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Evaluation of a Reproduction Technique for the Study of the Enamel Composite/Bracket Base Area

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Abstract. *The objective of the study was to evaluate a reproduction method that would enable the study of the enamel/bracket/composite interface* in vivo*, and consisted of* in vitro *assessment of two different impression materials to compare reproduction of brackets bonded to extracted teeth followed by* in vivo *assessment of the superior material.*

In vitro *standard edgewise brackets were bonded to two extracted teeth and impressions were taken using two different types of low viscosity silicone-based impression materials. A medium viscosity silicone impression material was used to support the original impression. Three impressions of both the gingival and occlusal aspect of the bracket base region were obtained using each of the impression materials. Replicas were then prepared for SEM viewing and these compared to SEMs of the real teeth for reproduction of detail. A 3-point Reproducibility Index was used to compare the SEM photographs of the comparable replicas.*

One impression material was clearly superior to the other and produced an acceptably accurate representation of the true clinical situation in three out of four samples. This material also performed well in the in vivo *situation.*

The technique described is satisfactory for the production and analysis of SEM pictures of the enamel/composite/bracket base interface in vivo.

Index words: Orthodontic Bonding, Replication Techniques, Scanning Electron Microscope.

Introduction

One of the major iatrogenic problems associated with fixed appliance orthodontic treatment is decalcification around the periphery of the bracket (Mitchell, 1992). Decalcification may be manifest as a small white patch or line on the enamel surface, which corresponds to the boundary of the bracket. It is generally agreed the decalcification is related to the presence of certain strains of bacteria that colonize the tooth surface and whose by-products cause loss of mineral from the enamel (Chang *et al*., 1997; Fournier *et al*., 1998). It is also recognized that bacteria will readily colonize the surface of rough materials such as composites (Quirynen *et al*., 1990).

A previous paper has shown that *in vitro* the surface of orthodontic composite adhesive is particularly rough and theoretically would provide the ideal nidus for the initiation bacterial colonization (Oliver and Howe, 1989). Further *in vitro* work has shown that significant growth of bacteria occurs on orthodontic bonding adhesives and this is related to surface roughness (O'Kane *et al*., 1993; Blunden *et al*., 1994). The clinical experience of decalcification around the bracket periphery is, therefore, hardly surprising. In fact, it is more surprising that this phenomenon occurs as infrequently as it does.

To date, there has been no work carried out to examine the actual roughness of the composite surface around the bracket periphery *in vivo*. In part, this may be due to the technical difficulties related to obtaining accurate information on this small area in the mouth.

Direct observation using optical instruments or photography may, with a co-operative patient, be just about possible on maxillary anterior teeth. It will, however, be impossible to perform on posterior teeth.

An indirect technique is therefore necessary to enable the enamel/composite/bracket base area to be studied in detail. In previous studies the use of scanning electron microscopy (SEM) has proven ideal for the *in vitro* assessment of this area, (Oliver and Howe, 1989). A search of the literature reveals that an *in vivo* reproduction technique for specimen production of this nature does not appear to have been attempted before in orthodontics. Other dental disciplines have overcome the problem of accurate reproduction of the oral structures in fine detail by the use of low viscosity impression materials. Replication techniques for the *in vivo* study of tooth surfaces have been investigated by Ekfeldt *et al*. (1985). He carried out a pilot study to screen several combinations of impression materials finding that satisfactory replica techniques for clinical application could readily be found with various combinations of impression and model materials.

In clinical use such impressions are cast in fine stone. Stone is inappropriate for use with the SEM as it cannot withstand the procedures necessary for specimen production. This problem may be overcome by casting using a low viscosity cold cure acrylic resin (Stycast®), which is capable of withstanding the desiccation and vacuum necessary for SEM viewing.

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Thus, there are two stages involved in the preparation of a specimen obtained *in vivo*,

(1) the impression stage;
(2) the casting of the impression.

Loss of detail may occur at both stages and render the eventual SEM photograph useless as a research tool. Replication techniques for the scanning electron microscope have been thoroughly reviewed by Barnes (1978, 1979), who suggested that the quality of resolution is limited by the primary impression, rather than the replica casting medium. High resolution is not required for all dental studies and such replication techniques provide adequate accuracy for viewing up to ×500.

This project has two stages. The first is an *in vitro* study to compare the ability of two different low viscosity silicone-based impression materials to reproduce the enamel/composite/bracket base area. The second is to test the best performing material in the *in vivo* situation.

