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Root resorption and its association with alterations in physical properties, mineral contents and resorption craters in human premolars following application of light and heavy controlled orthodontic forces

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Structured Abstract

Authors – Darendeliler MA, Kharbanda OP, Chan EKM, Srivicharnkul P, Rex T, Swain MV, Jones AS, Petocz P

Objectives – To study the effect of different orthodontic force levels on cementum, investigating from the point of view of its physical properties, alterations in the mineral components, type and location of the resorption craters and the exploration in 3D of space.

Design – *In vivo* human premolars subjected to heavy and light forces were employed for this study. After a period of movement they were analyzed for hardness and elasticity. Also, the mineral composition measuring Ca, P and F of the cementum root surface was investigated. A new method for volumetric analysis of resorption craters was developed.

Results – There were no significant differences for hardness and elastic modulus between the light and heavy force groups and no significant effects for different tooth positions. Significant inter-individual variation in the Ca, P and F concentrations was noted. Force-related data showed that mean volume of the resorption crater in light-force group was 3.49-fold greater than the control group, and the heavy-force group 11.59-fold more than control group. The heavy force group had 3.31-fold greater total resorption volume then light force group. Buccal cervical and lingual apical regions demonstrated significantly more resorption craters than the other regions. The 2D measurements were strongly correlated to 3D measurements.

Conclusion – The application of light and heavy forces did not show any statistically significant differences in hardness and elastic modulus when compared with untreated teeth. The inconsistent increase or decrease of Ca, P and F contents between control and experimental teeth at sites of compression and tension were difficult to explain. There was more resorption by volume in the heavy force group as compared with the light group and controls. Our data also suggested that the high-pressure zones might be more susceptible to resorption after 28 days of force application.

Key words: calcium; cementum; elastic modulus; EPMA; fluoride; hardness; light and heavy forces; phosphate; resorption craters; root resorption; scanning electron microscope; stereo imaging

Introduction

Root resorption has been used as a universal term that describes a pathologic process that clinically and radiologically manifests itself as surface thinning or shortening of tooth root for which no single etiologic component has been isolated. Historically the resorption of permanent teeth was first reported by Bates in 1856 (1). Becks and Marshall (2) defined it as the destruction of formed dental tissue. Root resorption was recently defined as the active removal of mineralized cementum and dentine (3). Studies of this phenomenon over the years have directed that root resorption could be an idiopathic and unpredictable adverse effect of orthodontic treatment. While some degree of root resorption occurs in almost every patient (4), 93% of treated adolescents demonstrated some form of root resorption (5). Nearly 15% (12–17% of orthodontically treated patients) show moderate to severe apical root resorptions (6, 7) and 10–20% of cases have been reported to have severe resorption of >3 mm (8, 9). However in most patients, root resorption is minor and insignificant (8).

Several biological and mechanical factors have been identified and have been indicated to cause increased susceptibility to root resorption. The most commonly affected teeth in decreasing frequency are: maxillary laterals, maxillary canines, mandibular incisors, mandibular first molars, mandibular second premolars and maxillary second molars (10). Teeth with radiographic signs of resorption prior to treatment have been reported to develop more extensive areas of resorption than initially intact teeth (4, 11, 12). A previously induced trauma and loss of vitality (13), could subsequently lead to severe root resorption and/or total loss of tooth/teeth.

Other predisposing factors that have been indicated are: gender of the patient (4), duration of the orthodontic treatment itself (14), type and mechanics of force delivery (4, 12, 15), magnitude and duration of the force (4, 15–18) and appliance type. Bone density (15, 19), endocrine disorders and asthma (20, 21) have also been suggested as predisposing factors.

Reitan (15, 18, 22) has advocated the use of light orthodontic forces in order to enhance cellular activity in the frontal areas of surrounding tissues and reduce the risk of root resorption. King and Fischlschweiger (23) found minimal root resorption with light forces, whereas intermediate or heavy forces resulted in substantial crater formation in rats. This was in agreement with earlier findings, both in animals (24–26) and in humans (17).

