A, Drevenšek M, Drevenšek G 2008 The endothelin system mediates bone modeling in the late stage of orthodontic tooth movement in rats. Bone 43: 740–747

Sprogar Š, Volk J, Drevenšek M, Drevenšek G 2007 The effects of TBC3214, a selective endothelin ET_Areceptor antagonist, on orthodontic tooth movement in rats. European Journal of Orthodontics 29: 605–608

Tatrai A, Foster S, Lakatos P, Shankar G, Stern P H 1992 Endothelin-1 actions on resorption, collagen and noncollagen protein synthesis, and phosphatidylinositol turnover in bone organ cultures. Endocrinology 131: 603–607

von Böhl M, Maltha J C, Von Den Hoff J W, Kuijpers-Jagtman A M 2004 Focal hyalinization during experimental tooth movement in beagle dogs. American Journal of Orthodontics and Dentofacial Orthopedics 125: 615–623

von Schroeder H P, Veillette C J, Payandeh J, Qureshi A, Heersche J N 2003 Endothelin-1 promotes osteoprogenitor proliferation and differentiation in fetal rat calvarial cell cultures. Bone 33: 673–684

Yin J J *et al.* 2003 A causal role for endothelin-1 in the pathogenesis of osteoblastic bone metastases. Proceedings of the National Academy of the Sciences of the United States of America 100: 10954–10959

Yoshimatsu M *et al.* 2006 Experimental model of tooth movement by orthodontic force in mice and its application to tumor necrosis factor receptor-deficient mice. Journal of Bone and Mineral Metabolism 24: 20–27

Zeballos G A, An S J, Wu J M 1991 Endothelin-1 secretion by human fibroblasts in culture: effects of cell density and IFN-beta. Biochemistry International 25: 845–852

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Emotional stress and orthodontic tooth movement: effects on apical root resorption, tooth movement, and dental tissue expression of interleukin-1 alpha and calcitonin gene-related peptide immunoreactive nerve fibres in rats

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SUMMARY The aim of the study was to investigate the effect of emotional stress on apical root resorption (ARR) and tooth displacement during orthodontic tooth movement in rats. A further area of interest was to evaluate if the expression of interleukin-1 alpha (IL-1α) as well as the density and distribution of peptidergic nerve fibres immunoreactive to calcitonin gene-related peptide (CGRP) in the periodontal ligament (PDL) are associated with possible stress-induced changes in root resorption and tooth movement.

A total of 52 male Wistar rats, aged 6 weeks, were divided in three experimental and one control group (n=4). Group 1 had orthodontic tooth movement and received foot shocks (OTMS; n=16), group 2 had orthodontic tooth movement but received no foot shocks (OTMNS; n=16), and group 3 had no orthodontic tooth movement and received foot shocks (NOTMS; n=16). Each group was further divided into four subgroups (n=4), corresponding to the period of the experiment, i.e. 3, 7, 13, and 21 days. At the end of each experimental period, the blood samples were taken, the animals were sacrificed, and the jaws excised, demineralized, and processed for immunocytochemistry. One-way analysis of variance was used to detect inter-group differences for all investigated variables. CGRP immunopositive nerve fibres were evaluated qualitatively.

All the experimental groups demonstrated higher corticosterone levels than the control group, suggesting a stress-induced experience by orthodontic treatment *per se*. The OTMS group had the least amount of cellular cementum throughout the experimental periods and showed significant reduction in tooth displacement, especially at 3 and 7 days. No obvious changes were observed in the dental tissue expression of IL-1α and CGRP immunoreactive nerve fibres between the stressed and non-stressed orthodontically treated groups.

Introduction

The aetiology and predictability of root resorption have been a matter of extensive research and a number of review articles summarize the knowledge on this topic (Brezniak and Wasserstein, 1993, 2002; Killiany, 1999; Hartsfield *et al.*, 2004; Segal *et al.*, 2004; Krishnan, 2005). Taking into account that the risk of apical root resorption (ARR) with fixed appliances is significantly greater than with removable appliances (Linge and Linge, 1983), numerous studies have attempted to identify the mechanotherapy that would reduce, or even eliminate root resorption. Different types of appliances, treatment modalities, and force regimens have been associated with different amounts of resorption and the results are often conflicting (Linge and Linge, 1983; Beck and Harris, 1994; Janson *et al.*, 2000; Mavragani *et al.*, 2000; Sameshima and Sinclair, 2001; Maltha *et al.*, 2004). A meta-analysis of the treatment-related factors of ARR revealed a strong correlation between root resorption and total apical displacement and treatment duration (Segal *et al.*, 2004). Age, gender, malocclusion type, and root shape have also been positively or negatively associated with root resorption (Brezniak and Wasserstein, 1993, 2002).

The systemic condition of the patient has received less attention in the literature. A few studies have shown an increased risk of root resorption in patients with chronic asthma and/or allergies (McNab *et al.*, 1999; Owman-Moll and Kurol, 2000). Endocrine disturbances and immune responses have also been mentioned as possible contributing factors (Goultschin *et al.*, 1982; Ng *et al.*, 1990). So far, emotional stress has not been addressed as a potential factor, although it is quite clear that stress situations are closely related to and affect both the endocrine and immune systems. Stressful events cause fear and anxiety which are regarded as risk factors for a variety of diseases, including periodontal (Peruzzo *et al.*, 2007). Hence, the aim of this study was to investigate, primarily, the effect of emotional stress on root resorption and tooth displacement during experimental orthodontic tooth movement in rats. A further area of interest was to evaluate if the expression of interleukin-1 alpha (IL-1α) at protein level as well as the density and distribution

of peptidergic nerve fibres immunoreactive to calcitonin gene-related peptide (CGRP) in the periodontal ligament (PDL) are associated with possible stress-induced changes in root resorption and tooth movement.

Materials and methods

The study was authorized by the Norwegian Animal Research Authority and conducted in accordance with the animal welfare act.

Animals

The material comprised 52 six-week-old male rats (mol:WIST Han), with a mean body weight of 243 ± 22.7 g. The animals were housed in polycarbonate cages with standard 12-hour light–dark cycles, temperature 21°C, and fed a standard pellet diet with tap water *ad libitum*. The animals were acclimatized for approximately 5 days before the start of the experiment.

The experimental animals ($n=48$) were divided into three groups as follows: group 1 had orthodontic tooth movement and received foot shocks (OTMS; $n=16$), group 2 had orthodontic tooth movement but received no foot shocks (OTMNS; $n=16$), and group 3 had no orthodontic tooth movement and received foot shocks (NOTMS; $n=16$). Each of the groups was further divided into four subgroups ($n=4$), corresponding to the number of days the experiment lasted, i.e. 3, 7, 13, and 21 days. Four animals served as an untreated control group.

Experimental procedures

The operations were carried out under general anaesthesia, using a subcutaneous injection of fentanyl–fluanison—midazolam, 0.2 ml/100 g body weight. As previously described (Vandevska-Radunovic *et al.*, 1997). An orthodontic appliance consisting of a coil spring ligated to the first right maxillary molar and connected to a band cemented on the incisors was inserted in the animals undergoing experimental tooth movement. The coil exerted a mesial force to the molar of approximately 0.5 N. The animals subjected to stress received 0 to 10 foot shocks daily in a specially designed apparatus (Overmier and Murison, 1991). The shocks were given with random inter-shock intervals to avoid learning effects, each shock being at 1 mA intensity and of 1 second duration. All animals were weighed before the start of the experiment and before sacrifice. Blood samples were taken under general anaesthesia from the jugular vein at 3, 7, 13, and 21 days after the initiation of the experiment. Samples were centrifuged, and the plasma was drawn off and frozen for later fluorometric analysis of corticosterone. The animals were then sacrificed, the jaws excised, and fixed in 4 per cent paraformaldehyde for 24 hours and then demineralized in 10 per cent ethylenediaminetetraacetic acid (EDTA) plus 7.5 per cent polyvinylpyrrolidone for 4 weeks. After demineralization, the jaws were sectioned sagittally at 20 μm in a freezing microtome.

Histological and immunohistochemical procedures

Alternate serial sections from the central part of experimental and control first maxillary molars were used for further analysis. Sections intended for evaluation of root resorption and tooth displacement were stained with haematoxylin and eosin on gelatin-coated slides. However, immunoreaction was performed on free-floating sections in tissue culture wells, 12 alternate sections for each antibody. These sections were incubated for 72 hours at 4°C in anti-rat IL-1α (1:400 dilution; Endogen, Cambridge, Massachusetts, USA) and CGRP (1:6000 dilution; Diagnostika, Falkenberg, Sweden) polyclonal antibodies raised in rabbits. Antigen–antibody complexes were detected by the avidin–biotin–peroxidase (ABC) method, using a commercially available kit (Vectastain ABC kit; Vector Laboratories, Burlingame, California, USA), and visualized by 3,3′-diaminobenzidine (Sigma, St Louis, Missouri, USA) in the presence of 0.2 per cent $(NH_4)_2Ni(SO_4)_26H_2O$ to enhance immunostaining. Finally, the sections were mounted on gelatine-coated slides and counterstained with methylene blue/azure II in 1 per cent sodium borate and distilled water. They were then dehydrated in a graded alcohol series, cleared in xylene, and coverslipped with Eukitt (Kindler, Freiburg, Germany). The specificity of the immune reaction was tested by omitting the primary antibody. In these sections, no immunospecific staining was observed.

Evaluation and statistical procedure

Both tooth displacement and cellular cementum volume were measured on 12 central sections per molar in a Cue-3 Image Analyzer (Galai Production, Migdal HaeMek, Israel). Cellular cementum volume was measured for both the mesial and the distal roots. The values from each root were pooled and then all sections summarized to obtain the relative volume for each molar. The amount of tooth displacement was measured between the first and second maxillary molars by drawing a line perpendicular to the long axis of the distal root of the first molar and connecting the most distant crown points just above the junctional epithelium (Figure 1). All values were summarized and divided by the number of sections in order to obtain the mean distance. Cells immunopositive to IL-1α were counted in a light microscope (×25 objective ×10 ocular; Labophot-2, Nikon, Tokyo, Japan) throughout the PDL of the most mesial and distal roots of all investigated molars, below the alveolar bone crest.

Statistical analysis

Means and standard deviations were calculated for corticosterone levels, tooth displacement, cellular cementum volume, and number of cells immunopositive to IL-1α in all

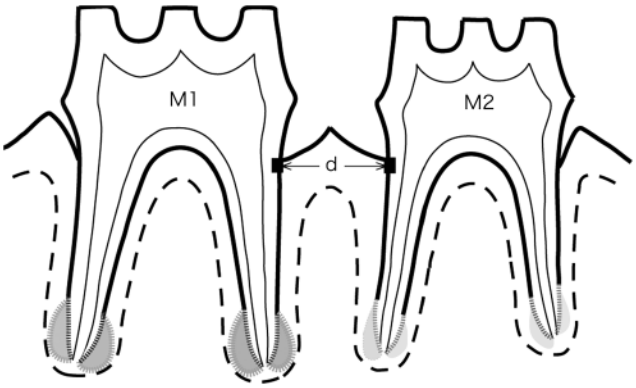

Figure 1 Measurement of the distance (d) between the first (M1) and the second (M2) maxillary molars. The grey periapical area on M1 denotes the cellular cementum.

investigated animals. One-way analysis of variance (ANOVA) with a least significant *post hoc* test was used to detect statistically significant inter-group differences. CGRP immunopositive nerve fibre morphology and distribution was assessed qualitatively, while nerve fibre density was graded as equal to (0) and increased (+) compared with the controls.

Results

There was a mean weight loss of approximately 3–5 per cent in the 3 day experimental period in the groups with orthodontic appliances. However, there was an overall gain in weight of approximately 10–15 per cent at the end of the experimental periods in all groups.

Corticosterone levels

Corticosterone levels and their distribution throughout the experimental periods showed individual variations both in the control and experimental groups (Figure 2A). In the control group, corticosterone values were pooled and their mean value was used for statistical analysis. All experimental groups showed a statistically significant ($P < 0.01$) increase in corticosterone levels at day 3 compared with the control group but not between each other. At 7 days, the increase was significant ($P < 0.01$), but only for the OTMS group compared with the control group. At days 13 and 21, there were no statistically significant differences between the groups although, on average, corticosterone levels were higher in the experimental groups than in the control group. When the experimental groups were used as a dependent variable, the only statistically significant change was in the OTMS group, where corticosterone levels decreased from 7 to 13 days.

Cellular cementum/ARR

The relative volume of cellular cementum was taken as a measure for ARR. In general, the OTMS group showed the least amount of cellular cementum, which was significantly less ($P \leq 0.05$) than the control group at 3, 7, and 13 days (Figure 2B) and significantly less ($P \leq 0.05$) than the NOTMS group at 7, 13, and 21 days. The OTMNS group also showed significantly less cellular cementum ($P \leq 0.01$) compared with the NOTMS and control groups but only at 7 and 13 days (Figure 3). At 21 days, the OTMNS group approached the values of the control group. No significant differences in cellular cementum were observed between the OTMS and OTMNS groups.

Tooth movement

At 3 days, the first maxillary right molar in both the OTMS and OTMNS groups showed significantly greater mesial displacement than the molars in the NOTMS and control groups ($P < 0.05$; Figure 2C). Mesial tooth movement of the right first maxillary molar in the OTMNS group gradually increased through days 7 and 13, thus gaining statistical significance when compared with the OTMS group ($P < 0.01$; Figure 2C) and maintaining significance when compared with the control and NOTMS groups ($P < 0.01$). At 21 days, mesial tooth movement of the maxillary first molar in the OTMNS group was still significantly greater than in the NOTMS and control groups ($P < 0.05$) but not when compared with the OTMS group. The right first maxillary molar in the OTMS group demonstrated greater mesial movement than previously; this was on the border of being statistically significant but only when compared with the NOTMS group ($P = 0.051$).

