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SCIENTIFIC SECTION

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A novel *in vitro* culture model to investigate the reaction of the dentine–pulp complex to orthodontic force

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Objective: To develop a novel mandible slice organ culture model to investigate the effects of externally applied force on the dentine–pulp complex.

Design: In vitro organ culture.

Setting: School of Dentistry, Birmingham, UK.

Materials and methods: Transverse 2 mm thick sections were cut from the mandibles of five 28-day-old male Wistar rats. Serial sections were used for control and test pairs. Springs made from 0.016-inch and 0.019×0.025-inch stainless steel wires were used to apply a 50 g tensile or compressive force, respectively, to test specimens. Control and test specimens were cultured for 5 days in a humidified incubator with 5% CO_2 at 37°C and processed for routine histological investigation. Nine more rats were used to provide control and compression test pairs where the pulps were extirpated after 3 days culture and total RNA isolated for gene expression analysis by reverse transcriptase polymerase chain reaction (RT-PCR).

Results: Histology showed the dental and supporting tissues maintained a healthy appearance in the control cultures after culture. Histomorphometric analysis revealed a 20–27% increase in pulp fibroblast density in test specimens compared with controls. Gene expression analyses revealed up-regulation in the test groups of PCNA, c-Myc, Collagen 1α, TGF-β1 and alkaline phosphatase, whilst expression of osteocalcin was reduced.

Conclusions: The results demonstrated that the present organ culture technique provides a valuable *in vitro* experimental model for studying the effects of externally applied forces. These forces stimulated a cellular response in the pulp chamber characterized by altered gene expression and proliferation of fibroblasts; the latter being unaffected by the nature of the force in terms of compression or tension.

Key words: Organ culture, orthodontic force, dentine–pulp complex

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Introduction

Continuing advances in orthodontic technology have highlighted a need to better understand the responses of the dental tissues to orthodontic forces. In particular, the effects on the dentine pulp complex have not yet been significantly investigated. Some workers[1] have found a mild inflammatory type response in the pulp of teeth that have been subjected to orthodontic forces, but specific cellular responses have not been investigated.

Orthodontic forces cause tooth movement, which results from a complex series of biological tissue reactions in response to the applied force. The resultant effect on the alveolar bone and other supporting structures of the tooth socket has been vigorously investigated at both the cellular and molecular level.[2–4]

Relatively few studies have looked at reactions within the dental pulp.[5–7] Those that have been documented mainly show a trend towards the predominance of vascular changes. For example, application of an intrusive force to monkey incisors has been reported to cause necrosis of the pulp tissues.[8] The effects of intrusive forces on human teeth have also been examined histologically.[9,10] The study sample consisted of 35 premolar teeth scheduled for orthodontic extraction from children aged 10–13 years. Sectional fixed appliances applied intrusive forces (35–250 g) for up to 35 days. Control teeth were usually obtained from

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different individuals to the test ones. Test teeth were extracted either immediately after the intrusive force was stopped or up to 104 days after force removal. The main pulp changes observed included vacuolization (inter- and intra-cellular spaces) and circulatory disturbances, (including enlarged and engorged blood vessels), local haemorrhage and brown haemosiderin deposits from red blood cell degradation. Root and dentine resorption were seen, as was a reduction of pre-dentine width in experimental groups, which was suggestive of a general degenerative change in the pulps. Pulp changes were not directly related to force magnitude, although a tendency towards greater severity with increased force was seen. Factors other than the magnitude of the force appeared to play a role with the developmental stage of the root being the most prominent. Other workers have noted vascular changes consequent upon orthodontic force application.[1]

Not all workers have found degenerative changes in the pulps of teeth subjected to orthodontic forces. Nixon *et al.*[11] found a force-dependent increase in pre-dentine width at a time corresponding to the peak of the tooth movement cycle in rats. Indeed, even in the study by Stenvik and Mjör, where the overall results suggested that degenerative changes predominated,[9] there were a few experimental teeth that showed increased activity in the odontoblast layer. This was accompanied by an increase in number of cells in the layer of Weil and a broader than usual pre-dentine layer.

There appears to be considerable variation in the observed pulpal response to orthodontic forces. It is difficult to compare different studies in view of the considerable variations in methodology, sample sizes and types. The nature and magnitude of force, however, as well as state of development of the root appear to be important variables that influence the response. In particular, many of the effects described above may be attributed to effects of circulatory changes brought about in response to applied forces. The specific biological mechanisms responsible for these vascular changes are as yet unclear. Enhanced angiogenesis has been reported to be due to diffusible angiogenic growth factors released from human dental pulp explants of orthodontically moved teeth.[12–14] Dentine matrix also contains growth factors, which may have angiogenic effects when released.[15]

Technological advances have continued to produce new materials, such as rectangular nickel titanium wires, and current approaches to orthodontic therapy may apply greater forces to teeth at earlier stages in treatment than was formerly the case. The need to understand the cellular and molecular mechanisms that protect the integrity, or affect behavior, of the dentine–pulp complex after such potentially iatrogenic insults thus assumes greater importance. An improved understanding of the mechanisms by which the integrity of the pulp is protected during orthodontic tooth movement may help provide a biologically sound basis for the optimization of clinical treatment strategies by harnessing the cellular activity of the dental pulp.