Because of the fact that different types of orthodontic appliances, different force systems and force vectors and different types of tooth movement were utilized in these previous studies, a direct comparison of results could not be performed. It has been reported that resorption craters appear mainly on the pressure side (15, 18, 22, 27, 28) and rarely on the tension side (29). Resorption could be also a naturally occurring physiologic phenomenon (30).

Although a number of studies have investigated root resorption from several different aspects, the exact mechanism and etiology of root resorption still remains elusive. The team at the University of Sydney looked at the resorption process from a different perspective. We focused on the effect of different orthodontic force levels on cementum, investigating from the point of view of its physical properties, alterations in the mineral components, type and location of the resorption craters and their exploration in 3D.

Lately, research is being directed to study cellular, molecular and genetic control of the resorption process, genetic susceptibility, repair of cementum following induction of resorption and possibilities of preventing resorption through availability of trace elements through systemic route. The aim of this paper is to report on the understanding of root resorption encompassing several investigations on effects of orthodontic force levels, physical characteristics of cementum and enamel, mineral content of cementum and volumetric quantification of root resorption. The studies are summarized in order as follows.

Materials and methods

Initial experiments with the first sample

1. A new method for three-dimensional evaluation (31).
2. The effect of different storage methods (32).

Studies on root cementum with second core sample of 36 premolars

1. Changes in physical properties of human premolar cementum after the application of controlled orthodontic forces (33).
2. A quantitative analysis of the mineral composition of human premolar cementum – an EPMA study (34).
3. A comparative quantitative analysis of the mineral composition of human premolar cementum after the application of orthodontic forces – an EPMA study (Rex et al, unpublished data).
4. Volumetric analysis of root resorption craters after application of light and heavy orthodontic forces (35).
5. Validation of 2D vs. 3D measurements of root resorption craters on human premolars after application of light and heavy orthodontic forces (Chan et al, unpublished data).

Studies on genetics and on molecular control in expression of root resorption (not discussed in this paper)

1. Cellular and molecular control of root resorption following tooth movement in rats (36).
2. Assessing the genetic link of orthodontic root resorption (Ngan et al, unpublished data).

Initial experiments

A new method for three-dimensional evaluation (31)

Cementum is a non-uniform connective tissue that covers the roots of human teeth. Investigation of the physical properties of cementum may help in understanding or evaluating any possible relationship and/or susceptibility to root resorption. A variety of engineering tests are available to investigate these properties. However, the thickness of the cementum layer varies, and this limits the applicability of these techniques in determining the physical properties of cementum. Hardness testing with Knoop and Vickers indentation techniques overcame some of these limitations (37), but the process of embedding and tooth preparation prohibited retrieval and retesting of the sample and therefore the testing was restricted to one area or section of the tooth. In previous studies teeth were embedded, sectioned and polished (37–40). This procedure is time consuming, may alter the physical properties because of dehydration and limits the testing areas (only either cross-sectional or longitudinal cuts). Measurement spots are difficult to be relocated for re-measurements. There also exists a hybrid layer consisting of acrylic and cementum interface. It is often difficult to truly identify the true cementum and this hybrid layer may be measured instead. There were also published papers on the adverse effects of storage media of these samples over time (41).

Premolars were harvested from orthodontic patients requiring extractions (Fig. 1A) and then mounted on a newly designed surveyor that allowed sample retrieval and 3D rotation (Fig. 1B). A new method to investigate the physical properties of human premolar cementum was developed to obtain a three-dimensional (3D) map of these properties with the Ultra Micro Indentation System (UMIS-2000; Commonwealth Scientific and Industrial Research Organization, Lindfield, Australia). UMIS-2000 is a nano-indentation instrument for investigation of the properties of the near-surface region of materials (Fig. 1C). The UMIS-2000 was programmed at 5 mN of contact force (1/2 g) with a 20 μm spherical diamond indenter penetrating the surface with a maximum force of 500 mN (50 g) in 20-indent increments. Each coordinate comprised five individual indents in a 100 μm square array.