IL-1α immunopositive cells and CGRP immunopositive nerve fibres

The number of IL-1α immunopositive cells was highest in the OTMS group both at 3 and at 7 days (Figure 2D). However, this was not statistically significant when compared with the OTMNS group. The majority of the immunopositive cells were observed in the distal PDL of the distal root corresponding to the tension area and in the gingival papillae mesial and distal to the maxillary first molars (Figure 3). At 13 and 21 days, almost no IL-1α-positive cells were seen in the PDL below the alveolar crest of the investigated molars. The control and NOTMS groups showed almost no IL-1α-positive cells in the PDL, but individual positive cells could be observed in the gingival papillae. Large individual differences were present in the experimental groups undergoing orthodontic tooth movement.

The CGRP immunopositive fibres were short, mainly located in the periapical area and close to the alveolar bone, although individual fibres extended towards the cellular cementum. No obvious differences in the number and distribution of immunopositive nerve fibres were observed among the various groups at 3 and 21 days. An increase in nerve fibre density was noted in the apical and cervical half of the PDL of the OTMNS and OTMS groups at 7 days when compared with the NOTMS and control groups (Table 1). At

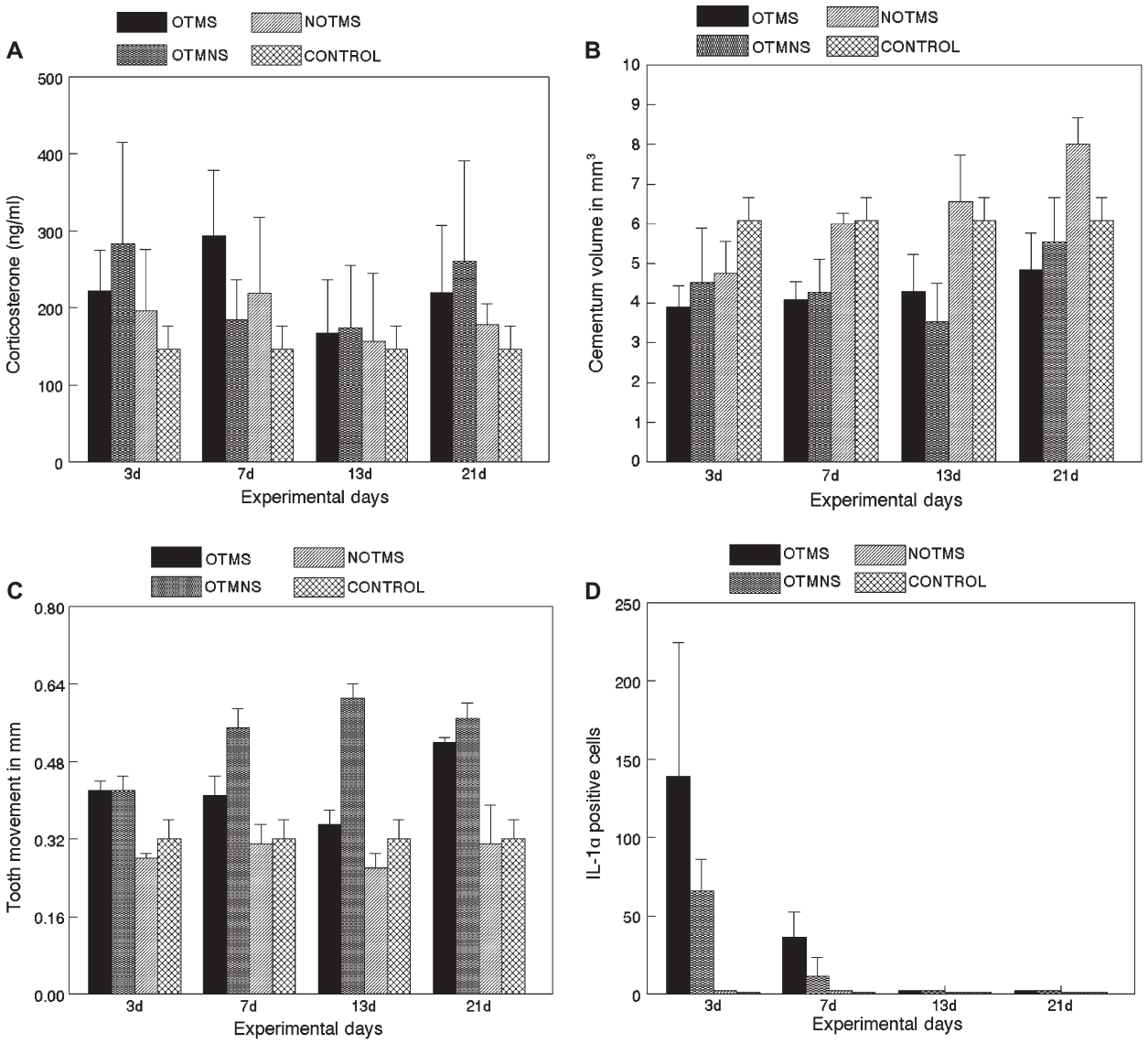

Figure 2 Means and standard deviations of (A) corticosterone levels (ng/ml), (B) cellular cementum volume (mm^3) taken as a measure for apical root resorption of the first maxillary molar, (C) mesial tooth displacement (mm) of the first maxillary molar, and (D) interleukin-1 alpha immunopositive cells in the periodontal ligament of the first maxillary molar in orthodontically treated and stressed (OTMS), orthodontically treated non-stressed (OTMNS), non-treated stressed (NOTMS), and control groups at 3, 7, 13, and 21 days.

13 days, these changes were obvious only in the apical area. No apparent differences in nerve fibre distribution and density could be seen between the experimentally moved teeth of the OTMS and OTMNS groups.

Discussion

Emotional or psychological stress has been recognized as a risk factor in the aetiology and pathogenesis of a number of diseases. Chronic inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, psoriasis, and inflammatory bowel disease are some of the conditions that can be initiated or exacerbated by stressful events (Straub *et al.*, 2005; Mawdsley and Rampton, 2006; Kemeny and Schedlowski, 2007). Moreover, periodontal disease, bruxism, orofacial pain, and even relapse tendency after orthodontic treatment have been shown to have positive relationship with stress (Fried, 1976; Rosales *et al.*, 2002; Peruzzo *et al.*, 2007). The results from the present study provide evidence that emotional stress is also associated with orthodontic tooth movement. Animals subjected to stress and experimental orthodontic treatment demonstrated reduced amounts of tooth movement when compared with controls and non-stressed orthodontically treated animals. They also showed the greatest amount of root resorption throughout the experimental period.

Glucocorticoids, such as cortisol and corticosterone, are the main hormones released after stress stimuli and their high plasma levels indicate a positive stress response. All animals

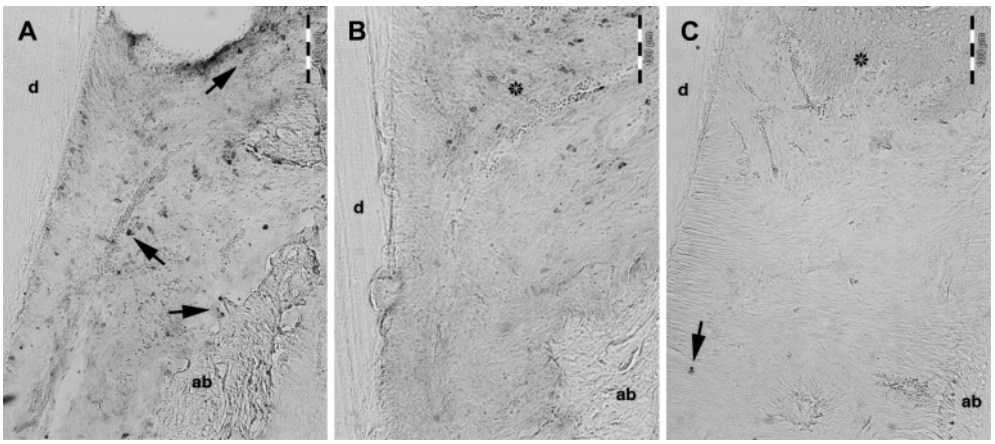

Figure 3 Distal periodontal area of the distal root of the first maxillary molar at 3 (A), 7 (B), and 13 (C) days of an orthodontically treated and stressed [orthodontic tooth movement and received foot shocks (OTMS)] rat. (A and B) Interleukin-1 alpha immunocompetent cells (arrows) are seen in the periodontal ligament close to dentine (d) and alveolar bone (ab) and in the interdental papilla (*). At day 13 (C), only individual immunopositive cells can be seen (arrow). Bar = 100 μm.

in the experimental groups in the present study showed higher corticosterone levels at all experimental periods when compared with the control group. The groups having orthodontic tooth movement, with and without foot shocks, demonstrated similar temporal distribution and attained high corticosterone levels 3 weeks after the placement of the orthodontic appliance. This suggests that orthodontic tooth movement *per se* elicits stress effects and that adjoining psychological stress does not have an additional effect on corticosterone plasma levels. Behavioural testing of animals subjected to experimental tooth movement has confirmed that emotional stress can be evoked after the placement of an orthodontic appliance and ahead of pain-related animal behaviour (Yozgatian *et al.*, 2008). This is most probably due to occlusal disharmony and stimulation of the periodontal receptors, which in turn affect corticosterone plasma levels and lead to stress-related behaviour (Yoshihara *et al.*, 2001). To date, there are no human investigations that evaluate the effect of orthodontic treatment on corticosterone levels. However, behavioural responses to mechanical tooth movement associated with pain and even anxiety of the procedure have been well documented and indicate possible stress effects in humans (Giddon *et al.*, 2007).

If orthodontic treatment itself evokes stressful responses, the question that arises is if additional emotional stress has confounding effects on root resorption and tooth movement. Severe cementum resorption in mammalian teeth, with and without repair, has been reported in connection with stressful experiences including starvation, temperature extremes, injury, and fear (Myrick, 1988). That author suggested that these stimuli lead to hypersecretion of glucocorticoids and in turn evoke hypocalcaemia which results in dental tissue resorption. Experiments on hypocalcaemic rats subjected to orthodontic tooth movement showed that the occurrence and severity of root resorption was increased when compared with normal animals, and that these changes could be related to an increase in alveolar bone turnover (Engström *et al.*, 1988). The present findings are partly in agreement with

Table 1 Evaluation of the density of the calcitonin gene-related peptide immunopositive nerve fibres in different areas of the periodontal ligand (PDL) of the experimental groups: orthodontic tooth movement and shock (OTMS), orthodontic tooth movement and no shock (OTMNS), and no orthodontic tooth movement and shock (NOTMS).

Tissue location	Experimental period (days)											
	3			7			13			21		
	OTMS	OTMNS	NOTMS	OTMS	OTMNS	NOTMS	OTMS	OTMNS	NOTMS	OTMS	OTMNS	NOTMS
Apical half of the PDL	0	0	0	+	+	0	+	+	0	0	0	0
Cervical half of the PDL	0	0	0	+	+	0	0	0	0	0	0	0

The control group is taken as having zero (0) value. 0, equal to the control group; +, increased density compared with the control group.

those results which show that stressed animals with orthodontic treatment (OTMS) had significantly less cellular cementum throughout the experimental periods when compared with the control and stressed animals without orthodontic treatment (NOTMS). However, the differences between the two orthodontic treatment groups were small at 3, 7, and 13 days, but increased at 21 days, at which time the stressed animals displayed the least amount of cellular cementum or the greatest amount of root resorption. These data can be interpreted as a delay in the reparative processes in the stress group, which should have taken place 3 weeks after the onset of orthodontic tooth movement. It may also be that the measuring of the apical cellular cementum underscored the amount of root resorption and that group differences would have been greater if lateral resorption lacunae had been included. This has been confirmed in a group of animals treated with corticosteroids (Verna *et al.*, 2006). The relative volume of cellular cementum, and not lateral root resorption, was taken as an indirect measure of root resorption mainly because of its clinical relevance. Root shortening and not lateral root surface resorption is considered to be detrimental to orthodontically treated teeth. On the other hand, precise delineation of resorptive lacunae in the cellular cementum area, particularly in rats, is difficult. This problem was avoided by measuring the relative volume of cellular cementum as a quantitative indicator of resorbed cementum.

A clear linear increase in tooth movement was noted in the non-stressed orthodontically treated group. The displacement was significantly greater than that observed in the stressed and orthodontically treated group at 7 and 13 days. These periods correspond to the pro-inflammatory processes triggered by force application and encompass increased vascular permeability, cellular infiltration, and secretion of cytokines and neuropeptides (Vandevska-Radunovic, 1999; Krishnan and Davidovitch, 2006). Stress-induced elevation of glucocorticoids can transiently suppress cytokine production and decrease leukocyte proliferation and mobilization (Mercado *et al.*, 2002; Dhabhar, 2003). Knowing that these mediators serve central roles in the process of tooth movement, the decrease in tooth displacement in the stressed group was not unexpected. However, longer experimental periods might have given different results, as chronic administration of corticosteroids has been shown to increase tooth movement in orthodontically treated rats (Kalia *et al.*, 2004).