Materials and Methods

In Vitro Assessment

Standard edgewise brackets were bonded to two extracted, prepared and etched teeth using either a heavily-filled chemically-cured composite adhesive (Concise® 3M, USA) or a light-cured adhesive (Transbond® 3M, USA). These two materials have been shown previously to display different surface roughness characteristics, (O'Kane, 1991). Excess composite was removed from around the periphery of the bracket before the material had set using a probe.

Two different types of low viscosity silicone based impression materials were tested. Material 1 (Extrude® Kerr Corp., USA) was supplied in cartridge form and was dispensed by placing the two cartridges (base and catalyst) in a 'gun'. Mixing was achieved by squeezing the trigger of the gun which propelled the two pastes along a special mixing nozzle. The mixed material was then applied to the bracket periphery directly from the end of the mixing nozzle via a narrow tip.

Material 2 (President Jet® Coltène AG, Switzerland) was supplied, mixed, and applied in a similar manner to Material 1.

A medium viscosity silicone impression material (Provil Soft® Heraeus, Germany) was used to support the original impression and was applied before the original material had reached the full recommended setting time, mimicking standard restorative impression techniques. The complete impression was then carefully removed from the tooth and stored in a plastic bag until ready for pouring using the low viscosity cold cure acrylic.

Three repeat impressions of the gingival aspect and three repeat impressions of the occlusal aspect of the bracket base region were obtained using each of the two impression materials. Thus each tooth had a total of 12 impressions.

The casting of the specimens and preparation for viewing using SEM was carried out by a technician skilled in these tasks (see Appendix for details of the preparation of the specimens).

Relatively low power (between ×25 and ×150) SEM photographs of enamel/composite/bracket base area of the real tooth were developed and printed. These were then used so that, as far as possible, views of the acrylic casts could be obtained of the same area, at similar magnification, and taken from the same perspective as those of the real tooth. It was found that a magnification of ×50 was most suitable for this exercise.

An independent observer was then asked to compare the SEM photograph of the real tooth with an SEM photograph of the acrylic cast of the same area and rate the latter on a 3-point Reproducibility Index scale according to the following criteria: 0 = poor reproduction, little detail visible (Figure 1); 1 = adequate reproduction, moderate detail visible (Figure 2); 2 = good reproduction, fine detail visible (Figure 3).

This procedure was repeated on two separate occasions by the same observer with an interval of 1 week between assessments and no reference to the earlier results.

In Vivo Assessment

A patient was selected for this evaluation of the technique by fulfilment of the following criteria;

(1) Required extraction of four premolars prior to orthodontic treatment.
(2) These premolars were sound, unrestored, and with no macroscopically apparent enamel defects.
(3) The extractions were considered to be straight-forward and were to be carried out within the hospital under local anaesthesia.
(4) Consent obtained.

Procedure

The maxillary and mandibular first premolars were isolated by means of cheek retractors and saliva ejector. The teeth were prepared for etching by prophylaxis using an oil-free paste and rubber cup in a slowly rotating handpiece.

The bonding system used for etching and attachment was Concise®. Each of the teeth were then etched using a gel on the buccal surface for 30 seconds, rinsed for 20 seconds with water from a syringe, and dried.

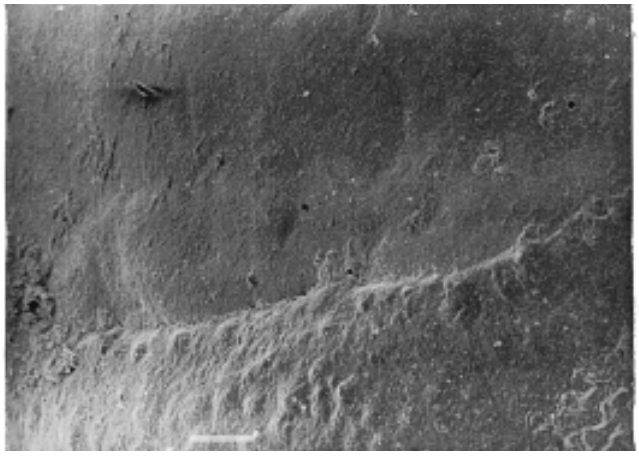

FIG. 1 Scanning electron microscope photograph of replica cast. Score 0—poor reproduction/little visible detail.