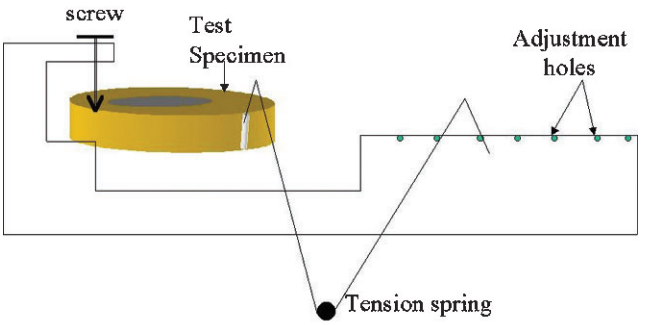
A tooth slice organ culture model has been previously described[16] and applied to cytotoxicity testing for biomaterials.[17] The aim of the present study was to develop a novel mandible slice organ culture model to investigate the effects of externally applied force on the dentine–pulp complex.

Materials and methods

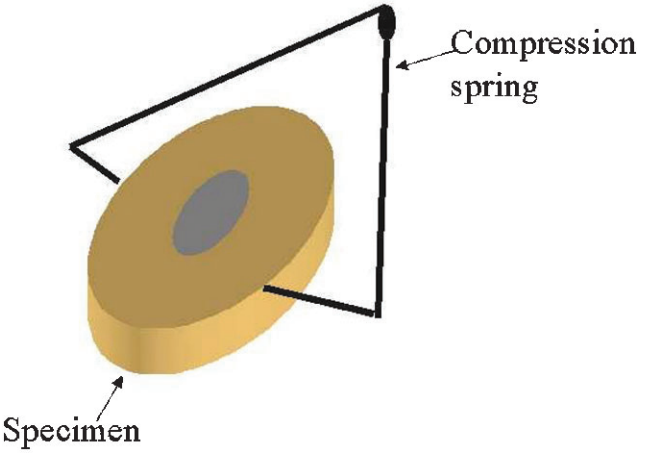
The mandibles from freshly killed 28-day-old male Wistar rats were dissected out and immediately placed in sterile transport medium (Dulbecco's Modified Eagle's Medium, DMEM, Sigma Chemical Co., UK) at 37°C. Transverse sections of approximately 2 mm thickness were cut manually from each mandible using a diamond edged rotary saw (Isomet, Bühler Ltd., Germany) cooled with transport medium and pre-sterilized by washing with 70% aqueous ethanol. Each section was washed several times with medium and placed in sterile transport medium. Serial sections were labeled appropriately in order to provide comparable control and test pairs. To provide meaningful sample sizes, two animals were used for the tension experiment and three for the compression group, on the basis that each animal provides between 6 and 8 good sections.

Method for applying tension

A '½ round' steel rose-head bur in a rotary slow speed drill was used to create a hole in the bone of each test specimen using transport medium as coolant. The hole was positioned in the widest portion of the mandible at the root analogue end of the section and was used to allow one arm of the tension spring to be attached to the specimen (Figure 1a). Springs were constructed from 0.016-inch Australian stainless steel wire and both the springs and the test apparatus were sterilized by autoclaving using a 135°C cycle prior to use. After several washes in transport medium, the crown analogue end of the specimen was positioned and fixed to one end of the apparatus by means of a custom made clamp. The other arm of the tension spring was attached to one of a number of holes on the opposite side of the test apparatus. Force application was adjusted by varying the hole position employed. Springs were calibrated against a strain gauge to deliver a force of 50 g when 2 cm separated the two arms during the study.



(a)



(b)

Figure 1 Showing (a) tension spring set-up and (b) compression spring set-up

Method for applying compression

Compression springs were constructed from 0.019 × 0.025-inch stainless steel wire and sterilized by autoclaving with a 135°C cycle prior to use. They were applied to the test specimens approximately half way between the root and crown analogue ends of the section (Figure 1b). Each spring was calibrated to produce a 50 g force when opened by 3 mm, corresponding to the average width of a section.

Organ culture technique

The culture medium consisted of DMEM containing 0.584 g/l glutamine and 1% penicillin/streptomycin solution (containing 100 units/ml of penicillin and 100 µg/ml of streptomycin), 20% heat inactivated fetal calf serum and 0.15 mg/ml ascorbic acid.

Each specimen was placed in an appropriate sized petri dish, immersed completely in the culture medium and cultured in a humidified incubator at 37°C in an atmosphere of 5% CO_2 in air. Daily changes of medium were carried out during the culture period.