IL-1α is a highly inflammatory multifunctional cytokine localized, among others, in mononuclear cells, osteoclasts, osteoblasts, and fibroblasts (Lossdörfer *et al.*, 2002, Kamolmatyakul *et al.*, 2004). Increased production has been reported during inflammatory infectious and autoimmune diseases, while corticosteroids are shown to suppress its production (Dinarello, 1996). Plasma corticosterone levels did not seem to have such an effect in the early experimental periods in the current study. Similar responses were observed in the density changes of nerve fibres immunoreactive to CGRP, where the stressed and non-stressed animals with orthodontic treatment showed almost no differences. The local inflammatory processes induced by orthodontic force seemed to override the possible suppressive effects of systemic corticosterone and caused a transient increased IL-1α expression and CGRP fibre density in the dental tissues. On the other hand, evidence exists that the cytokine suppressive effect of glucocorticoids is observed at supraphysiological ranges and *in vitro*, while experiments with basal or stress-related doses enhance cytokine production *in situ* (Wilckens and De Rijk, 1997). Furthermore, stress hormones, e.g. corticosterone and catecholamines, can sometimes have a different if not opposing effect, and different concentrations and combinations of these hormones may lead to different responses (Dhabhar, 2003). Glucocorticoids may also induce different systemic and local tissue-specific effects for the same cytokines (Gibb *et al.*, 2008). These data coupled with the large individual differences can explain the observed effect of joint emotional stress and orthodontic treatment on local dental tissue expression of IL-1α and CGRP immuoreactive nerve fibres. In contrast, their confounding effect on cellular cementum resorption and tooth displacement was more obvious, although individual differences were also present.

Finally, it is important to note that the investigation was undertaken in accordance with the three 'R's of animal testing, i.e. reduction, refinement, and replacement. In view of the reduction principle, the number of animals in each subgroup was minimal and yet suitable for statistical analysis. The variables investigated were assumed to be normally distributed and therefore a one-way ANOVA was used. Obtaining significant results in these circumstances clearly shows that there are inter-group differences. It is possible that the differences could have been greater if the number of animals was greater, but this would have undermined the basic principles of animal experimentation.

Conclusions

Orthodontic tooth movement *per se* can evoke emotional stress. Additional emotional stress leads to increased cellular cementum resorption and particularly to decreased tooth movement in the early experimental periods but has no confounding effect on the local dental tissue expression of IL-1α and CGRP immuoreactive nerve fibres.

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References

Beck B W, Harris E F 1994 Apical root resorption in orthodontically treated subjects: analysis of edgewise and light wire mechanics. American Journal of Orthodontics and Dentofacial Orthopedics 150: 350–361

Brezniak N, Wasserstein A 1993 Root resorption after orthodontic treatment: part 2. Literature review. American Journal of Orthodontics and Dentofacial Orthopedics 103: 138–146

Brezniak N, Wasserstein A 2002 Orthodontically induced inflammatory root resorption. Part I: the basic science aspects. Angle Orthodontist 72: 175–179

Dhabhar F S 2003 Stress, leukocyte trafficking, and the augmentation of skin immune function. New York Academy of Sciences 992: 205–217

Dinarello C A 1996 Biologic basis for interleukin-1 in disease. Blood 15: 2095–2147

Engström C, Granström G, Thilander B 1988 Effect of orthodontic force on periodontal tissue metabolism. A histologic and biochemical study in normal and hypocalcemic young rats. American Journal of Orthodontics and Dentofacial Orthopedics 93: 486–495

Fried K H 1976 Emotional stress during retention and its effect on tooth position. Angle Orthodontist 46: 77–85

Gibb J, Hayley S, Gandhi R, Poulter M O, Anisman H 2008 Synergistic and additive actions of a psychosocial stressor and endotoxin challenge: circulating and brain cytokines, plasma corticosterone and behavioral changes in mice. Brain, Behavior, and Immunity 22: 573–589

Giddon D B, Anderson N K, Will L A 2007 Cognitive, affective, and behavioural responses associated with mechanical tooth movement. Seminars in Orthodontics 13: 212–219

Goultschin J, Nitzan D, Azaz B 1982 Root resorption. Review and discussion. Oral Surgery, Oral Medicine, and Oral Pathology 54: 586–590

Hartsfield J K, Everett E T, Al-Quawasmi R A 2004 Genetic factors in external apical root resorption and orthodontic treatment. Critical Reviews in Oral Biology and Medicine 15: 115–122

Janson G R, De Luca Canto G, Martins D R, Henriques J F, De Freitas M R 2000 A radiographic comparison of apical root resorption after orthodontic treatment with 3 different fixed appliance techniques. American Journal of Orthodontics and Dentofacial Orthopedics 118: 262–273

Kalia S, Melsen B, Verna C 2004 Tissue reaction to orthodontic tooth movement in acute and chronic corticosteroid treatment. Orthodontics and Craniofacial Research 7: 26–34

Kamolmatyakul S *et al.* 2004 IL-1alpha stimulates cathepsin K expression in osteoclasts via the tyrosine kinase-NF-kappaB pathway. Journal of Dental Research 83: 791–796

Kemeny M E, Schedlowski M 2007 Understanding the interaction between psychosocial stress and immune-related diseases: a stepwise progression. Brain, Behaviour, and Immunity 21: 1009–1018

Killiany D M 1999 Root resorption caused by orthodontic treatment: an evidence-based review of literature. Seminars in Orthodontics 5: 128–133

Krishnan V 2005 Critical issues concerning root resorption: a contemporary review. World Journal of Orthodontics 6: 30–40

Krishnan V, Davidovitch Z 2006 Cellular, molecular, and tissue-level reactions to orthodontic force. American Journal of Orthodontics and Dentofacial Orthopedics 129: 469.e1–e32

Linge B O, Linge L 1983 Apical root resorption in upper anterior teeth. European Journal of Orthodontics 5: 173–183

Lossdörfer S, Götz W, Jäger A 2002 Localization of IL-1alpha, IL-1 RI, TNF, TNF-RI and TNF-RII during physiological drift of rat molar teeth an immunohistochemical and *in situ* hybridization study. Cytokine 7: 7–16

Maltha J C, van Leeuwen E J, Dijkman G E, Kuijpers-Jagtman A M 2004 Incidence and severity of root resorption in orthodontically moved premolars in dogs. Orthodontics and Craniofacial Research 7: 115–121

Mavragani M, Vergari A, Selliseth N J, Bøe O E, Wisth P L 2000 A radiographic comparison of apical root resorption after orthodontic treatment with a standard edgewise and a straight-wire edgewise technique. European Journal of Orthodontics 22: 665–674

Mawdsley J E, Rampton D S 2006 The role of psychological stress in inflammatory bowel disease. Neuroimmunomodulation 13: 327–336

McNab S, Battistutta D, Taverne A, Symons A L 1999 External apical root resorption of posterior teeth in asthmatics after orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopedics 116: 545–551

Mercado A M, Padgett D A, Sheridan J F, Marucha P T 2002 Altered kinetics of IL-1 alpha, IL-1 beta, and KGF-1 gene expression in early wounds of restrained mice. Brain, Behaviour, and Immunity 16: 150–162

Myrick A C 1988 Is tissue resorption and replacement in permanent teeth of mammals caused by stress-induced hypocalcemia?. In: Davidovitch Z (ed.). Biological mechanisms of tooth eruption and root resorption. EBSCO Media, Birmingham, pp. 379–389

Ng K T, King G J, Courts F J 1990 Humoral immune response to active root resorption with a murine model. American Journal of Orthodontics and Dentofacial Orthopedics 98: 456–462

Overmier J B, Murison R 1991 Juvenile and adult footshock stress modulate later adult gastric pathophysiological reactions to restraint stresses in rats. Behavioral Neuroscience 105: 246–252

Owman-Moll P, Kurol J 2000 Root resorption after orthodontic treatment in high- and low risk patients: analysis of allergy as a possible predisposing factor. European Journal of Orthodontics 22: 657–663

Peruzzo D C *et al.* 2007 A systematic review of stress and psychological factors as possible risk factors for periodontal disease. Journal of Periodontology 78: 1491–1504

Rosales V P, Ikeda K, Hizaki K, Naruo T, Nozoe S, Ito G 2002 Emotional stress and brux-like activity of the masseter muscle in rats. European Journal of Orthodontics 24: 107–117

Sameshima G T, Sinclair P M 2001 Predicting and preventing root resorption: part II. Treatment factors. American Journal of Orthodontics and Dentofacial Orthopedics 119: 511–515

Segal G R, Schiffman P H, Tuncay O C 2004 Meta analysis of the treatment-related factors of external apical root resorption. Orthodontics and Craniofacial Research 7: 71–78

Straub R H, Dhabhar F S, Bijlsma J W, Cutolo M 2005 How psychological stress via hormones and nerve fibers may exacerbate rheumatoid arthritis. Arthritis and Rheumatism 52: 16–26

Vandevska-Radunovic V 1999 Neural modulation of inflammatory reactions in dental tissues incident to orthodontic tooth movement. Review of literature. European Journal of Orthodontics 21: 231–247

Vandevska-Radunovic V, Kvinnsland S, Hals Kvinsland I, Jonsson R 1997 Immunocompetent cells in periodontal ligament of rat molars incident to orthodontic tooth movement. European Journal of Oral Science 105: 36–44

Verna C, Hartig L E, Kalia S, Melsen B 2006 Influence of steroid drugs on orthodontically induced root resorption. Orthodontics and Craniofacial Research 9: 57–62

Wilckens T, De Rijk R 1997 Glucocorticoids and immune function: unknown dimensions and new frontiers. Immunology Today 18: 418–424

Yoshihara T, Matsumoto Y, Ogura T 2001 Occlusal disharmony affects plasma corticosterone and hypothalamic noradrenaline release in rats. Journal of Dental Research 80: 2089–2092

Yozgatian J H, Zeredo J L, Hotokezaka H, Koga Y, Toda K, Yoshida N 2008 Emotional stress- and pain-related behaviors evoked by experimental tooth movement. Angle Orthodontist 78: 487–494

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Prostaglandin E_2 levels in gingival crevicular fluid during tooth- and bone-borne expansion

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SUMMARY The purpose of this study was to compare Prostaglandin E_2 (PGE_2) levels in gingival crevicular fluid (GCF) of young adults with maxillary constriction during tooth- and bone-borne expansion. Thirty patients, 15 females and 15 males, with a mean age of 17.3 ± 2.8 years were divided into three groups. Group I consisted of 10 patients, five females and five males, treated by transpalatal distraction (TPD) as a bone-borne device, group II 10 patients, five females and five males, with a Hyrax appliance as a tooth-borne device, and a control group of 10 patients, five females and five males, without any expansion appliances. GCF samples were collected with filter paper strips at six observation periods in order to evaluate the effect of heavy orthopaedic forces in both groups. In group II, the samples were additionally collected at two pre-treatment time points in order to evaluate the effect of the forces generated by the separators. An automated enzyme immunoassay was used to measure PGE_2 in the GCF. The differences within the groups were evaluated with a pairwise *t*-test and the differences between the groups were determined by the Mann–Whitney U-test.

The mean PGE_2 level was significantly elevated on day 4 after placement of the separators in group II ($P < 0.05$). The PGE_2 values in group II were significantly different to those in group I and the controls at all observation periods. Lower PGE_2 levels were observed in group I compared with group II and the controls. Expansion using the TPD method could potentially enhance the prognosis of the teeth by inducing more skeletal dental changes when compared with the Hyrax appliance.

Introduction

Bone remodelling is a complex process that is regulated by local factors such as cytokines, and growth and systemic factors such as hormones (Rossi *et al.*,1996). An acute inflammatory response is initiated with orthodontic forces. Osteoblastic and osteoclastic activities occur as a result of the inflammatory response of the surrounding tissues (Proffit *et al.*, 1986; Grieve *et al.*, 1994; Sarı *et al.*, 2004). Prostaglandins, produced by deformed osteoblasts and gingival fibroblasts, have been implicated in the cytokines of this inflammatory reaction (Saito *et al.*, 1991) Among the subclasses of prostaglandins, Prostaglandin E_2 (PGE_2) is closely related to bone resorption (Norrdin *et al.*, 1990).

Maxillary transverse deficiencies can be corrected with slow or rapid palatal expansion (RPE), surgically assisted RPE (SARPE), or a two piece Le Fort I osteotomy (Mommaerts, 1999). Slow expansion is indicated for dental transverse discrepancies in young subjects, while RPE is indicated in adolescents with transverse discrepancies, whether skeletal and/or dental (Bishara and Staley, 1987). Although it may be possible to expand the maxilla in older patients, the results are neither as predictable nor as stable. In order to overcome this, RPE may be accompanied by corticotomies to release the areas of bony resistance. In SARPE, tooth-borne conventional devices are still the preferred appliance type. However, they have the same disadvantages including periodontal ligament compression, buccal root resorption, dehiscences, and tooth tipping (Moss, 1968; Barber and Sims, 1981; Bishara and Staley, 1987; Mommaerts, 1999). Lanigan and Mintz (2002) reported temporary partial paralysis of the oculomotor nerve as another complication of SARPE.

Recently, bone-borne transpalatal distractors, such as the Rotterdam palatal distractor, have been developed in order to eliminate the undesired effects of RPE and SARPE (Koudstaal *et al.*, 2006). These devices are either screwed to the palatal vaults on each side of the palate or have pins that automatically stabilize the device without the need for screw fixation. Due to the necessity of palatal flap surgery, both techniques could be considered as invasive. Expansion of the posterior anchor teeth has been shown to result in more expansion of the anterior anchor teeth in both RPE (Adkins *et al.*, 1990) and SARPE (Bays and Greco, 1992) procedures. Pinto *et al.* (2001) reported that an increase in posterior expansion width during tooth-borne expansion reflects buccal tilting of the appliance carrying anchor teeth. They also reported that the expansion maintained by transpalatal distraction (TPD) is orthopaedic with minimal buccal tilting of the bony segments. Harzer *et al.* (2006) developed a procedure for bone-borne expansion using a Hyrax screw

fixed to both halves of the maxilla. They observed that bone-borne fixation induced greater bodily movement of the maxillary halves during expansion. Chung and Goldman (2003) evaluated the effect of dental tipping and dental rotation immediately after SARPE. They found that SARPE resulted in slight mesiobuccal rotation and significant buccal tipping of the first premolars and first molars.