Using a pilot sample of nine maxillary and/or mandibular human first premolars from two males and four

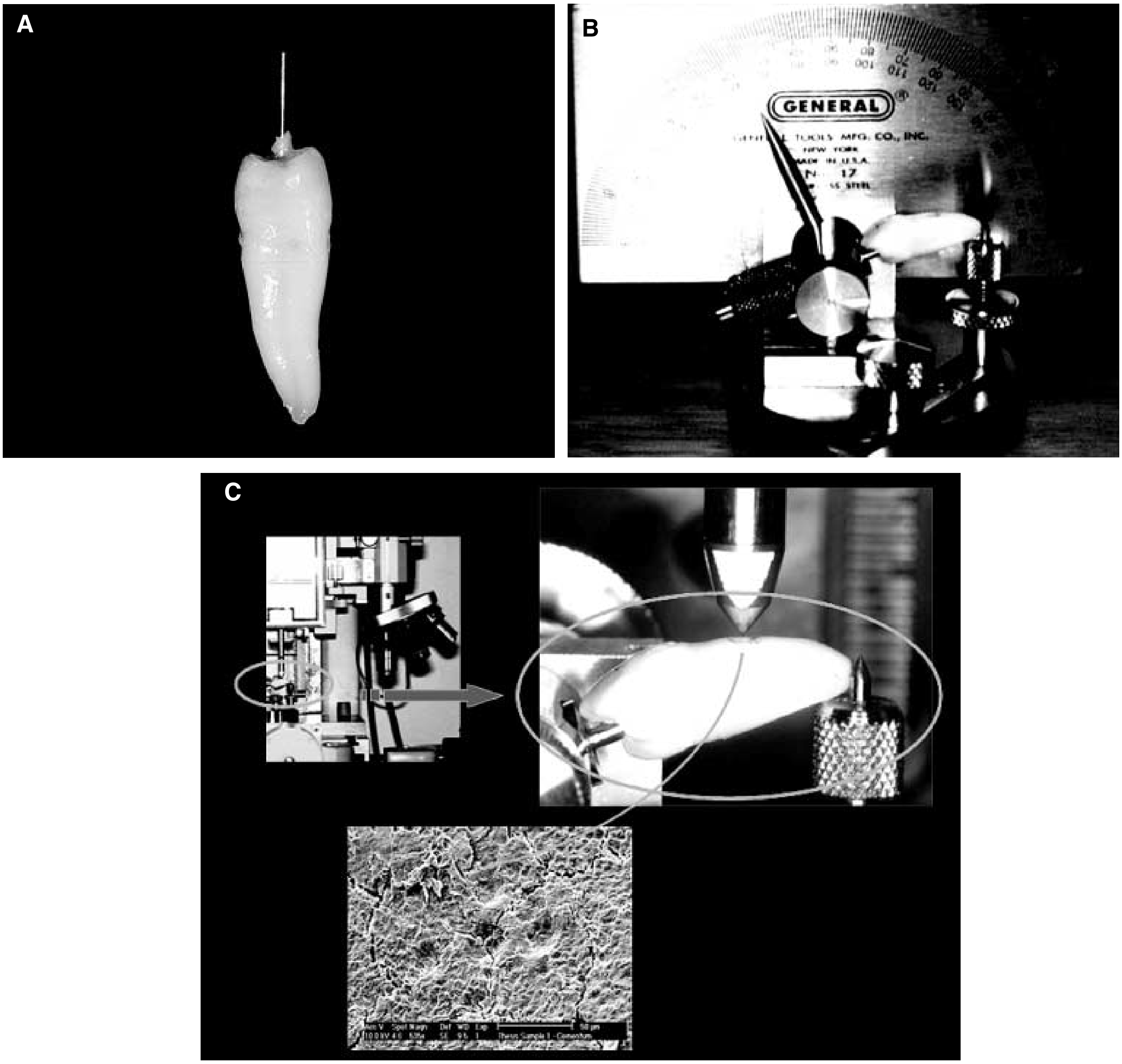


Fig. 1. (A) After extraction of the premolars, a long shank diamond bur was inserted into the pulp chamber and canal parallel to the long axis of the tooth and cemented with glass–ionomer cement to support the tooth during testing. (B) Newly designed surveyor that allowed sample retrieval and three-dimensional rotation, which also enable non-destructive testing and re-testing of the root surface. (C) Ultra Micro Indentation System (UMIS) – 2000 developed at the Commonwealth Scientific and Industrial Research Organization, Lindfield, Australia. Each coordinate comprised five individual indents in a 100 μm square array.

female subjects, a 3D mapping of hardness and elasticity of the surface cementum and enamel of the teeth was performed at 62 sites (Fig. 2A) on each tooth using the UMIS-2000 and the method described earlier. This sample served as a baseline value for our subsequent studies and 3D colored digital maps were reproduced for cementum hardness and elastic modulus (Fig. 2B). There was a significant increase in the hardness from the apical to the cervical regions (Fig. 2C).

This method permitted the quantification of the hardness and elastic modulus along the root surface as well as on the buccal and lingual enamel surfaces of a minimally prepared tooth. As a result of the minimal preparation, the sample was easily retrievable for further investigations. This method, used with the location device, allowed the relocation of a given test site and therefore enabled repeated testing at that site.

The effect of different storage methods (32)

Ideally, teeth to be tested for their physical properties should be stored in a medium that is a replicate of *in vivo* conditions (42). Storage requires maintaining