After culture, each specimen was fixed for 24 hours in 10% (w/v) neutral buffered formalin (Surgipath, UK) and subsequently demineralized in 10% (v/v) formic acid (Sigma, UK) for 5 days before being processed for routine histological examination and embedded in paraffin wax (Tissue Tek III). Serial sections of 5 µm thickness were cut, and stained in Mayer's hematoxylin and eosin (H&E). Five slides from each specimen in the compression experiment groups were stained with hematoxylin only to highlight the nuclei for the purposes of automated image analysis.

Histomorphometric analysis

Cell numbers in the pulps of cultured rat mandible slices were counted on 5 µm thick H&E-stained sections. Randomized manual cell counts were carried out under a ×40 objective lens using a 10 × 10 square ocular graticule. Only one central row of 10 squares from the graticule was utilized for the cell counts and the graticule was placed on each slide overlying the midline of the central portion of the pulp at positions corresponding to the top, middle and bottom of the specimen. The total area inside the ten squares counted was 0.009 mm^2 (0.33 × 0.03 mm). Only clearly defined, well-stained nuclei were counted and partial nuclei were included in the count only if they were on the left or top of the grid. Nuclei were only counted once if they fell within two squares in the counting area. Mean cell counts were expressed as density per unit area.

For the tension test groups, five H&E-stained sections per specimen were selected for analysis using random number tables and six cell counts were carried out on each section, according to the protocol specified above.

Manual cell counts were performed initially for the compression group to determine whether there was a difference in cell numbers between test and control groups prior to confirmatory quantification using digital image analysis software. For this purpose three counts per H&E-stained section were carried out on one random slide per specimen.

Image analysis

Five haematoxylin-only stained slides from each compression group specimen were taken from the centre of the respective specimen blocks. A digital monochrome image of each slide was captured using Image Pro Plus image analysis software (Media Cybernetics, USA) with a ×20 objective. At this magnification, the image represented most of the core area of the pulp for each slide. For this reason, only one cell count per slide was considered necessary when using image analysis. The

software was programmed to count only those 'nuclei' that registered above a certain threshold level of density (intensity of staining) and within preset size parameters.

Statistics

The mean cell counts for control and test specimens were examined with a one-way analysis of variance (ANOVA) to determine if there was a statistical difference between the test and control groups.

To examine reproducibility of the manual counting technique, 10 of the tension group and 5 of the compression slides were re-counted after a period of 3 weeks. No significant difference was found between the two sets of counts using one-way ANOVA.

The mean areas for the structures recorded as 'nuclei' with image analysis in the compression test and control groups were compared using ANOVA, and no statistically significant difference was found. This confirmed that the software parameters had been correctly set to record nuclei.

All statistical analyses were carried out using the Minitab software package (Minitab Inc., USA).

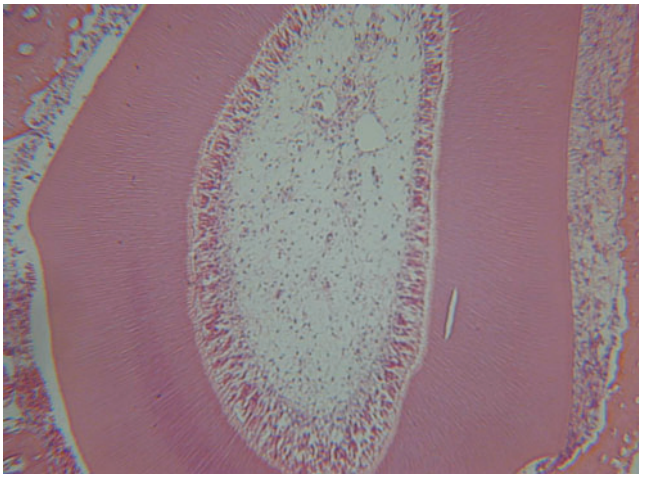
Gene expression studies

RNA isolation and cDNA synthesis. Thirty-five paired control and test mandible sections were cut from 9 freshly killed 28-day-old male Wistar rats and cultured for 3 days. Test specimens were each subjected to a 50 g force from a compression spring during the culture period. The pulp from all control and test sections were extirpated with a blunt excavator instrument at room temperature and pooled into two separate groups. It was necessary to pool the extirpated pulps in this manner as preliminary studies showed that it was impossible to extract workable quantities of RNA from smaller samples. Total RNA was extracted from these samples using the RNeasy Mini Kit (Qiagen, UK), as recommended by the manufacturer. Subsequently, the DNase digested total RNA was used for oligo-dT reverse transcription to generate single stranded cDNA using the Omniscript kit (Qiagen, UK). Both RNA and cDNA concentrations were determined using a BioPhotometer (Eppendorf, UK).