Interleukin-1 beta (IL-1β) is known to be an inducer for Prostaglandin E_2 (PGE_2). Norrdin *et al.* (1990) and Sandy *et al.* (1993) reported that IL-1β synergistically up-regulates the formation of prostaglandins in periodontal cells under mechanical stress. Tzannetou *et al.* (1999) detected IL-1β and beta-glucuronidase in the gingival crevicular fluid (GCF) during RPE. They also found that orthodontic and orthopaedic forces evoked changes in the levels of the inflammatory mediators in the periodontal tissues, which might trigger biological processes associated with remodelling of the alveolar bone surrounding the roots.

The purpose of this study was to compare the PGE_2 levels in GCF, during maxillary expansion with bone-borne TPD and tooth-borne RPE devices.

Subjects and methods

A total of 30 adult orthodontic patients, 15 females and 15 males, mean age of 17.3 ± 2.8 years, with constricted maxillary arches who fulfilled the following criteria were included in the study:

1. All premolars present and fully erupted.
2. No history of systemic diseases.
3. Good periodontal health with no radiographic evidence of bone loss, i.e. periodontal probing depths equal to or less than 3 mm and no signs of gingival inflammation.
4. No history of antibiotic or anti-inflammatory drug use in the month preceding the study.
5. The female subjects were not pregnant.

Informed consent was obtained from all patients. The subjects were instructed to brush their teeth once in the morning after breakfast and once at night before bedtime for a minimum of 3 minutes in order to maintain periodontal prophylaxis and oral hygiene. Each patient was asked to use 0.5 ml of 0.2 per cent chlorhexidine gluconate mouth rinse following brushing.

The patients were divided into three groups. Group I included 10 patients, five females and five males, who received a TPD device. Distraction was delivered with the guidance of a TPD transporter as previously described by Sarı *et al.* (2007). Group II comprised 10 patients, five females and five males, who underwent SARPE. Care was taken to ensure that the arms of the Hyrax appliance were parallel to the palatal mucosa. The second premolars were not included in the appliance design. The control group included 10 patients, five females and five males, who did not receive any orthodontic treatment.

The patients in group II were instructed to turn the Hyrax appliances once in the morning and once at night for a total activation of 0.5 mm/day. Expansion of the TPD occurred at a rate of 0.33 mm/day, starting 1 week after surgery. Both Hyrax expansion and TPD were continued until the required expansion was achieved. Maxillary expansion was obtained in 2 weeks in both groups. At the end of that period, the screws were locked in place. The patients were observed every 7 days during the activation period of the appliance and for 28 days during passive wear.

Surgical procedure for groups I and II

All surgical procedures were carried out under general anaesthesia as described by Cureton (1998) and Atac e*t al.* (2006). The incisions were bilateral at the depth of the vestibule from the first molar area to the distal aspect of the lateral incisor. The mucoperiosteum was elevated, and the maxillary bone was exposed from the piriform aperture anteriorly to the pterygomaxillary fissure posteriorly. Osteotomy was performed horizontally above the apices of the teeth, including the release of the pterygoid junction. A thin osteotome was used to mallet between the central incisors just below the anterior nasal spine. Antibiotics, analgesia, and an oroantral regimen were prescribed for all patients.

GCF sample collection

GCF samples were obtained from the mesiobuccal and mesiopalatal gingival sulci of the maxillary permanent first molars, maxillary first premolars, and maxillary permanent central incisors at six observation periods, O1, O2, O3, O4, O5, and O6. Additionally, two samples, S1 and S2, were collected from group II in order to evaluate the effect of light forces generated by the separators prior to banding of the maxillary permanent first molars and first premolars. Note that in S1, GCF samples were collected from all participants prior to placement of the separators and the S2 samples were obtained at the fourth day after S1 before fitting the molar and premolar bands. This extra information was used to compare the changes in the prostaglandin levels within group II. The GCF samples obtained from groups I and II were used to evaluate the effects of heavy orthopaedic forces generated by the Hyrax and the TPD and to compare the differences between the groups. The sample collection was continued for a period of 28 days. The details of the observation periods are shown below.

O1: Initial samples were obtained prior to the start of expansion. In group I, TPDs were placed and expansion was initiated 1 week following surgery. GCF samples were obtained before the start of expansion in group I. In group II, expansion was initiated immediately after insertion of the Hyrax appliances.

O2 (24 hours): Activation of the screws in both groups measuring GCF at this time point.

O3: At day 7 following appliance activation.
O4: Following 14 days of active appliance wear. The screws of both groups were locked in place at this time point.
O5: After 7 days of retention (21 days).
O6: On day 28 of retention.
S1 (Baseline: 0): GCF samples were collected from all subjects in group II prior to placement of the separators. Loose 4.4 mm S2 modul separators (3M Unitek Ortodontic Products, Monrovia, California, USA) were placed between the mesial and distal interproximal areas of the maxillary permanent first molars and the maxillary first premolars.
S2 (4 days after S1): GCF samples were obtained from group II. Molar and premolar bands were inserted in order to fabricate the Hyrax appliances. Alginate impressions were taken, and separators were replaced. Following S2, all patients in group II underwent a Le Fort I osteotomy.

GCF sampling took place in a temperature-controlled area, maintained at 20°C and 40 per cent relative humidity between 09:00 and 10:00 a.m. This was done with six filter paper strips for GCF, which were housed in a single Eppendorf tube. All filter papers were autoclaved and weighed on a digital scale (Mettler AT-210; Mettler-Toledo Inc., Columbus, Ohio, USA) before use. The sites under investigation were isolated with cotton rolls. Supragingival plaque was removed, and the region was dried with an air syringe. Two filter papers for the mesiobuccal of the maxillary permanent first molars, two for the mesiobuccal of the first premolars, and two for the mesiobuccal of the maxillary permanent central incisors were inserted into the gingival crevice for 30 seconds. Samples containing blood were discarded. Acceptable filter papers were placed in the eppendorf tubes and weighed again to determine the volume of fluid collected.

A sterilized saline solution (250 μl) was added to the eppendorf tubes and the samples were centrifuged for 1 minute. All cytokines were recovered from the paper strips after 5 minutes of centrifugal elution. The papers were then removed and the solutions stored at −70°C until immunoassay.

GCF samples of the control group were also prepared following the same protocol.

PGE_2 assay

A commercial PGE_2 enzyme-linked immunosorbent assay kit (BioSource International, Camarilo, California, USA) was used to determine PGE_2. The standards for preparation of PGE_2 required eight eppendorf tubes that were numbered from one to eight. An aliquot of 900 μl automated enzyme immunosorbent (EIA) buffer was added to tube one. Tubes two to eight were filled with 500 μl EIA buffer. Then, 100 μl of the bulk standard was transferred to tube one and mixed thoroughly to make a 1 pg/ml standard. Then, 500 μl of the standard solution from tube one was placed in tube two and mixed thoroughly. Next, 500 μl from tube two was placed in tube three and mixed thoroughly. This procedure was repeated for tubes three to eight. EIA 100 μl buffer was added to non-specific binding and 50 μl EIA buffer to maximum binding (N_0) wells. Fifty microlitres of each diluted standard solution and 50 μl of each sample was then transferred to the appropriate wells. Finally, PGE_2 acetylcholinesterase (AChE) conjugate (PGE_2 Tracer; 50 μl) and then PGE_2 monoclonal antibody (50 μl) was transferred to each well according to the instructions in the PGE_2 assay protocol (Cayman Chemical Company, Ann Arbor, Michigan, USA). The plate was then covered and incubated for 18 hours at 4°C. At the end of incubation, the wells were washed five times with Wash Buffer (i.e. ultra pure water-free organic contaminants). Ellman's Reagent (which contains the substrate to AchE; 200 μl) was added to each well followed by the addition of 5 μl of the tracer, Total Activity. The plate was covered with plastic film and shaken on a microtiter EIA shaker for 60 minutes in the dark. The plate was then read at 450 nm on an EIA assay reader (EL 312e Biotek Instruments, Winooski, Vermont, USA) within 2 hours.

Statistical analysis

The data were analysed using the Statistical Package for Social Sciences Version 13.0 (SPSS Inc., Chicago, Illinois, USA). Within-group differences of PGE_2 levels between O1 and O6 and between S1 and O2 were evaluated by the pairwise *t*-test. The differences between the PGE_2 levels of the groups at the beginning and at O2, O3, O4, O5, and O6 were determined by the Mann–Whitney U-test. Statistical significance was set at $P < 0.05$.

Results

PGE_2 levels in group II were significantly increased on day 4 following the insertion of separators when compared with S1 values ($P = 0.000$). However, no statistical differences were observed between S1 and O1 (7 days after the separators were removed; $P = 0.165$). PGE_2 levels in GCF were increased after 24 hours of activation (O2) with the Hyrax screw. This increase was found to be significantly different when compared with baseline values ($P = 0.000$) as well as with the values at S2 ($P = 0.002$; Table 1).

Following activation of the screws (O1), a significant increase in PGE_2 levels was observed in groups I and II ($P < 0.05$). Despite this significant increase, a decrease was observed during O5 and O6 in group I, which resulted in significantly different PGE_2 levels of GCF compared with O1 ($P = 0.231$). In group II, PGE_2 levels of GCF at O2, O3, O4, O5, and O6 were significantly greater than those observed at O1 while PGE_2 levels in group I at O2 were not statistically different compared with the levels at O5

($P = 0.024$). PGE_2 values in the control group did not show any significant increases during the study. The changes in the PGE_2 levels are shown in detail in Tables 2 and 3.

Group comparisons at O2 demonstrated a significant increase in PGE_2 levels following activation in groups I and II ($P = 0.000$) compared with those of the control group. In addition, the increase in PGE_2 in group II was found to be significantly different from group I at O2. In general, the mean PGE_2 levels in group II were significantly higher than in group I at both activation periods, O3 and O4, and both retention periods, O5 and O6 ($P = 0.000$). On the other hand, significant elevation in the PGE_2 levels was observed in group II for all observation periods ($P = 0.000$) compared with the control group. When the mean PGE_2 level in group I was compared with that of the control group, significant differences were observed between activation of the screws, O2, and first week retention periods O5 ($P < 0.05$). However, no statistically significant differences were noted in the PGE_2 levels in GCF ($P = 0.445$) between group I and the control group at O6. The group comparisons are shown in Table 4 and Figure 1.

Discussion

Orthodontic forces induce inflammatory events in the periodontium that result in bone resorption and orthodontic tooth movement (Grieve *et al.*, 1994). At the bimolecular level, prostaglandins, growth factors, and cytokines are

Table 1 Evaluation of the effects of light and heavy orthopaedic forces on tooth movement in group II (surgically assisted rapid palatal expansions) at baseline (S1), 4 days after S1, initial expansion (O1), and following 24 hours of expansion (O2).

	S1	S2	*P*	O1	*P*	*P*	O2	*P*	*P*
			S1–S2		S1–O1	S2–O1		S1–O2	S2–O2
Group II ($n = 10$)	40.56 ± 2.92	59.11 ± 4.76	0.000	39.11 ± 1.27	0.165	0.000*	67.67 ± 4.74	0.000*	0.002*

Significance level $\alpha = 0.05$. Values are mean ± standard deviation.
*$P < 0.05$.

Table 2 Comparison of Prostaglandin E_2 levels (picogram/microlitre) at 24 hours (O2), 7 days (O3), 14 days (O4), 21 days (O5), and 28 days (O6) with O1 (initial samples).

Groups	O1	O2	*P*	O3	*P*	O4	*P*	O5	*P*	O6	*P*
			O1–O2		O1–O3		O1–O4		O1–O5		O1–O6
Group I ($n = 10$)	38.22 ± 2.54	45.89 ± 2.32	0.000*	47.89 ± 1.69	0.000*	50.2 ± 2.59	0.000*	44.22 ± 2.54	0.000*	39.56 ± 1.51	0.231
Control ($n = 10$)	37.67 ± 2.24	37.89 ± 2.32	0.594	37.78 ± 2.49	0.865	38.56 ± 3.09	0.086	38.00 ± 3.16	0.500	38.56 ± 2.30	0.069
Group II ($n = 10$)	39.78 ± 1.22	67.67 ± 1.72	0.000*	80.56 ± 4.22	0.000*	90.00 ± 3.77	0.000*	78.67 ± 6.40	0.000*	93.11 ± 3.86	0.000*

Significance level $\alpha = 0.05$. Values are the mean ± standard deviation.
*$P < 0.05$.

Table 3 Statistical evaluation of Prostaglandin E_2 (picogram/microlitre) levels between the transpalatal distraction (group I), surgically assisted rapid palatal expansion (group II), and controls at 24 hours (O2), 7 days (O3), 14 days (O4), 21 days (O5), and 28 days (O6).