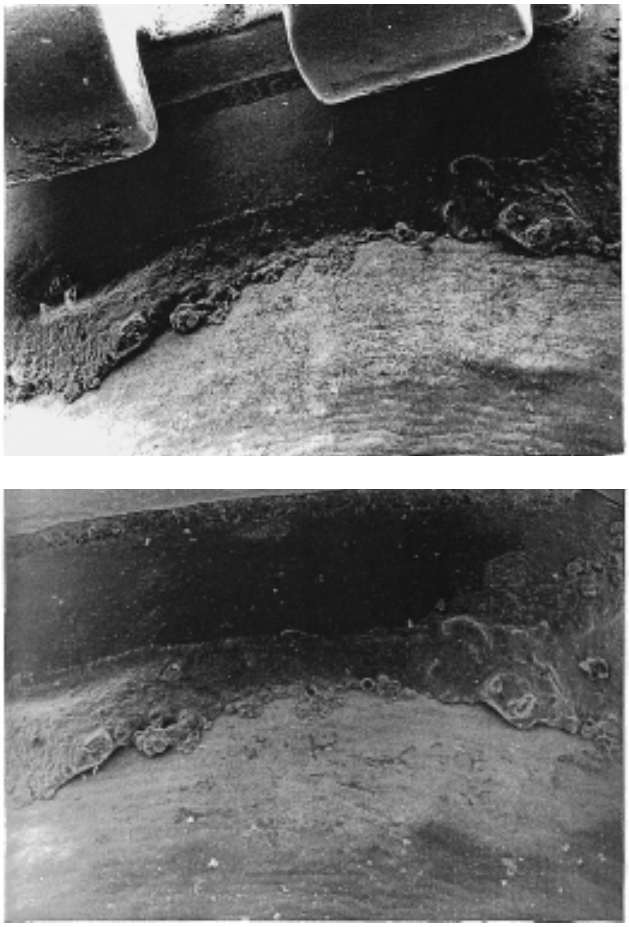

FIG. 2 a) Scanning electron microscope photograph of enamel/composite/bracket interface of extracted tooth. b) SEM photo of replica cast of same area of tooth as in Fig 2a. Score 1—adequate reproduction/moderate detail visible.

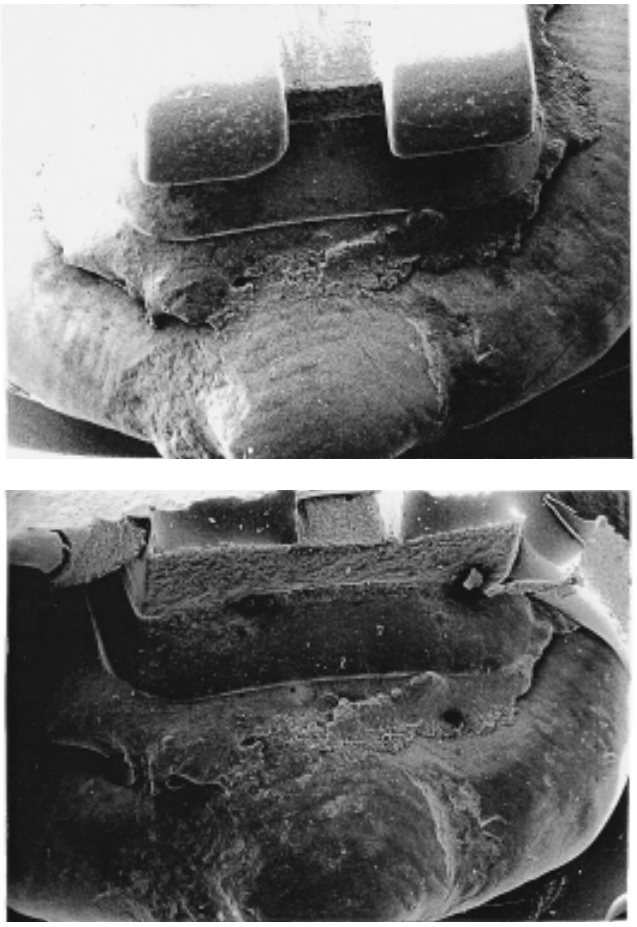

FIG. 3 (a) SEM photo of enamel/composite/bracket interface of extracted tooth. (b) SEM photo of replica cast of same area of tooth as in Fig 3a. Score 2—good reproduction/fine detail visible.

Appropriate pre-adjusted edgewise brackets (Roth prescription, A Company® Johnson Johnson, USA) were then overloaded with the composite (mixed to manufacturer's instructions) and placed on the FA points (Andrews, 1989) of the premolars (as assessed by the operator). The excess uncured composite was then carefully removed from the periphery of the bracket using a dental probe. The composite mixing and bracket loading was carried out by a dental nurse skilled in these procedures.