Semi-quantitative reverse transcriptase polymerase chain reaction (Sq-RT-PCR analysis). Sq-RT-PCR analysis was performed for the genes PCNA, c-Myc, TGF-β1, collagen 1α, alkaline phosphatase and osteocalcin. PCNA and c-Myc were examined as indicators of cell proliferation, TGF-β1 and collagen 1α as indicators of cellular activity and alkaline phosphatase and osteocalcin as genes involved in mineralization. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. A 50 ng sample of single stranded cDNA was used to seed a 50 µl PCR. Samples were amplified for between 22 and 30 cycles in an Eppendorf Mastercycler thermal cycler (Eppendorf, UK). Table 1 lists the primer sequences and cycle conditions used. Following the designated number of cycles, 8 µl of the reaction were removed and the product separated and visualized on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. Scanned gel images were imported into AIDA image analysis

Table 1 Primer sequences and cycling conditions used for gene analyses

Gene	Primer sequence (5'→3')	Annealing temperature (°C)	Cycles
PCNA (Proliferating Cell Nuclear Antigen)	Forward TTG GAA TCC CAG AAC AGG AG Reverse CGA TCT TGG GAG CCA AAT AA	60	30
c-Myc	Forward TGT CCG TTC AAG CAG ATG AG Reverse GCC CTA TGT ACA CCG GAA GA	60	28
TGF-β1 (Transforming Growth Factor-β1)	Forward TGA GTG GCT GTC TTT TGA GC Reverse TTC TCT GTG GAG CTG AAG CA	60	28
Collagen 1α	Forward TAA AGG GTC ATC GTG GCT TC Reverse ACT CTC CGC TCT TCC AGT CA	60	28
Alkaline phosphatase	Forward CTC CGG ATC CTG ACA AAG AA Reverse ACG TGG GGG ATG TAG TTC TG	60	24
Osteocalcin	Forward TCC GCT AGC TG TCA CAA TTG Reverse CCT GAC TGC ATT CTG CCT CTC T	60	28
GAPDH (Glyceraldehyde-3-phosphate dehydrogenase)	Forward CGA TCC CGC TAA CAT CAA AT Reverse GGA TGC AGG GAT GAT GTT CT	60	22



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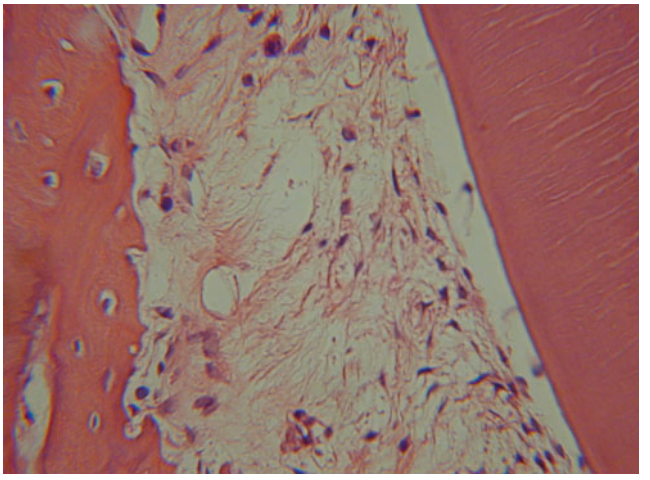
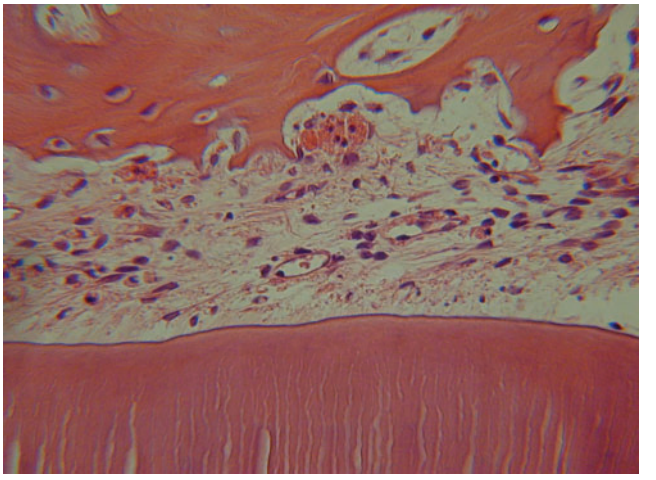


(b)

Figure 2 (a) H&E section showing viable bone, periodontal ligament and pulp in a 5 day cultured mandibular slice (×25). (b) Region of 'normal' periodontal ligament (×40)