Groups	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*
	O2–O3	O2–O4	O2–O5	O2–O6	O3–O4	O3–O5	O3–O6	O4–O5	O4–O6	O5–O6
Group I ($n = 10$)	0.002*	0.000*	0.024	0.000*	0.006*	0.000*	0.000*	0.000*	0.000*	0.000*
Control ($n = 10$)	0.886	0.347	0.834	0.111	0.452	0.791	0.288	0.366	1.	0.302
Group II ($n = 10$)	0.000*	0.000*	0.000*	0.000*	0.000*	0.239*	0.000*	0.000*	0.012*	0.000*

Significance level $\alpha = 0.05$. Values are the mean ± standard deviation.
*$P < 0.05$.

Table 4 Intergroup comparisons of Prostaglandin E_2 (picogram/microlitre) levels at the different time points for the transpalatol distraction (group I), surgically assisted rapid palatal expansion (group II), and the controls at initial expansion (01) and at 24 hours (02), 7 days (03), 14 days (04), 21 days (05), and 28 days (06) after expansion.

Time	*P*	*P*	*P*
	Group I–control ($n = 10$)	Group I–group II ($n = 10$)	Group II–control ($n = 10$)
O1	0.754	0.117	0.057
O2	0.000*	0.000*	0.000*
O3	0.000*	0.000*	0.000*
O4	0.000*	0.001*	0.000*
O5	0.001*	0.000*	0.000*
O6	0.445	0.000*	0.000*

Significance level $\alpha = 0.05$. Values are the mean ± standard deviation
*$P < 0.05$.

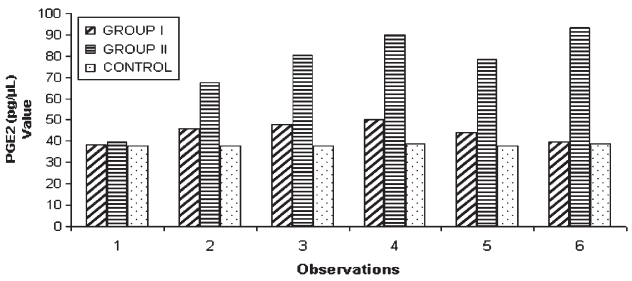

Figure 1 Prostaglandin E_2 (PGE_2; picogram/microlitre) levels in group I (transpalatal distraction), group II (surgically assisted rapid palatal expansion), and the controls at the different observation periods.

released from periodontal ligament cells (Sandy *et al.*, 1993). Previous studies have shown that when an orthodontic force is applied to periodontal tissues, inflammatory cytokines and prostaglandins are expressed (Saito *et al.*, 1991; Grieve *et al.*, 1994; Sarı *et al.*, 2004). In this study, PGE_2 levels in GCF showed changes dependent on the activation periods in both groups.

Maxillary expansion was performed using orthopaedic forces in both groups in the study. It was observed that PGE_2 levels in GCF collected from the first molars of healthy adolescents increased after separator placement (S2) and 24 hours following activation of the Hyrax appliance (O2). These results indicate that either light or heavy forces can induce a biochemical response in the periodontium. The intragroup findings of the present study were similar to those of Tzannetou *et al.* (1999) who evaluated the IL-1β (synergistic with PGE_2) and beta-glucuronidase in GCF around molars during RPE.

Variations in PGE_2 levels in GCF in both study groups were observed. After the initial peaks of PGE_2 at 24 hours, PGE_2 levels were higher compared with baseline values during the following 2 weeks in both groups. PGE_2 levels decreased at the end of the expansion periods. However, the differences between the PGE_2 levels at O2 (24 hours of activation) and O5 (1 week retention) were not significantly different, whereas the differences in the levels of PGE_2 at O6 (2 weeks of retention) and O5 were statistically significant from the PGE_2 levels at O4, in group II. Although a significant decrease in PGE_2 values at O5 and O6 was observed compared with those at O4, PGE_2 levels at O3 and at O4 were maintained at significantly higher levels than those at O2 in group I. Additionally, the PGE_2 values at O6 were not significantly different from the baseline values in group I. The dissipation of fibroblast activation and orthodontic force decay were most likely responsible for this change in PGE_2 in both groups.

The stability of SARPE has been examined in previous studies (Bays and Greco, 1992; Pogrel *et al.*, 1992). Those investigators evaluated the relapse rate of palatal expansion after SARPE and found a mean relapse rate of 8.8 mm at the canines and 7.7 mm at the molars. However, Phillips *et al.* (1992) found that relapse in the second molar region was higher than in the first premolar region. For prevention of relapse, overexpansion in SARPE cases has been recommended (Moss 1968; Atac *et al.*, 2006; Tausche *et al.*, 2007). In the present study, the high values of PGE_2 at O5 and O6 in group II could be attributed to the relapse tendency of the maxillary segments due to continuous fibroblast activation. Although PGE_2 levels at O6 were nearly the same as baseline values in group I, the relapse could be due to tension of the palatal tissues. Contrary to tooth-borne expansion, Kraut (1984) reported that distraction devices can be used with an overcorrection of as little as 0.5–1.5 mm.

Dental tipping with surgically assisted tooth-borne appliances has been shown to be greater than that with bone-borne appliances. Tausche *et al.* (2007) evaluated three-dimensional changes with the Dresden distractor on dental, skeletal, and alveolar structures and found that bone-borne expansion appliances protect teeth by inducing more skeletal and less dental change. With conventional tooth-borne appliances such as the Hyrax that was used in the present study, expansion force was transmitted via anchor teeth to the alveolar bone and thus dental tipping was always greater, or at least equal to, alveolar ridge tipping. On the other hand, skeletal tipping during bone-borne expansion is more than dental tipping as a result of the forces transferred directly to the bone. The higher levels of PGE_2 recorded in group II coincide with the results of Tausche *et al.* (2007). However, following activation of the screws in group I, PGE_2 levels at O2, O3, O4, and O5 were found to be significantly higher than those in the control group. This could be explained by adaptive orthodontic tooth movement during orthopaedic palatal expansion in group I.

When post-expansion PGE_2 level changes were evaluated, group II showed higher PGE_2 levels than group I. This could

be explained by the ongoing bone remodelling with fibroblast activation around the anchor teeth during the 2 week retention phase. This result indicates that both maxillary segments need to be retained to avoid the relapse tendency in SARPE cases.

Conclusion

This study investigated PGE_2 in GCF. Increases in PGE_2 levels were observed in both experimental groups. Both light orthodontic and heavy orthopaedic forces resulted in an increase in PGE_2 levels. This was found to be higher in the SARPE cases than in the TPD cases following activation of the screws and may be due to greater dental effects with the SARPE procedure. The low PGE_2 levels in group I could indicate that using TPD might have prevented unfavourable sequela, such as root resorption, bony dehiscences, and buccal tipping of the anchor teeth.

Further studies, investigating different factors present in GCF, are recommended for a thorough clinical comparison of tooth- and bone-borne expansion appliances.

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References

Adkins M D, Nanda R S, Currier F G 1990 Arch perimeter changes on rapid palatal expansion. American Journal of Orthodontics and Dentofacial Orthopedics 97: 194–199

Atac A A T, Karasu H A, Aytac D 2006 Surgically assisted rapid maxillary expansion compared with orthopedic rapid maxillary expansion. Angle Orthodontist 76: 353–359

Barber A F, Sims M R 1981 Rapid maxillary expansion and external root resorption in man: a scanning electron microscope study. American Journal of Orthodontics 79: 630–652

Bays R A, Greco J M 1992 Surgically assisted rapid palatal expansion: an outpatient technique with long-term stability. Journal of Oral and Maxillofacial Surgery 50: 110–113

Bishara S E, Staley R N 1987 Maxillary expansion: clinical implications. American Journal of Orthodontics and Dentofacial Orthopedics 91: 3–14

Chung C H, Goldman A M 2003 Dental tipping and rotation immediately after surgically assisted rapid palatal expansion. European Journal of Orthodontics 25: 353–358

Cureton S L 1998 A significant transverse discrepancy: a case with a high mandibular plane angle, a severe maxillary arch length deficiency, and significant transverse discrepancy,. American Journal of Orthodontics and Dentofacial Orthopedics 114: 307–310

Grieve W G, Johnson G K, Moore R N, Reinhardt R A 1994 Prostaglandin E (PGE) and interleukin-1β (IL-1β) levels in gingival cervicular fluid during human orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 105: 369–374

Harzer W, Schneider M, Gedrange T, Tausche E 2006 Direct bone placement of the Hyrax fixation screw for surgically assisted rapid palatal expansion (SARPE). Journal of Oral and Maxillofacial Surgery 64: 1313–1317

Koudstaal M J, van der Wal K G, Wolvius E B, Schulten A J 2006 The Rotterdam palatal distractor: introduction of the new bone-borne device and report of the pilot study. International Journal of Oral and Maxillofacial Surgery 35: 31–35

Kraut R A 1984 Surgically assisted rapid maxillary expansion by opening the midpalatal suture. Journal of Oral and Maxillofacial Surgery 42: 651–655

Lanigan D, Mintz M S 2002 Complications of surgically assisted rapid palatal expansion: review of the literature and report of a case. Journal of Oral and Maxillofacial Surgery 60: 104–110

Mommaerts M Y 1999 Transpalatal distraction as a method of maxillary expansion. British Journal of Oral and Maxillofacial Surgery 37: 268–272

Moss J P 1968 Rapid expansion of the maxillary arch. II. Indications for rapid expansion. The Journal of Practical Orthodontics 2: 215–223

Norrdin R W, Jee W S S, High W B 1990 The role of prostaglandins in bone *in vivo*. Prostaglandins, Leukotrienes, and Essential Fatty Acids 41: 139–149

Phillips C, Medland W H, Fields Jr H W, Proffit W R, White Jr R P 1992 Stability of surgical maxillary expansion. International Journal of Adult Orthodontics and Orthognathic Surgery 7: 139–146

Pinto P, Mommaerts M, Wreakes G, Jacobs W 2001 Immediate post expansion changes following the use of the transpalatal distractor. Journal of Oral and Maxillofacial Surgery 59: 994–1000

Pogrel M A, Kaban B, Vargervik K, Baumrind S 1992 Surgically assisted rapid maxillary expansion in adults. International Journal of Adult Orthodontics and Orthognathic Surgery 7: 37–41

Proffit W, Fields H W, Ackerman J, Thomas P, Tulloch J F 1986 The biological basis of orthodontic therapy. In: Rudolph P (ed.). Contemporary orthodontics, 2nd edn. Mosby, St Louis, pp. 296–325

Rossi M, Whitcomb S, Lindemann R 1996 Interleukin-1β and tumor necrosis factor- α production by human monocytes cultured with L-thyroxine and thyrocalcitonin: relation to severe root shortening. American Journal of Orthodontics and Dentofacial Orthopedics 110: 399–404

Saito M, Saito S, Ngan P W, Shanfeld J, Davidovitch Z 1991 Interleukin 1 beta and prostaglandin E are involved in the response of periodontal cells to mechanical stress *in vivo* and *in vitro*. American Journal of Orthodontics and Dentofacial Orthopedics 99: 226–240

Sandy J R, Farndale R W, Meikle M C 1993 Recent advances in understanding mechanically induced bone remodeling and their relevance to orthodontic theory and practice. American Journal of Orthodontics and Dentofacial Orthopedics 103: 212–222

Sarı E, Olmez H, Gürton Ü 2004 Comparison of some effects of acetylsalicylic acid and rofecoxib during orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 125: 310–315

Sarı E, Uçar C, Ceylanoglu C 2007 Transpalatal distraction in a patient with a narrow maxilla. Angle Orthodontist 77: 1126–1131

Tausche E, Hansen L, Hietschold V, Lagravère M O, Harzer W 2007 Three-dimensional evaluation of surgically assisted implant bone-borne rapid maxillary expansion: a pilot study. American Journal of Orthodontics and Dentofacial Orthopedics 131: 92–99

Tzannetou S, Efstratiadis S, Nicolay O, Grbic J, Lamster I 1999 Interleukin-1 beta and beta-glucuronidase in gingival crevicular fluid from molars during rapid palatal expansion. American Journal of Orthodontics and Dentofacial Orthopedics 115: 686–696

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Maintenance of a deep bite prior to surgical mandibular advancement

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SUMMARY Adult patients with a Class II skeletal base are often treated by a combined orthodontic and surgical approach. Advancement of the mandible, most often including a bilateral sagittal split osteotomy (BSSO), is preceded by orthodontic alignment and frequently the curve of Spee is levelled. When the chin is prominent, there is a risk of accentuating this as a result of surgery. An option to prevent this is to maintain a deep curve of Spee before surgical advancement. This will result in an opening rotation of the mandible during surgery and thus, a less prominent chin.

The aim of this study was to compare, retrospectively, two orthodontic treatment approaches in patients treated by a BSSO. In one group (4 males, 20 females; mean age pre-surgery 29.3 years), the deep bite was maintained (deep bite group) while in the other (3 males, 10 females; mean age pre-surgery 27.1 years) the overbite was normal prior to surgery (level group). Lateral skull radiographs were taken before orthodontic treatment (T0), prior to surgery (T1), and at the end of treatment (T2). Differences between the groups as measured on lateral skull radiographs at T1 and T2 were analysed and quantified using an independent *t*-test.

The results showed that soft tissue pogonion moved significantly further forward in the level than in the deep bite group ($P < 0.05$). Lower anterior face height and the cranial base-mandibular plane angle increased more in the deep bite than in the level group ($P < 0.05$ and $P = 0.001$, respectively).

The maintenance of a deep bite prior to mandibular advancement surgery induces an opening rotation of the mandible reducing chin prominence and increasing lower anterior face height post-surgically.