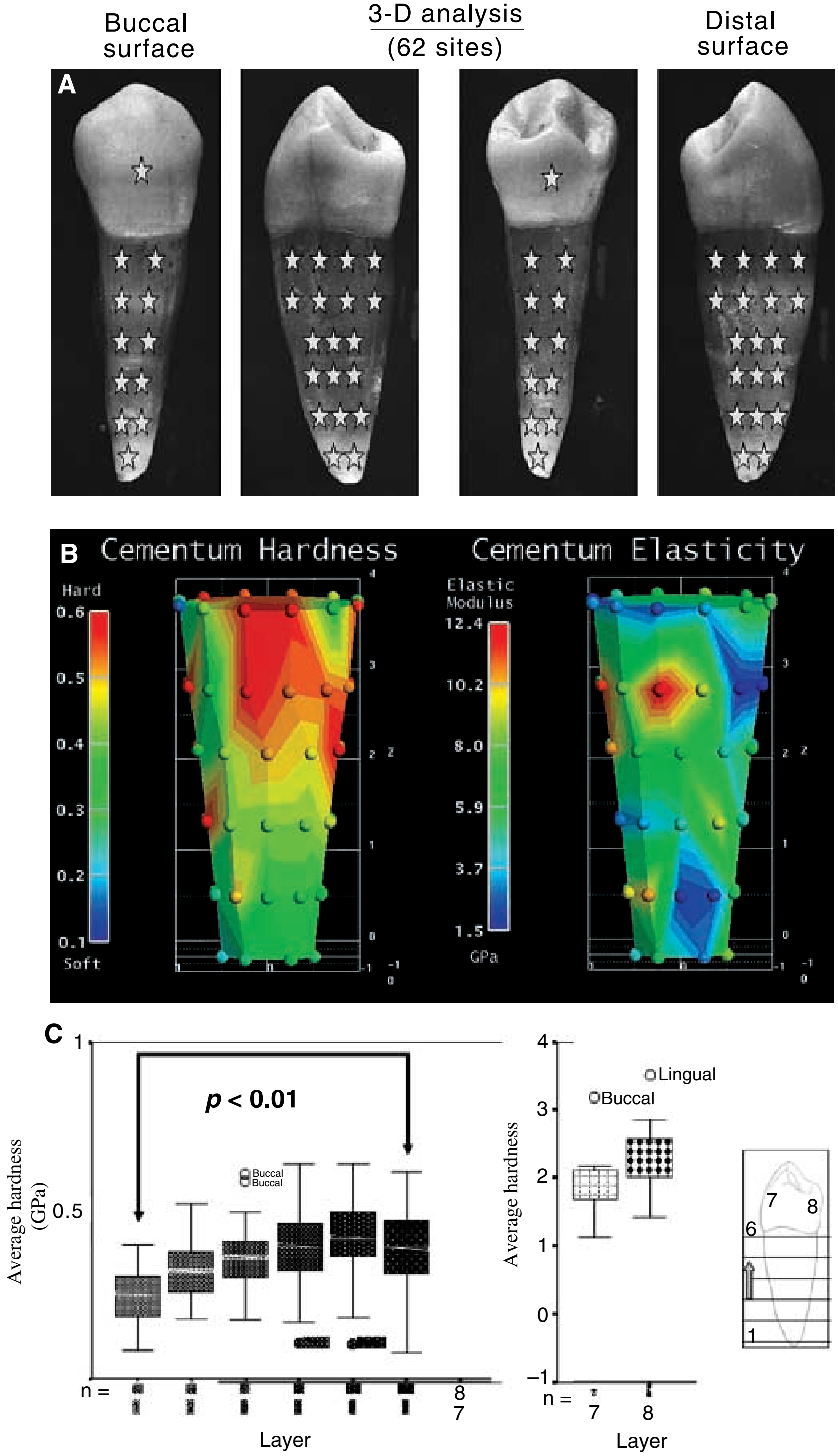


Fig. 2. (A) The number and location of the indentation spots on different surfaces of the premolar tooth. There were a total number of 60 spots on the root surface and two on the enamel. (B) Color-coded map of hardness and elastic modulus of a premolar tooth. Hardness and elastic modulus increases from blue to red. (C) Hardness values at different layers around the root are shown. There is a significant increase in the hardness from apical to cervical regions.

hydration of the tissues until after testing (42). Over the years researchers have used a variety of methods to store the teeth from dry air storage (41–44), water and its modifications, Saline and its modifications and preservatives like formalin and body fluids like saliva. Tap water was used by LeFevre and Manly (45), Rautiola and Craig (39), distilled water by Mahoney et al. (46), Addy and Mostafa (47), Aquilino et al. (48), de-ionized water by Poolthong (37) and distilled water with thymol by White et al. (49). Saline with some modifications was used by a number of researchers; this included aqueous sodium chloride (48), and saline solutions including Krebs–Ringer phosphate buffer (50, 51), phosphate-buffered saline (42, 49, 52), phosphate-buffered saline with sodium azide (51, 53) or thymol (54, 55), physiologic saline (43, 56, 59), formal-saline (43), isotonic saline with sodium azide (57–60) and HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) saline (61). Other investigators have tested specimens after storing in formalin (49, 56), ethanol (49, 56), and saliva (62).

Human tooth structure is mainly calcified material with a variable proportion of water and/or organic matrix. The nature of the storage method, its chemical composition, pH and duration of the storage period has the ability to affect properties of adsorption, diffusion and dissolution, and therefore, possibly alter the physical properties of cementum/dental hard tissues. Most studies in the literature have investigated the effect of various sterilization/disinfection and storage protocols on enamel and dentine (37, 39, 42, 55); however, very few studies have examined the effect of such protocols on the hardness and elastic modulus of cementum (39, 41).