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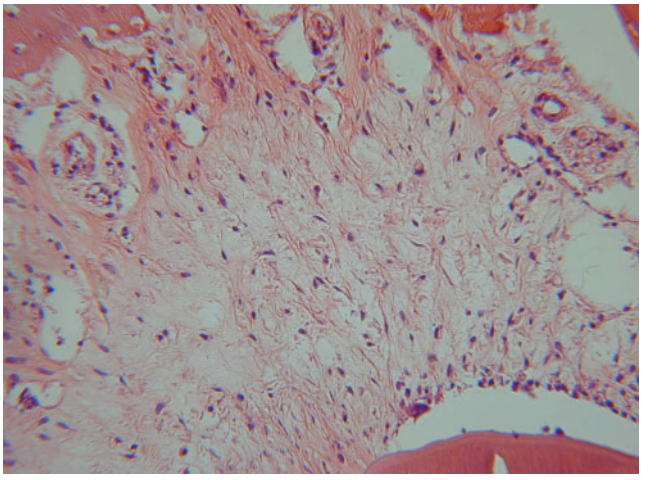


(b)

Figure 3 Showing a. compressed region of periodontal ligament and b. region where periodontal fibres are stretched / under tension (×40)

software (Fuji, UK) and the volume density of amplified products calculated and normalized against GAPDH control values. Each gene analysis was repeated twice and the average density value calculated was used in the final analysis.

Results

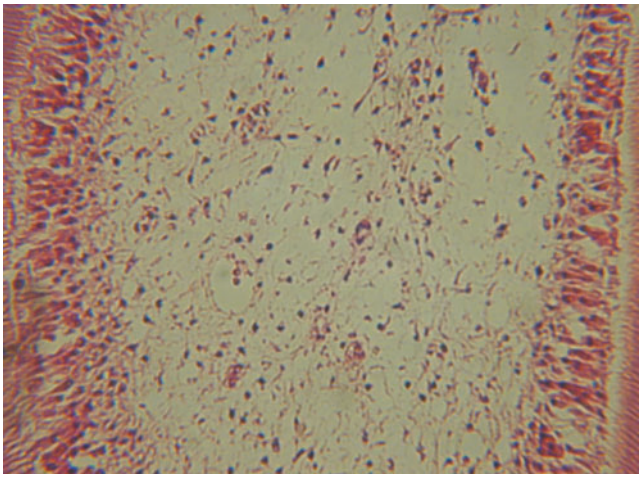
Histological investigation

Representative H&E sections from the cultured mandible slices are shown in Figures 2–5. Figure 2 shows (a) the normal histological appearance and organization of the sections after 5 days of culture and (b) the appearance of control (not stretched or compressed) periodontal ligament. Figure 3 shows the appearance of (a) compressed and (b) stretched areas of ligament. Figure 4 shows a comparison of a control and test (compression spring) pair to show the visual difference in pulp cell density, whilst Figure 5 illustrates the 'mosaic' like appearance of the extra cellular matrix seen in the test pulps.

Histomorphometric analysis

Table 2 provides comparison of the summary cell counts of pulp fibroblast numbers in control and test specimens subjected to tension. These data reveal a statistically significant ($p=0.003$) 26% increase in fibroblast numbers in the test group compared with controls.

Table 3 compares summary data for manual cell counts of paired control and compression test



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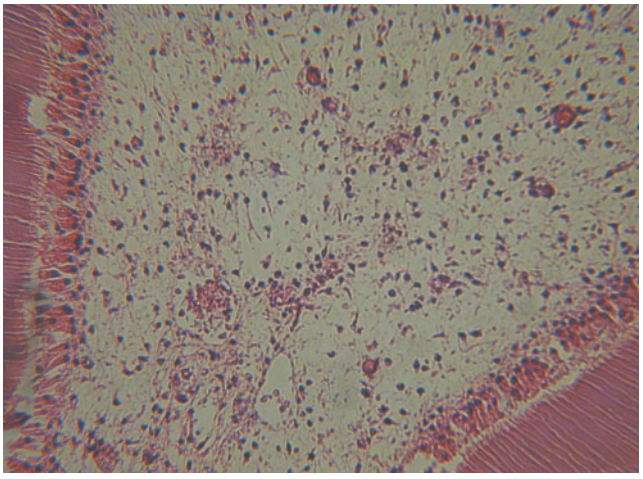


(b)

Figure 4 Showing comparison of pulp fibroblast density in control (a) and test (b) specimens (×25)

specimens. A 20% increase in fibroblast numbers was found in the test group (P=0.003).

Table 4 records the mean cell density calculated for the compression experiment specimens using image analysis.