Introduction

In adult patients with skeletal Class II anomalies, a combined orthodontic and surgical approach is often necessary to obtain a satisfactory treatment outcome. The combined aim of the orthodontist and surgeon is to establish a good dental occlusion, optimal function, and harmonious facial aesthetics. Generally, the dentition is aligned prior to mandibular advancement and the surgical procedure to advance the mandible is most frequently a bilateral sagittal split osteotomy (BSSO). The post-surgical chin position is an important factor to consider in treatment planning. If the dentition is levelled in the pre-surgical orthodontic phase, the mandible will merely move horizontally during surgery, guided by the occlusal plane. This moves the chin point maximally forward, which is undesirable in patients with an already prominent chin. There is a risk of accentuating a prominent chin, which may necessitate further camouflage surgery, such as a reduction genioplasty or a Le Fort I osteotomy with dorsal impaction (Jacobs and Sinclair, 1983; Poulton and Ware, 1985; McCollum *et al.*, 1989). Maintaining a deep curve of Spee in the pre-surgical orthodontic phase has been suggested in order to prevent the chin from being brought further forward (Zetz *et al.*, 1984; Tuinzing *et al.*, 1989, 2005; Mommaerts *et al.*, 2004). The possible influence of this procedure on chin prominence and lower profile changes has not previously been determined. Therefore, the aim of the present study was to quantify the differences in chin point prominence and lower anterior face height after pre-surgical levelling of the bite (creating an overbite less than or equal to 3 mm) as opposed to maintaining a deep overbite (greater than 3 mm) prior to BSSO.

Subjects and methods

Subjects

The records of all skeletal Class II patients who had undergone orthodontic treatment combined with orthognathic surgery from November 1992 until November 2002 were assessed. All were treated orthodontically by one orthodontist (MWJB) and underwent surgery in the Oral and Maxillofacial Surgery Department of the University Medical Center Groningen.

Patient records were included according to the following inclusion criteria: skeletal Class II anomaly; (non-growing) adult patient; orthodontic treatment followed by BSSO and no other surgical interventions; lateral skull radiographs with clear soft tissue representation available before

orthodontic treatment (T0), prior to surgery (T1), and at the end of treatment (T2); availability of orthodontic treatment records; and BSSO preferably performed by same surgeon (JJ).

From a total of 190 Class II combined orthodontic surgery-treated cases, 37 subjects (7 males, 30 females) met the inclusion criteria. The radiographs taken at T0 were used to confirm Class II severity.

Two groups were formed on the basis of the depth of the vertical overbite on the tracing at T1. When the overbite was equal to or less than 3 mm at T1, the patients were assigned to the 'level' group and those with a vertical overbite of more than 3 mm to the 'deep' bite group.

The resulting study sample consisted of 24 subjects (4 males, 20 females) with a deep bite that was maintained during surgery and 13 subjects (3 males, 10 females) with a normal overbite prior to surgery. The mean age at T1 in the deep bite group was 29.3 years (range 15.8–53.0 years) and 27.1 years (range 17.2–37.1 years) in the level group. The radiographs at T2 were taken an average of 18.0 months (range 4–63 months) after T1 in the deep bite group and after 11.6 months (range 5–22 months) in the level group.

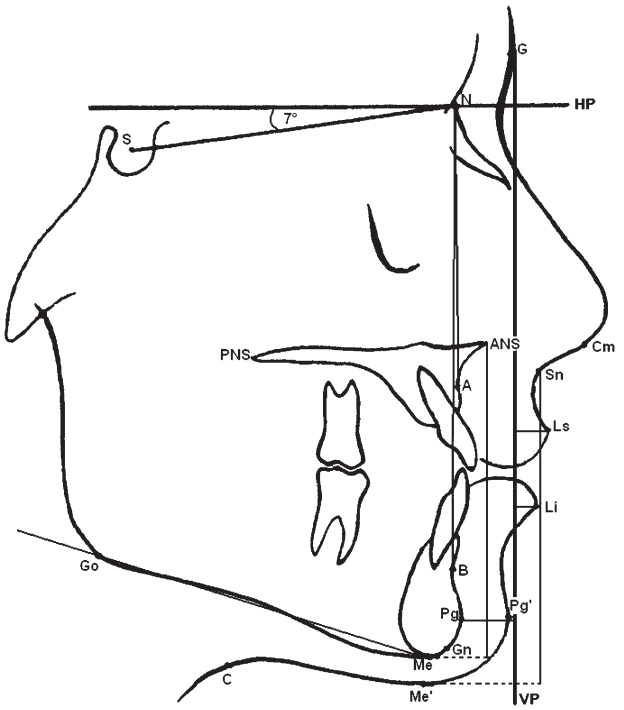

Figure 1 Landmarks used in the study and measurements. Lanmarks: A, point-A; ANS, anterior nasal spine; B, point-B; Cm, columella; G, glabella; Gn, gnathion; Go, gonion; HP, horizontal plane; 7 degrees from SN-line through nasion (Legan and Burstone, 1980); Li, labrale inferior; Ls, labrale superior; Me, menton; Me', soft tissue menton; N, nasion; Pg, pogonion; Pg', soft tissue pogonion; PNS, posterior nasal spine; S, sella; Sn, subnasale; VP, vertical plane; perpendicular to HP through glabella; (Legan and Burstone, 1980). Measurements: SNB (°), antero-posterior position of the mandible; ANB (°), sagittal relationship maxilla/mandible; Wits (mm), sagittal relationship maxilla/mandible to the occlusal plane; Overjet (mm), distance of the upper incisor edge to the lower incisor buccal surface; Overbite (mm), distance of the upper incisor edge to the lower buccal incisor edge; SN/GoGn (°), vertical relationship cranial base/mandibular plane; PP/GoGn (°), vertical relationship between the spinal plane/mandibular plane; APFH index, anterior posterior face height ratio(S–Go/N–Me); AFH index, anterior face height index parallel to VP(ANS–Me/N–Me // VP); LAFH, lower anterior face height parallel to the VP [ANS–Me // VP (mm)]; stLAFH, soft tissue lower anterior face height parallel to the VP [Sn–Me' // VP (mm)]; Pg–VP // HP (mm), chin protrusion parallel to the HP; Pg'–VP // Hp (mm), soft tissue chin protrusion parallel to the HP; Ls–VP // HP (mm), upper lip protrusion parallel to the HP (UL–VP); Li–VP // HP (mm), lower lip protrusion parallel to the HP (LL–VP).

Cephalometric analysis

Conventional radiographs taken at T0, T1, and T2 of all patients were scanned with a digital scanner (Canon Epson Expression 1680 pro; Seiko Epson Corp., Nagano-Ken, Japan). Registration was performed in Viewbox (Version 3.1.1.7© dHal Software, Kifissia, Greece). Tracings were made on the T1 and T2 lateral skull radiographs by one examiner (FOdC) in a darkened room.

The measurements were performed using the reference grid from the soft tissue analysis of Legan and Burstone (1980). For this analysis, a horizontal plane (HP) was constructed by drawing a line through nasion 7 degrees from the sella–nasion line. Perpendicular to this horizontal line a vertical reference line (VP) was constructed through glabella. The landmarks and distances used in this study as well as the angular and linear measurements are presented in Figure 1. For superimposition, both radiographs (T1 and T2) were orientated with maximum coincidence of the cribriform plate, trabecular pattern of the superior portion of the ethmoid bone, and the lower portion and anterior wall of the sella turcica. Nasion and sella points were then transferred from the T1 radiograph to the T2 tracing. In this manner, the HP/VP reference grid that was created was identical on the T1 and T2 tracings. Structure-based differences caused by treatment and/or growth could be measured and described with reference to this grid. Linear distances were measured either parallel to the horizontal plane or parallel to the vertical plane so treatment changes were represented as a horizontal or vertical vector. The mean treatment change of the variables was calculated by subtracting the outcomes at T2 from T1.

Reliability

To determine intraexaminer reliability, 10 randomly chosen radiographs from the sample were retraced by the same examiner after a 6 week interval.

Statistical analysis

For reliability testing, a paired *t*-test was used to analyse the difference between measurements using the Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois, USA version 12). Group consistency at T1 was assessed by applying an independent *t*-test to all variables and comparing the deep bite with the level group. Comparison of changes

during treatment between the groups (T2–T1) were likewise analysed using an independent *t*-test.

Results

Reliability

There were no significant differences between the two tracings ($P > 0.05$). The standard error of the mean ranged from 0.12 mm for overjet to 0.33 mm for Wits (Houston, 1983).

Cephalometric analysis

At T1, there was a significant difference in overbite ($P < 0.001$) between the deep bite and the level groups (Table 1). Likewise, there were significant differences in lower anterior face height (LAFH; $P < 0.01$), soft tissue LAFH ($P < 0.01$), and anterior face height index ($P < 0.05$).

Changes between T1 and T2 in the deep bite group were compared with changes in the level group (Table 2). The change in overbite was significantly different ($P < 0.001$) between the groups. In the deep bite group, there was a 3.2 mm reduction compared with a 0.2 mm reduction in the level group. Soft tissue pogonion moved 2 mm further forward in the level group than in the deep bite group ($P < 0.05$). Hard tissue pogonion also moved 2 mm further forward but this was not significantly significant ($P = 0.07$). The angle formed between sella–nasion and the mandibular plane angle (SN–GoGn) increased 2.1 degrees more ($P = 0.001$) and the palatal plane–mandibular plane (PP–GoGn) angle increased 1.9 degrees more in the deep bite group ($P = 0.001$). The anterior to posterior face height ratio decreased significantly more in the deep bite group ($P < 0.01$). The mean LAFH increased by 2.2 mm in the level group while this was 3.7 mm in the deep bite group ($P < 0.05$).

No significant differences could be found in sagittal movement of the upper or lower lips.

Table 1 Pre-surgery differences between the groups.

Measurement	Deep bite (SD) $n = 24$	Level bite (SD) $n = 13$	Significance
SNB (°)	75.2 (±3.3)	73.7 (±5.3)	ns
ANB (°)	6.5 (±2.6)	7.6 (±2.3)	ns
Wits (mm)	8.0 (±3.0)	8.0 (±4.1)	ns
Overjet (mm)	8.4 (±1.6)	7.9 (±2.9)	ns
Overbite (mm)	6.3 (±1.6)	1.5 (±1.1)	$P < 0.001$
SN/GoGn (°)	29.6 (±5.7)	34.1 (±10.9)	ns
PP/GoGn (°)	22.8 (±4.9)	28.9 (±10.1)	ns
APFH index	67.3 (±5.0)	64.9 (±8.9)	ns
AFH index	56.1 (±2.3)	58.3 (±1.6)	$P < 0.05$
LAFH (mm)	67.8 (±4.9)	73.1 (±3.4)	$P < 0.01$
stLAFH (mm)	67.2 (±5.1)	72.0 (±3.0)	$P < 0.01$
Pg–VP (mm)	−11.2 (±7.1)	−17.0 (±13.2)	ns
Pg'–VP (mm)	1.6 (±7.3)	−3.6 (±12.3)	ns

ns, non-significant.

Table 2 Treatment change differences between completion of treatment (T2) and pre-surgery (T1) in the level and deep bite group.

Difference T2–T1	Group	Mean (±SD)	Significance
SNB (°)	Level	2.7 (1.3)	ns
	Deep	2.6 (0.9)	
ANB (°)	Level	−2.7 (1.3)	ns
	Deep	−2.6 (1.1)	
Wits (mm)	Level	−5.1 (2.9)	ns
	Deep	−6.2 (1.5)	
Overjet (mm)	Level	−4.8 (3.0)	ns
	Deep	−5.0 (1.8)	
Overbite (mm)	Level	−0.2 (1.4)	$P < 0.001$
	Deep	−3.2 (2.5)	
SN/GoGn (°)	Level	0.9 (0.9)	$P = 0.001$
	Deep	3.0 (1.9)	
PP/GoGn (°)	Level	1.2 (0.9)	$P = 0.001$
	Deep	3.1(1.9)	
APFH index	Level	−1.9 (1.2)	$P < 0.01$
	Deep	−3.3 (1.6)	
AFH index	Level	0.8 (0.4)	$P < 0.05$
	Deep	1.3 (0.8)	
LAFH (mm)	Level	2.2 (1.5)	$P < 0.05$
	Deep	3.7 (2.2)	
stLAFH (mm)	Level	2.4 (1.5)	$P = 0.05$
	Deep	3.8 (2.1)	
Pg–VP (mm)	Level	−4.7 (2.4)	ns
	Deep	−3.1 (2.5)	
Pg'–VP (mm)	Level	−5.2 (3.1)	$P < 0.05$
	Deep	−3.2 (2.5)	
UL–VP (mm)	Level	0.1 (1.2)	ns
	Deep	0.3 (1.2)	
UL–VP (mm)	Level	3.2 (3.0)	ns
	Deep	2.3 (2.1)	

ns, non-significant.

Discussion

Maintenance of a deep curve of Spee is a common procedure performed in order to prevent the chin from being brought too far forward in patients treated by a BSSO (Zetz *et al.*, 1984; Tuinzing *et al.*, 1989, 2005; Mommaerts *et al.*, 2004).

The results of the current study confirmed that after surgery and completion of treatment, there was less forward movement of the chin when a deep bite was maintained prior to BSSO compared with a normal overbite. Additionally, the deep bite group demonstrated a significant increase in LAFH and a more marked opening rotation of the mandible. The explanation for this phenomenon lies in the fact that when the deep bite is maintained during orthodontic therapy, the mandible will not only move forward during surgery but, because of the deep bite, will also rotate downwards and backwards (Rubenstein *et al.*, 1991). Following surgery, the dentition will only have contact in the incisor region and at the second molars. The open bite in the premolar region is then closed within a few months using vertical elastic traction (Zetz *et al.*, 1984).