The full setting time for the composite material, in accordance with manufactures recommendations, were allowed prior to impressions using President Jet®. Models from the impressions were prepared for SEM viewing as described previously. The teeth were extracted carefully under local anaesthesia, using only elevators, by a skilled surgeon. Great care was taken during the extractions to avoid contact and disturbance to the buccal surfaces of the teeth and the brackets by the elevators. The teeth were then prepared for SEM viewing as already described.

The SEM photographs of the real teeth and their reproductions (Figure 4) were then assessed using the 3-point scale described above.

Results

In Vitro

Intra-examiner reproducibility was assessed using the Sign Test. There was good agreement between the two assessments:

Sign Test

Variable 1 = time 1
Variable 2 = time 2
Total no. of readings = 45
No. with variable 2 > 1 5
No. with variable 2 < 1 3
2 = 1 37
$P = <0{\cdot}22$ (not significant).

A bar chart (Figure 4) visually highlights the differences in detail achieved between the impression materials. It can be seen that the combined time 1 and time 2 Reproducibility Index scores for President Jet® were such that approximately 75 per cent of the prepared acrylic replicas showed values of 1 and 2 (moderate/fine detail reproduction). In contrast to this only approximately 25 per cent of the Extrude® samples showed moderate/fine detail reproduction.

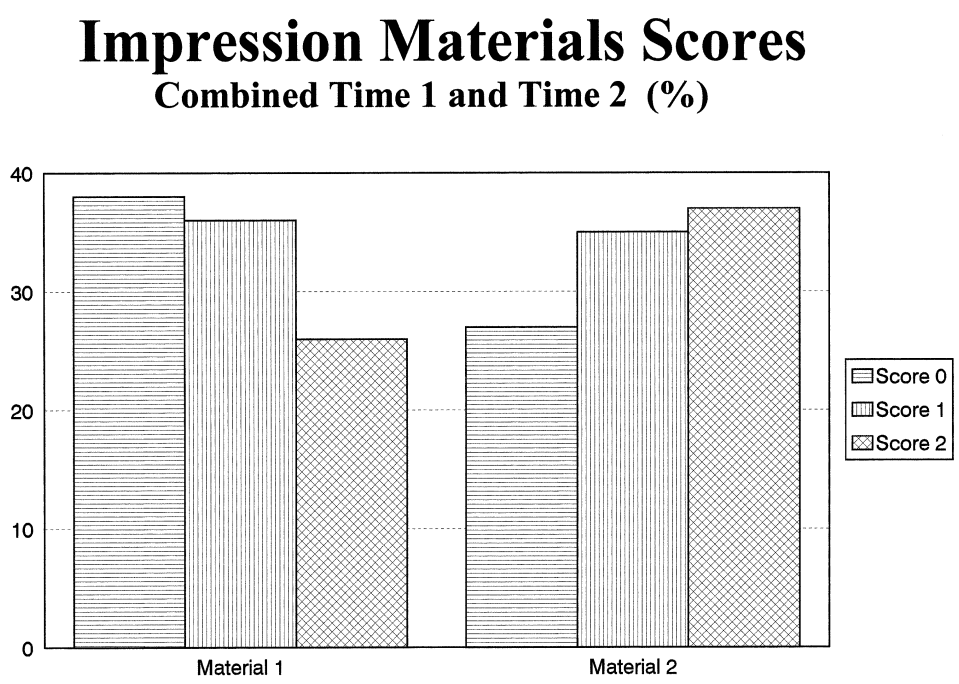

FIG. 4 Bar chart of Reproducibility Index score of the two impression materials for the *in vitro* experiment.

Thus, it would appear that replicas made from impressions using President Jet® are superior to Extrude® and produce an acceptably accurate representation of the true clinical situation in approximately three out of four samples.

In Vivo

Using the 3-point scale described earlier the SEM photographs of the real teeth and those of the replicas obtained from impressions using President Jet® were compared. All replicas (occlusal and gingival aspects of each tooth) showed moderate/fine detail reproduction as compared to the real teeth,

Poor reproduction, little detail visible	Number of replicas = 0
Adequate reproduction, moderate detail visible	Number of replicas = 3
Good reproduction, fine detail visible	Number of replicas = 5

Discussion

In Vitro

A review of replication technique and the scanning electron microscope has been presented by Barnes (1978, 1979) and Ekfeldt *et al.* (1985). Artefacts are frequently seen when replicas are studied under the SEM and, unless recognized, may lead to misinterpretation.