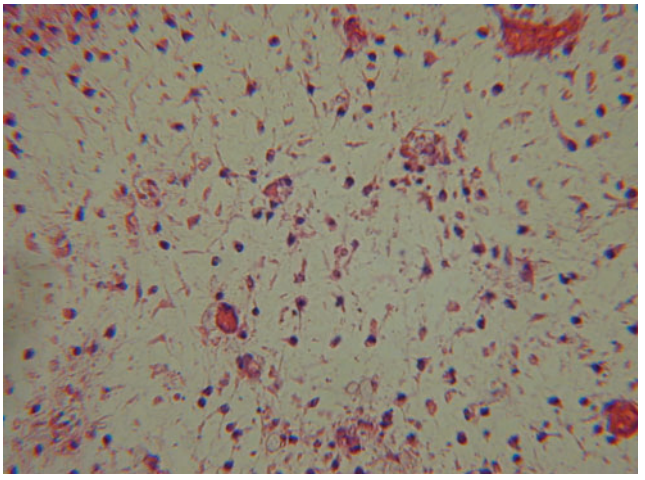


Figure 5 Mosaic type appearance of test group pulps (×40)

A statistically significant 27% increase in mean cell density was found in the test group compared with controls (P=0.005).

The cell density data given in Table 4 are summarized in Figure 6.

Figure 7(a–c) shows the gene expression data from Sq-RT-PCR and summarizes the findings of the

Table 2 (b) One-way analysis of variance between mean cell counts for control and test specimens

Source	DF	SS	MS	*F*	*p*
Factor	1	209.4	209.4	13.46	0.003

DF, degrees of freedom; SS, sum of squares; MS, mean square; *F*, *F* value; *p*, probability.

Table 2 (c) Overall cell density calculation

Measurements of grid area $=0.33\times0.03$ mm^2=0.009 mm^2
Control mean=30.25/0.009=3361 mm^{-2}
Test mean=37.987/0.009=4221 mm^{-2}
Mean cell density increase=25.6%

Table 2 (a) Summary manual cell count data comparing pulp fibroblast numbers in control and test specimens subjected to tension

	Controls					Tension			
Specimen	*n*	Mean cell count	SD	SE	Specimen	*n*	Mean cell count	SD	SE
1	30	30.30	7.71	1.4	2	30	42.57	4.78	0.87
3	30	30.13	5.44	0.99	4	30	44.90	6.24	1.1
5	30	31.40	8.80	1.6	6	30	33.87	6.91	1.3
7	30	33.73	7.32	1.3	8	30	39.87	5.89	1.1
9	30	27.23	5.03	0.92	10	30	31.70	8.40	1.5
11	30	27.37	7.07	1.3	12	30	33.27	5.62	1.0
13	30	31.60	7.78	1.4	14	30	39.73	7.48	1.40

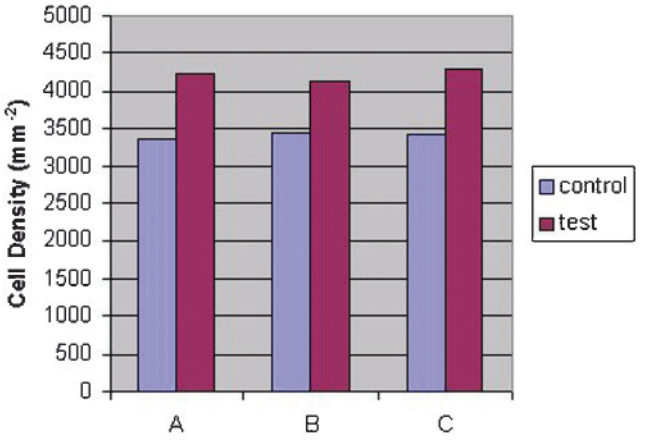


Figure 6 Summarizing cell density data. (A) Tension group with manual cell counts. (B) Compression group with manual cell counts. (C) Compression group with image analysis

densitometric analyses of gel images after importing into AIDA image analysis software (Fuji, UK). These results show up-regulation of expression of PCNA, c-Myc, Collagen 1α, TGF-β1 and alkaline phosphatase in the test groups compared with controls. Osteocalcin expression was reduced in the test group.

Discussion

Previous studies on the effects of orthodontic forces on dental tissues have concentrated on bone[18] and periodontal ligament[3] responses. The reaction of the dentine pulp complex is less well characterized and much of our current knowledge results from *in vivo* animal experimentation, where vascular effects have been seen to dominate.[11] However, methodological variations in such studies have limited interpretation.

Increasing awareness of animal welfare has led to alternatives to *in vivo* animal experimentation being sought. A robust *in vitro* experimental model for studying the effects of orthodontic forces could provide such an alternative and importantly, allow better control of experimental variables.

Histological evidence from the present study shows that our mandible organ culture technique is viable and can be used for studying the effects of orthodontic forces on dental tissues, since the viability of the dental and supporting tissues were well maintained during the 5 day period in culture (Figure 2). In addition, the appearance of the supporting tissues in the specimens subjected to force (Figure 3) was similar to that described in previous studies.[19] In particular, the areas of compressed or stretched periodontal ligament, which are central to the pressure–tension theory of orthodontic force transduction, were clearly visible. Whilst the present study concentrated on pulpal effects, one specific advantage of this *in vitro* approach is the ability to study all relevant dental and supporting structures together, including bone, periodontal ligament and pulp, and to examine their inter-relationships. In addition, the model

Table 3 (b) One-way analysis of variance between mean control specimen cell counts and mean test specimen cell counts

Source	DF	SS	MS	*F*	*p*
Factor	1	482.7	209.4	11.64	0.003

DF, degrees of freedom; SS, sum of squares; MS, mean square; *F*, *F* value; *p*, probability.