Several studies have investigated cephalometric changes after BSSO and found an increase in LAFH in patients with a low mandibular plane angle (Mobarak *et al.*, 2001; Berger *et al.*, 2005). Additionally, compared with medium and high angle cases, less forward movement of the chin point was found (Mobarak *et al.*, 2001). The pre-surgical orthodontic techniques, however, were not specified in these studies.

A significant drawback of the present investigation is that due to differences in magnification, pre-treatment (T0) lateral skull radiographs could not be used to perform linear cephalometric measurements. Therefore, changes occurring during the pre-surgical orthodontic phase (T0–T1) could not be quantified.

Levelling of the lower curve of Spee with continuous archwire mechanics is known to induce an opening rotation of the mandible as a result of (pre)molar extrusion (Levin, 1991; Parker *et al.*, 1995; Weiland *et al.*, 1996). It therefore may be argued that the opening rotation occurs in the pre-surgical orthodontic phase when the curve of Spee is levelled with continuous archwire mechanics. At the end of treatment, this would result in similar chin prominence and increase in LAFH, irrespective of the pre-surgical orthodontic technique. Further studies, including measurement of cephalometric changes occurring during pre-surgical orthodontic treatment, are needed to confirm the possible beneficial effects of maintaining a deep curve of Spee prior to BSSO on chin prominence and LAFH.

Conclusions

Maintenance of a deep bite prior to surgical mandibular advancement induces an opening rotation of the mandible reducing chin prominence and increasing LAFH post-surgically.

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References

Berger J L, Pangrazio-Kulbersh V, George C, Kaczynski R 2005 Long-term comparison of treatment outcome and stability of Class II patients treated with functional appliances versus bilateral sagittal split ramus osteotomy. American Journal of Orthodontics and Dentofacial Orthopedics 127: 451–464

Houston W J B 1983 The analysis of errors in orthodontic measurements. American Journal of Orthodontics 83: 382–389

Jacobs J D, Sinclair P M 1983 Principles of orthodontic mechanics in orthognathic surgery cases. American Journal of Orthodontics 84: 399–407

Legan H L, Burstone C J 1980 Soft tissue cephalometric analysis for orthognathic surgery. Journal of Oral Surgery 38: 744–751

Levin R I 1991 Deep bite treatment in relation to mandibular growth rotation. European Journal of Orthodontics 13: 86–94

McCollum A G H, Reyneke J P, Wolford L M 1989 An alternative for the correction of the Class II low mandibular plane angle. Oral Surgery, Oral Medicine, and Oral Pathology 67: 231–241

Mobarak K A, Espeland L, Krogstad O, Lyberg T 2001 Soft tissue profile changes following mandibular advancement surgery: predictability and long-term outcome. American Journal of Orthodontics and Dentofacial Orthopedics 119: 353–367

Mommaerts M Y, Shteif M, Hendrickx K, Laster Z 2004 Surgical options in deep-bite mandibular deficiency with prominent chin—aesthetic considerations. Journal of Cranio-Maxillo-Facial Surgery 32: 112–118

Parker C D, Nanda R S, Currier G F 1995 Skeletal and dental changes associated with the treatment of deep bite malocclusion. American Journal of Orthodontics and Dentofacial Orthopedics 107: 382–393

Poulton R P, Ware W H 1985 Increase in mandibular and chin projection with orthognathic surgery. American Journal of Orthodontics 87: 363–376

Rubenstein L K, Strauss R A, Isaacson R J, Lindauer S J 1991 Quantitation of rotational movements associated with surgical mandibular advancement. Angle Orthodontist 61: 167–173

Tuinzing D B, Greebe R, Dorenbos J 1989 Correction of deep overbite. A modified splint permitting rapid extrusion of posterior teeth. Journal of Cranio-Maxillo-Facial Surgery 17: 172–174

Tuinzing D B, Greebe R, Dorenbos J, Becking A 2005 Surgical orthodontics. Classification, diagnosis and treatment. Chapter 5. Treatment modalities. Elsevier, Maarssen, pp. 105–138

Weiland F J, Bantleon H P, Droschl H 1996 Evaluation of continuous arch and segmented arch leveling techniques in adult patients—a clinical study. American Journal of Orthodontics and Dentofacial Orthopedics 110: 647–652

Zetz M R, Dean C D, Burris A G, Griffith A R 1984 Correction of skeletal Class II malocclusions with deep bite by rapid extrusion of posterior teeth. Oral Surgery, Oral Medicine, and Oral Pathology 58: 631–636

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Treatment and post-treatment effects of facemask therapy on the sagittal pharyngeal dimensions in Class III subjects

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SUMMARY The purpose of this cephalometric study was to analyse the treatment and post-treatment craniofacial effects of a facemask (FM) combined with a bite block (BB) with specific regard to the sagittal pharyngeal dimensions in subjects with a Class III malocclusion when compared with an untreated Class III control group. The FM/BB group (22 subjects, 12 females and 10 males) had a mean age pre-treatment (T1) of 8.9 ± 1.5 years, at the end of active treatment (T2) of 10.5 ± 1.3 years, and post-treatment (T3) of 12.6 ± 1.9 years. The treated group was compared with a control group of 14 subjects (6 females and 8 males) with untreated Class III malocclusions that matched the FM/BB group as to age at T1, T2, and T3, observation periods and skeletal maturation. Comparisons of the T2–T1 and T3–T1 changes between the two groups were analysed with the Mann–Whitney test.

Significant favourable skeletal changes in the maxilla and mandible were observed in the treated group both after Ts2 and T3. No significant short- or long-term changes in the sagittal oropharyngeal and nasopharyngeal airway dimensions were induced by maxillary protraction in subjects with a Class III malocclusion when compared with untreated controls.

Introduction

Orthopaedic treatment of a Class III malocclusion with a facemask (FM) is able to produce favourable changes in growing subjects by both enhancement of maxillary growth and restraint and/or redirection of mandibular growth (De Toffol *et al.*, 2008). The use of lateral cephalograms appears adequate for the investigation of sagittal changes in the pharyngeal dimensions (Hiyama *et al.*, 2002; Sayinsu *et al.*, 2006; Kilinç *et al.*, 2008; Oktay and Ulukaya, 2008).

While some authors (Sayinsu *et al.*, 2006; Kilinç *et al.*, 2008) used a FM in combination with rapid maxillary expansion, Hiyama *et al.* (2002) and Oktay and Ulukaya (2008) treated Class III patients by means of maxillary protraction only. Significant changes of both oropharyngeal and nasopharyngeal dimensions have been reported following FM therapy (Kilinç *et al.*, 2008; Oktay and Ulukaya, 2008), while Hiyama *et al.* (2002) did not assess changes in the airway dimensions and Sayinsu *et al.* (2006) found significant changes only for nasopharyngeal size. The major limitation of all these studies is the lack of untreated controls with Class III malocclusions (with one exception; Kilinç *et al.*, 2008) and the short-term nature of the observations.

The aim of the present study was to evaluate the craniofacial changes induced by a FM combined with a bite block (BB) with special regard to the oro- and nasopharyngeal sagittal airway dimensions in subjects with dentoskeletal Class III malocclusions when compared with an untreated Class III control group immediately after therapy and at post-treatment observation.

Subjects and methods

Subjects

The treated group comprised 22 subjects (12 females and 10 males) with a Class III malocclusion, who were treated consecutively with an FM combined with a lower removable BB appliance by a single operator (PC) at the Department of Orthodontics, University of Rome 'Tor Vergata'. Lateral cephalograms were taken before treatment (T1), at the end of active treatment (T2), and at an average interval after the completion of FM/BB therapy of approximately 2 years (about 42 months after the initiation of treatment; T3). The average age of the FM/BB group was 8.9 ± 1.5 years at T1, 10.5 ± 1.3 years at T2, and 12.6 ± 1.9 years at T3. At T1, all patients had a Class III malocclusion in the mixed dentition characterized by Wits appraisal of −2 mm or less, an anterior crossbite or incisor end-to-end relationship, and a Class III molar relationship.

The patients were instructed to wear the FM at least 14 hours per day. The FM was attached to a double-arch structure cemented to the upper first molars. Forces of 600 g were used during protraction therapy. Cooperation was good for all patients. During FM treatment, a removable BB appliance was used full-time. The BB appliance was constructed in the form of a Schwarz plate for the lower arch with a vestibular arch, occlusal resin splints, and an expansion screw that was activated when needed. The splints were used to control molar eruption, limit intermaxillary divergence, and prevent clockwise mandibular rotation. The patients were instructed to wear the BB 24 hours a day, including during meals; cooperation was good for all subjects.

All patients were treated at least to a positive dental overjet before discontinuing treatment; most patients were overcorrected towards a Class II occlusal relationship (Westwood *et al.*, 2003). The T1−T2 interval comprised active therapy followed by 6–9 months with a Hawley retainer in the maxillary arch, whereas no appliance was worn during the post-treatment period (T2−T3).

The treated group was compared with an untreated group of 14 subjects (6 females and 8 males) with a Class III malocclusion selected from the records at the Department of Orthodontics, University of Florence. The average age was 7.6 ± 1.4 years at T1, 9.8 ± 1.9 years at T2, and 11.9 ± 1.2 years at T3. All the treated and control subjects showed a prepubertal stage of skeletal growth (CS 1; Baccetti *et al.*, 2005) at T1 and a post-pubertal stage (CS 4, CS 5, or CS 6) at T3.

Cephalometric analysis

A customized digitization regimen and analysis were used for all cephalograms examined in this study. Before the cephalometric analysis, the intraobserver measurement error was evaluated. Fifteen lateral cephalograms, selected from various subjects in the study, were traced and measured twice within a week by the same operator (MM). The measurements at both times for each patient were analysed with the intraclass coefficient correlation, which varied between 0.966 and 0.995. These values indicated a high level of intraobserver agreement. Lateral cephalograms for each subject in both the treatment and the control groups at T1, T2, and T3 were taken using a standardized radiographic protocol, with an 8 per cent magnification factor.

Cephalograms were traced for each subject at the three time points, and the following variables were measured:

1. Cranial flexure: NSBa angle;
2. Maxillary skeletal: A to nasion perp (point A to a line drawn perpendicular to Frankfort horizontal from nasion), Co–A;
3. Mandibular skeletal: Pg to nasion perp (point Pg to a line drawn perpendicular to Frankfort horizontal from nasion), Co–Go, Co–Gn;
4. Sagittal skeletal: Wits appraisal (distance between the two points of intersection of the two perpendicular lines from points A and B to the functional occlusal plane) and maxillo–mandibular difference (difference between Co–Gn and Co–A);
5. Vertical skeletal: palatal plane to mandibular plane angle, gonial angle (Ar–Go–Me angle).

Specific variables to evaluate the sagittal nasopharyngeal and oropharyngeal airway dimensions were chosen according to the definitions of McNamara (1984) and Martin *et al.* (2006; Figure 1).

The method error for all the cephalometric variables assessed on 20 sets of repeated measurements was calculated by means of Dahlberg's (1940) formula. The error for linear measurements ranged from 0.1 to 0.3 mm, while for angular measurements it varied by 0.2–0.4 degrees.

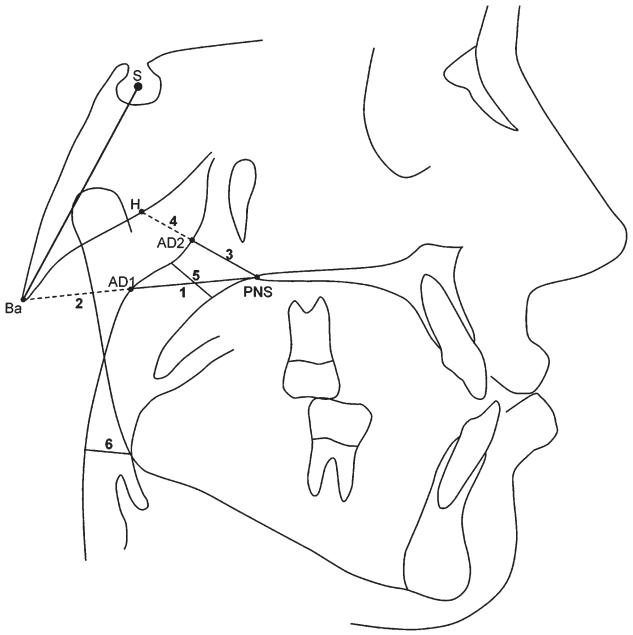

Figure 1 Cephalometric measurements for the analysis of airway dimensions. (1) PNS-AD1: lower airway thickness; distance between the PNS and the nearest adenoid tissue measured through the PNS-Ba line (AD1). (2) AD1-Ba: lower adenoid thickness; defined as the soft tissue thickness at the posterior nasopharynx wall through the PNS-Ba line. (3) PNS-AD2: upper airway thickness; distance between the PNS and the nearest adenoid tissue measured through a perpendicular line to S–Ba from PNS (AD2). (4) AD2-H: upper adenoid thickness; defined as the soft tissue thickness at the posterior nasopharynx wall through the PNS-H line (H, Hormion, point located at the intersection between the perpendicular line to S–Ba from PNS and the cranial base). (5) McNamara's upper pharynx dimension: the minimum distance between the upper soft palate and the nearest point on the posterior pharynx wall. (6) McNamara's lower pharynx dimension: the minimum distance between the point where the posterior tongue contour crosses the mandible and the nearest point on the posterior pharynx wall.