Loss of surface detail may be due to faulty impression techniques, inadequate mixing, or removal of the impression prior to complete set. These problems were limited as far as possible by using a standardized technique to obtain the impressions, and the use of a skilled and dedicated operator for specimen preparation of SEM viewing. Surface detail may be lost due to disturbance of the low viscosity impression material prior to its full set by the application of the medium body impression material. However, as the technique was standardized, and both low viscosity impression materials had equivalent setting times, it is unlikely that one material would be affected more than the other. The results confirm the clinical impression of the operator who took the impressions that President Jet® was less viscous than Extrude®, and would therefore be more likely to flow well and reveal fine detail.

Surface detail may also be lost in casting due to use of material beyond its shelf life, incorrectly proportioned mix of material or a mix containing air bubbles. This was controlled as far as possible by the SEM technician. Some difficulty was found in orientation of the specimens to achieve matching photographs in the same perspective, of the tooth and replica. This is a technical problem related to the positioning of the specimens in the SEM. Where possible, the tie wings of the brackets were used to assist with orientation, but on occasions these were not in the original impression and thus presented difficulties. In future *in vitro* work it may be prudent to provide additional orientation marks either on the bracket base or the enamel surface close to the bracket to assist with the task of specimen orientation.

In Vivo

The results of the *in vivo* study confirm the *in vitro* work that the use of a low viscosity silicone-based impression

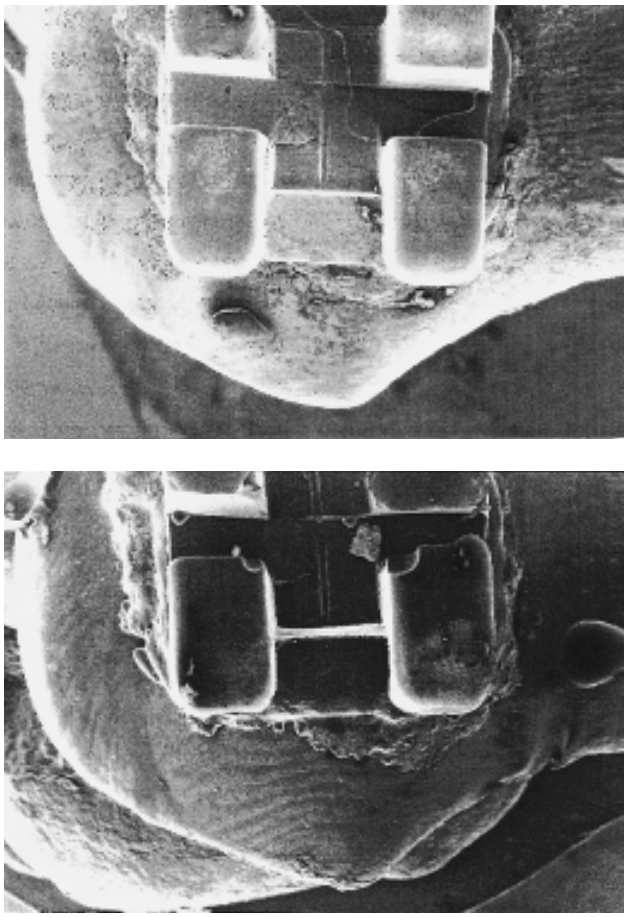

FIG. 5 (a) SEM photo of extracted premolars *in vivo*. (b) SEM photo of replica cast of the same area of the premolars *in vivo*.

material can provide an acceptably accurate reproduction of the bracket/composite/enamel boundary. It must be remembered, however, that this was undertaken when the teeth and their attachments were uncontaminated by accretions, such as a pellicle, plaque, and/or debris. If this technique were to be applied in clinical trials, careful attention to cleansing of the area to be examined would be necessary prior to taking the impression.

This procedure thus seems to provide a valid indirect technique for the study of the enamel/composite/bracket interface.

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Appendix

Production of the Replica

The impressions were prepared for casting by mounting upon a base of silicone impression material (Optosil® Coltène AG, Switzerland). This was moulded around the primary impression to provide a solid case and create walls to enclose the impression so as to contain the casting resin.

The casting resin used was Stycast 1266®, a clear low viscosity casting resin. The resin was used to manufacturers instruction:

1. 28 parts of constituent B were added to 100 parts by weight of constituent A. The liquid resin was then mixed thoroughly, whilst being agitated to eliminate air bubbles.
2. The castings were allowed to harden for at least 8 hours prior to removal from the impressions.
3. The Stycast replicas were then placed upon stubs and prepared for SEM by sputtering with gold to a thickness of 20–40 nm.

All specimens were viewed in an EBT 1 (Electron Beam Technology) Scanning Electron Microscope. SEM photographs were taken of the replicas under a variety of magnifications from ×15 to ×150. Photographs were also taken of the corresponding areas of the real teeth, matching as far as possible the magnifications.

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