Table 3 (c) Overall cell density calculation

Measurement grid area=0.33×0.03 mm^2=0.009 mm^2
Control mean=31.01/0.009=3446 mm^{-2}
Test mean=40.83/0.009=4125mm^{-2}
Mean cell density increase=20%

Table 3 (a) Summary data for manual cell counts for paired control and compression test specimens

	Controls					Test			
Specimen	*n*	Mean cell count	SD	SE	Specimen	*n*	Mean cell count	SD	SE
1	3	36.33	1.53	0.88	2	3	35.67	4.73	2.73
3	3	35.00	3.00	1.73	4	3	30.33	6.81	3.93
5	3	23.00	9.29	5.36	6	3	31.67	9.24	5.33
7	3	25.00	8.72	5.03	8	3	45.33	12.34	7.13
9	3	34.00	6.08	3.51	10	3	43.67	5.03	2.91
11	3	30.67	9.29	5.36	12	3	33.67	1.53	0.88
13	3	31.33	13.58	7.84	14	3	54.00	5.00	2.89
15	3	25.00	3.21	1.86	16	3	47.00	4.36	2.52
17	3	33.67	4.04	2.33	18	3	44.67	4.62	2.67
19	3	36.00	4.00	2.31	20	3	42.33	3.21	1.86

allows the tissues to be investigated in isolation from vascular effects. The *in vitro* organ culture model therefore shows considerable potential for use in future studies.

The histomorphometric analysis (Figure 6), which revealed an increase in cell numbers in the core of pulps of test specimens subjected to both tension and compression is the first evidence that forces applied externally may be transmitted to the dental pulp to produce a cellular effect. The increase in cell numbers was found in the core or central portion of the pulp, and by location and appearance, it appeared that the applied force resulted in proliferation of pulp fibroblasts. It appears that the response of these cells is similar, regardless of whether tensile or compressive forces are applied. Considering the mechanisms by which external forces are transmitted to the dentine–pulp complex, certain parallels can be drawn between bone and dentine and, in particular, between odontoblasts and osteocytes. The odontoblast process, like the osteocyte, is completely encased in mineralized tissue. It is therefore possible that deformation of dentine may be 'sensed' by the odontoblasts, acting as mechanosensors, leading to activation of signaling mechanisms that produce cellular responses within the dentine–pulp complex. Similar effects have previously been implicated in the regulation of bone turnover.[20]

Gene expression analysis was performed to investigate further the transduction mechanisms involved in the observed pulpal responses. In particular, the expression levels of proliferating cell nuclear antigen (PCNA) and c-Myc were examined. PCNA is a nuclear protein that is involved in DNA replication by acting as a cofactor for DNA polymerase δ and reaches its highest concentration during the S (DNA replication) phase of the cell cycle. It is an established marker of cell proliferation. c-Myc is an oncogene and altered expression of this gene generally results in alterations in the expression of its product, the Myc protein. Myc has been associated with the promotion of cellular growth and proliferation, as well as desensitization to growth inhibitory stimuli.[21] Expression levels of both genes were elevated in test samples subjected to compressive force compared with

Table 4 (b) One-way analysis of variance between mean control specimen cell density and mean compression test specimen cell density

Source	DF	SS	MS	*F*	*p*
Factor	1	4.570	4.570	10.25	0.005

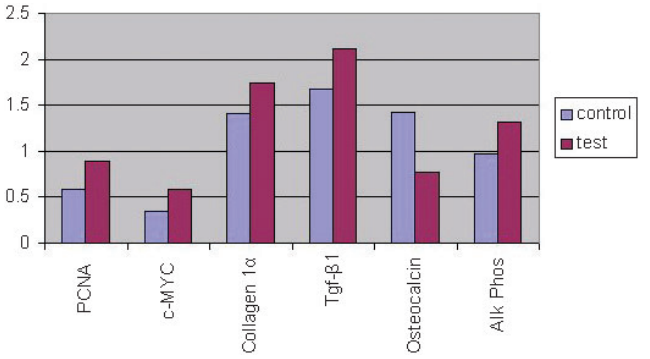
DF, degrees of freedom; SS, sum of squares; MS, mean square; *F*, *F* value; *p*, probability.