Statistical analysis

Descriptive statistics were calculated for all the cephalometric measurements in the two groups at T1, for the T2−T1 changes (active treatment changes) and for the T3−T1 changes (treatment and post-treatment changes). The preliminary assessment of sample size revealed that with the number of subjects included in the two groups, the power of the study exceeded 0.80. Shapiro Wilks' test revealed a lack of normal distribution for the data. The following comparisons were, therefore, performed by means of Mann–Whitney tests: comparison of craniofacial starting forms at T1, between the T2−T1 and T3−T1 changes between the treated and the control groups.

Logistic regression on the variables at T1 with T3−T1 change of the upper pharynx as the dependent variable was performed with the aim of identifying T1 predictive variables for individual response to treatment in terms of modification of airway size (method stepwise, with P to enter <0.05 and

P to remove >0.1). All statistical computations were performed with a statistical software (MedCalc 9.6.2.0, MedCalc Software, Mariakerke, Belgium).

Results

No significant differences between the treated and the control groups at T1 were found for any craniofacial variable or airway measurement (Table 1). Descriptive statistics and comparisons of the T2−T1 and T3−T1 changes between treated and untreated control groups are reported in Tables 2 and 3.

During active treatment (T2−T1), the treated group showed significant increments in maxillary skeletal variables, as well as significant improvements in the Wits appraisal and in the Max/Mand difference. A significant increase in the vertical intermaxillary relationships was also found. No statistically significant differences were observed for any of the analysed variables for upper and lower sagittal airway dimensions.

During the overall treatment and post-treatment period (T3−T1), the treated group exhibited a significant increase in A to nasion perp and a significant reduction in mandibular length (Co–Gn). Significant improvements in the Wits appraisal and in the Max/Mand difference were still present. No statistically significant differences were found for the vertical skeletal relationships or for the upper and lower sagittal airway dimensions.

Logistic regression with the T3−T1 change in upper pharynx as the dependent variable (greater than 4.8 mm versus less than 4.8 mm, as 4.8 mm is the average difference in T3−T1 change between treated and control groups, Table 3) on the variables at T1 did not reveal any predictive variable.

Discussion

The present study evaluated the treatment and post-treatment craniofacial changes produced by orthopaedic therapy of Class III malocclusions by means of an FM/BB protocol with special regard to the sagittal oropharyngeal and nasopharyngeal airway dimensions. The literature reports contrasting findings with regard to the possibility of improving the sagittal airway dimension by means of maxillary protraction (Hiyama *et al.*, 2002; Sayinsu *et al.*, 2006; Kilinç *et al.*, 2008; Oktay and Ulukaya, 2008).

The features of the present investigation were represented by:

1. The study evaluated both active and post-treatment outcomes, with the post-treatment observation approximately 2 years after the completion of FM/BB therapy; during the post-treatment period, the patients did not wear any orthodontic appliance.
2. A group of 14 subjects with untreated Class III malocclusions was used as a longitudinal control sample for both active treatment and post-treatment periods.
3. All subjects in both treated and control groups were at a prepubertal stage in skeletal development at initial observation and at post-pubertal stage at the final observation.

Table 1 Descriptive statistics and comparison of starting forms.

Cephalometric measures	Treated group *N* = 22		Control group *N* = 14		Difference	Significant
	Mean	SD	Mean	SD		
Cranial flexure						
NSBa (°)	128.4	5.1	128.0	5.4	0.4	NS
Maxillary skeletal						
A to nasion perpendicular (mm)	−0.8	2.7	−1.3	3.3	0.5	NS
Co–A (mm)	75.2	5.0	74.6	2.8	0.6	NS
Mandibular skeletal						
Pg to nasion perpendicular (mm)	−3.1	5.1	−4.9	5.6	1.8	NS
Co–Gn (mm)	99.7	5.3	98.8	5.3	0.9	NS
Co–Go (mm)	45.3	4.1	45.9	3.2	−0.6	NS
Skeletal difference						
Wits (mm)	−6.2	2.6	−7.2	4.1	1.4	NS
Max/Mand difference (mm)	24.5	2.6	24.1	3.7	0.4	NS
Vertical skeletal						
Palatal plane to mandibular plane (°)	29.6	6.4	30.5	4.0	−0.9	NS
Gonial angle (°)	134.1	6.3	135.5	4.4	−1.4	NS
Airway dimensions						
PNS-AD1 (mm)	20.2	3.2	19.1	4.0	1.1	NS
AD1-Ba (mm)	22.2	4.7	21.3	4.7	0.9	NS
PNS-AD2 (mm)	14.6	2.0	13.1	3.1	1.5	NS
AD2-H (mm)	15.8	3.0	15.1	3.4	0.7	NS
Upper pharynx (mm)	9.8	3.1	9.8	3.3	0.0	NS
Lower pharynx (mm)	15.4	3.3	14.0	4.1	1.4	NS

NS, not significant.

Table 2 Descriptive statistics and comparison of the pre-treatment and end of active treatment (T_1-T_2) changes between the treated and control group.

Cephalometric measures	Treated group $N = 22$		Control group $N = 14$		Difference	Significant
	Mean	SD	Mean	SD		
Cranial flexure						
NSBa (°)	−0.6	3.1	0.5	4.4	−1.1	NS
Maxillary skeletal						
A to nasion perpendicular (mm)	1.3	3.0	−1.2	2.0	2.5	**
Co–A (mm)	5.2	3.9	2.1	2.7	3.1	*
Mandibular skeletal						
Pg to nasion perpendicular (mm)	−1.3	6.1	0.2	4.3	−1.5	NS
Co–Gn (mm)	5.1	4.3	6.2	5.2	−1.1	NS
Co–Go (mm)	2.3	3.1	3.5	3.3	−1.2	NS
Maxillary/mandibular						
Wits (mm)	1.8	3.2	−0.7	3.8	2.5	*
Max/Mand difference (mm)	−0.2	2.2	4.1	3.5	−4.3	**
Vertical skeletal						
Palatal plane to mandibular plane (°)	2.1	2.2	−0.6	1.7	2.7	**
Gonial angle (°)	−0.2	3.9	−1.2	3.4	1.0	NS
Airway dimensions						
PNS-AD1 (mm)	2.8	3.2	3.5	4.7	−0.7	NS
AD1-Ba (mm)	−0.4	2.9	−1.8	4.8	1.4	NS
PNS-AD2 (mm)	3.2	2.8	1.8	3.2	1.4	NS
AD2-H (mm)	0.3	2.4	−0.9	2.2	1.2	NS
Upper pharynx (mm)	2.0	2.7	2.4	3.8	−0.4	NS
Lower pharynx (mm)	0.0	3.5	2.1	5.3	−2.1	NS

NS, not significant. *$P < 0.05$, **$P < 0.01$.

Table 3 Descriptive statistics and comparison of the pre- and post-treatment (T_1-T_3) changes between the treated and control group.

Cephalometric measures	Treated group $N = 22$		Control group $N = 14$		Difference	Significant
	Mean	SD	Mean	SD		
Cranial flexure						
NSBa (°)	0.0	2.3	−1.2	3.1	1.2	NS
Maxillary skeletal						
A to nasion perpendicular (mm)	1.0	3.1	−1.1	2.1	2.1	*
Co–A (mm)	6.7	4.8	4.8	4.1	1.9	NS
Mandibular skeletal						
Pg to nasion perpendicular (mm)	0.9	5.0	2.9	5.1	−2.0	NS
Co–Gn (mm)	9.5	5.4	12.2	5.7	−2.7	*
Co–Go (mm)	4.7	5.3	6.3	4.2	−1.6	NS
Maxillary/mandibular						
WITS (mm)	2.0	3.0	−0.2	3.0	2.2	*
Max/Mand difference (mm)	2.8	3.0	7.4	4.4	−4.6	**
Vertical skeletal						
Palatal plane to mandibular plane plane (°)	0.5	2.3	−0.6	3.3	1.1	NS
Gonial angle (°)	−1.0	4.2	0.4	3.7	−1.4	NS
Airway dimensions						
PNS-AD1 (mm)	5.1	3.1	2.3	6.2	2.8	NS
AD1-Ba (mm)	−1.6	2.6	−0.3	5.6	−1.3	NS
PNS-AD2 (mm)	5.6	2.5	3.6	5.2	2.0	NS
AD2-H (mm)	−1.0	3.7	−1.6	4.1	0.6	NS
Upper pharynx (mm)	4.8	3.1	3.2	5.0	1.6	NS
Lower pharynx (mm)	−0.1	3.8	0.2	3.9	−0.3	NS

NS, not significant. *$P < 0.05$, ** = $P < 0.01$.

The results of the present investigation showed significant favourable effects of FM therapy on the skeletal components of Class III malocclusion, which were limited to the maxilla (2.5 mm improvement for A to nasion perp and 3.1 mm improvement for Co–A) during the active treatment period and were extended to the mandible as well during the overall

treatment and post-treatment period (2.7 mm of reduction in the growth of the mandible along Co–Gn). These changes led to favourable outcomes for both the Wits appraisal (2.2 mm improvement over the controls in the long-term) and the Max/Mand difference (4.6 mm improvement over the controls in the long term). The significant increase in the vertical intermaxillary relationship during the active treatment period (2.7 degrees over the controls) was not present in the long term. It should also be noted that for the majority of craniofacial variables, the standard deviations were rather large compared with the mean changes, thus reflecting a wide range of interindividual variability.

In spite of the favourable skeletal changes in the maxillary bony structures, no significant differences between the treated and control group were observed for any sagittal airway dimension variable. These findings differ from those by Kilinç *et al.* (2008) and Oktay and Ulukaya (2008), who reported that maxillary protraction with (Kilinç *et al.*, 2008) or without (Oktay and Ulukaya, 2008) rapid maxillary expansion induced statistically significant increments in the airway dimensions. It should be emphasized that both these studies were short term in design and that the study of Oktay and Ulukaya (2008) did not include a Class III control group.

Logistic regression was carried out on the variables at T1 with the T3−T1 change in upper pharynx as the dependent variable (greater than 4.8 mm versus less than 4.8 mm, as 4.8 mm was the average difference in T3−T1 change between treated and control groups). This analysis was undertaken because of the great variability in the changes of the airway measurements that suggested the need for identification of better responders to treatment. Statistical evaluation did not reveal any pre-treatment predictive variable for individual changes in the pharyngeal dimension.

In the appraisal of the lack of significant treatment-induced airway modifications, the physiological changes in the lymphoid tissue on the posterior pharyngeal wall should also be considered. Handelman and Osborne (1976) reported that during the pre-school years, the adenoid area increases more than the bony nasopharyngeal area, resulting in a restriction of airway space. Linder-Aronson and Leighton (1983) analysed the development of the posterior nasopharyngeal wall between 3 and 16 years and found that the size of the soft tissue was greater at 5 years; thereafter, a decrease occurred from 6 to 10 years. In agreement with this physiological growth pattern of the oronasal lymphoid tissue. The results of the present study revealed a decrease of the lymphoid tissue on the posterior pharyngeal wall (AD1-Ba and AD2-H) both in the treated and control group during the overall observation period. Even when considering this decrease of pharyngeal lymphoid tissue between 7 and 10 years (an interval in age similar to the one in the present study), therapeutic intervention with maxillary protraction was not able to produce a significant increase in the airway dimensions.

Conclusions

The findings of the present study demonstrated the followings:

1. The FM/BB protocol produced significant favourable changes both in the maxillary and mandibular structures in Class III subjects when compared with untreated controls; these favourable changes were maintained at the post-treatment observation after puberty.
2. No significant changes for the oro- and nasopharyngeal sagittal airway dimensions were induced by FM/BB therapy when compared with untreated Class III subjects.

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References

Baccetti T, Franchi L, McNamara Jr J A 2005 The cervical vertebral maturation (CVM) method for the assessment of optimal treatment timing in dentofacial orthopedics. Seminars in Orthodontics 11: 119–129

Dahlberg G 1940 Statistical methods for medical and biological students. Interscience Publications, New York

De Toffol L, Pavoni C, Baccetti T, Franchi L, Cozza P 2008 Orthopedic treatment outcomes in Class III malocclusion, a systematic review. Angle Orthodontist 78: 561–573

Handelman C S, Osborne G 1976 Growth of the nasopharynx and adenoid development from one to eighteen years. Angle Orthodontist 46: 243–259

Hiyama S *et al.* 2002 Effects of maxillary protraction on craniofacial structures and upper airway dimension. Angle Orthodontist 72: 43–47

Kilinç A S, Arslan S G, Kama J D, Özer T, Dari O 2008 Effects on the sagittal pharyngeal dimensions of protraction and rapid palatal expansion in Class III malocclusion subjects. European Journal of Orthodontics 30: 61–66

Linder-Aronson S, Leighton B C 1983 A longitudinal study of the development of the posterior nasopharyngeal wall between 3 and 16 years of age. European Journal of Orthodontics 5: 47–58

Martin O, Muelas L, Vinas M J 2006 Nasopharyngeal cephalometric study of ideal occlusion. American Journal of Orthodontics and Dentofacial Orthopedics 130: 436.e1–e9

McNamara Jr J A 1984 A method of cephalometric evaluation. American Journal of Orthodontics 86: 449–469

Oktay H, Ulukaya E 2008 Maxillary protraction appliance effect on the size of the upper airway passage. Angle Orthodontist 78: 209–213

Sayinsu K, Isik F, Arun T 2006 Sagittal airway dimension following maxillary protraction: a pilot study. European Journal of Orthodontics 28: 181–189

Westwood P V, McNamara Jr J A, Baccetti T, Franchi L, Sarver D M 2003 Long-term effects of Class III treatment with rapid maxillary expansion and facemask therapy followed by fixed appliances. American Journal of Orthodontics and Dentofacial Orthopedics 123: 306–320