Table 4 (c) Overall cell density calculation

Mean cell density in controls=$3.4 \times 10^{-3}/u^2$=3400 mm^{-2}
Mean cell density in test groups=$4.3 \times 10^{-3}/u^2$=4300 mm^{-2}
Mean cell density increase=26.5%

Table 4 (a) Mean cell density calculated for compression experiment specimens using image analysis

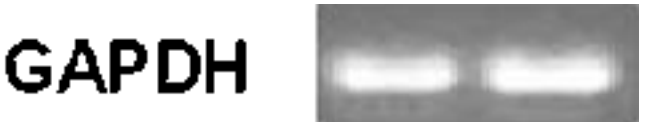
Specimen	Mean area(u^2)	Mean number (*n*)	Mean density ($\times 10^{-3}/u^2$)
1	64818.8	261	4.03
2	69852.6	197	2.82
3	66862.9	326	4.88
4	69372.6	235	3.39
5	72753.8	309	4.25
6	53780.6	171	3.18
7	72991.4	240	3.29
8	72301.2	332	4.59
9	60288.1	240	3.98
10	70573.1	182	2.58
11	54461.9	234	4.29
12	69461.5	342	4.92
13	72151.5	247	3.42
14	69915.5	193	2.76
15	74195.9	303	4.08
16	72579.9	383	5.28
17	59301.9	180	3.04
18	44455.0	203	4.57
19	71507.5	315	4.41
20	69067.7	292	4.22



(a)

Gene	Control / Test	% Change
PCNA		53% increase
c-MYC		66% increase
Collagen 1α		24% increase
TGFβ1		27% increase
Osteocalcin		47% decrease
Alkaline Phosphatase		27% increase

(b)



(c)

Figure 7 (a) Densitometric analysis of gel images after importing into AIDA image analysis software (Fuji, UK). (b) Gene expression data from Sq-RT-PCR analysis showing representative gel images and differential analysis of band intensity between control and test specimens expressed as percentage change. (c) All density calculations were relative to normalized GAPDH control values

controls (Figure 7). This result supports the initial histomorphometric findings and suggests that forces applied to teeth are capable of altering cellular behaviour at the molecular level. As initial results indicated that the response was independent of force direction, only compressive force was used, since it was easier to study.

Histological examination revealed a mosaic-like alteration of pulp extracellular matrix in teeth exposed to force (Figure 5) and gene expression data showed elevation of mRNA encoding collagen 1α (Figure 7). It is, however, unclear whether this altered appearance resulted from quantitative or conformational changes in the fibrous components. Up-regulation of collagen Iα gene expression in response to mechanical stress has been reported in other connective tissues.[22] In addition, it has been shown *in vitro* that the multifunctional cytokine TGF-β1 can stimulate collagen synthesis by fibroblasts.[23] The increased expression of TGF-β1 in the present study (Figure 7) could therefore be related to the matrix changes. Such a link between TGF-β1 and collagen synthesis after mechanical stress has been previously reported in anterior cruciate ligament fibroblasts.[22] TGF-β1 has also been implicated in tertiary dentinogenesis in response to injury.[24] Although not seen within the time span of the current study, an increase in pre-dentine width has been reported in rat teeth after sustained force application.[11] It is possible that the increased TGF-β1 expression seen in the present study may be associated with such effects. The up-regulation of the enzyme alkaline phosphatase (Figure 7), known to be important in mineralization events, may also be related. However, the concomitant finding that expression of the gene encoding the mineralized matrix component osteocalcin was down-regulated seems incongruous in this context and requires further investigation.

The gene expression studies were carried out on extirpated rat pulp tissue. Recent evidence suggests that, when rat pulp is extirpated at room temperature, the odontoblasts lining the pulp cavity are also removed with the pulp.[25] The heterogeneous nature of the cell population therefore makes it impossible to attribute effects to specific cell types. It may be, for example, that the up-regulation of TGF-β1 could be related to odontoblast activity, rather than to the pulpal fibroblasts.

The present study provides a novel *in vitro* experimental model for study of tissue responses to applied orthodontic forces, but care is required in direct extrapolation of these data to the clinical situation where force levels may differ. It is unclear as to what proportion of the applied force was actually transmitted to the dentine–pulp complex, but this study has highlighted the potential cellular effects of forces applied to the tissues and further investigations on the details of force transmission with this model should prove valuable.

Conclusions

A novel *in vitro* organ culture model has been developed to study the effects of orthodontic forces on dental tissues and its application to study of the effects of

external forces on the dentine pulp complex has been described. From the initial studies using this model, the following specific conclusions may be drawn:

- Externally applied forces can be transmitted to the dentine pulp complex.
- Externally applied forces can produce a cellular response in the pulp chamber, which is characterized by proliferation of fibroblasts and is unaffected by the nature of the force in terms of compression or tension.
- Force application alters gene expression within the dentine–pulp complex. In particular, genes associated with cellular proliferation (e.g. PCNA) and the extra cellular matrix component collagen 1α are up-regulated.

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Authors and contributors

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