This study was aimed at finding out the most suitable storage medium and protocol for testing of hardness and elastic modulus of human teeth. Five disinfection and/or storage protocols over different periods of time were investigated. A sample of 20 first premolars, which was divided into five groups of four teeth and stored in one of the following ways:

Group 1: Miltons™ solution (1% sodium hypochlorite) for 10 min.
Group 2: Miltons™ solution for 24 h.
Group 3: 70% alcohol.
Group 4: Desiccation.
Group 5: Milli Q® (de-ionized water, Millipore, Bedford, MA, USA).

Teeth in groups 1 and 2 were initially stored in Milli Q®, tested within 6 h from extraction, placed in their respective media and re-tested. Groups 3, 4 and 5 were tested within 6 h, then at 1 month, 2 months and 3 months after extraction. Group 5 was further investigated at 9 months and two teeth in group 4 were tested at 4 months. The hardness and elastic modulus of cementum was tested on unprepared specimens mounted on a 3D jig assembly using the UMIS.

The results showed a great variability of the effects in five different storage protocols. Desiccation caused a significant increase in both the hardness ($p = 0.02$) and elastic modulus ($p = 0.04$) from baseline to 3 months, with most changes occurring within the first month (Fig. 3) and hence this method should be abandoned. Storage in Miltons™ solution for 10 min had no significant effect while storage for 24 h caused a significant decrease in the hardness of cementum ($p = 0.03$). Hence use of Miltons™ solution for 10 min can be considered an appropriate method for disinfection and removal of fragments of the periodontal ligament without adversely affecting the hardness or elastic modulus of cementum.

Storage in 70% alcohol for up to 4 months and in Milli Q® for up to 9 months had minimal effects which were statistically not significant. We used and recommend Milli Q® for our future studies to store the teeth.

Physical properties of root cementum: second phase

The first phase of the studies have established a standard protocol for storage of teeth and methodology of use of 3D-jig and nano-indentation system (UMIS-2000) in mapping the hardness and elastic modulus of the cementum.

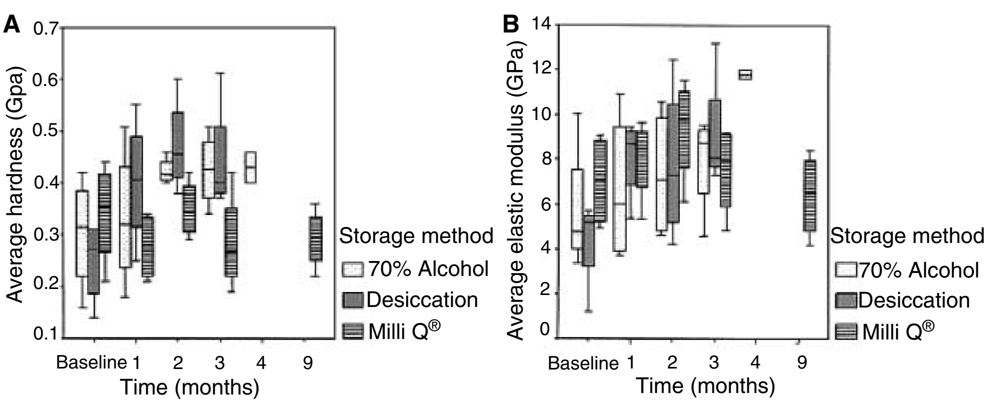


Fig. 3. Changes in hardness (A) and elastic modulus (B) in time with different storage media.

A series of further studies were executed on a core sample of human first premolars, which were subjected to light and heavy orthodontic forces. As the 3D-jig method and use of nano-indentation permit preservation of the tooth structures this sample could be used for future studies. They included studies on mineral composition, alteration in mineral contents and volumetric measurement of resorption craters.

Core sample

This sample consisted of 36 human maxillary and/or mandibular first premolars derived from sixteen orthodontic patients (10 males, 6 females) in the age range of 11.7–16.1 years (mean age 13.9 years) requiring at least bilateral first premolar extraction. (Ethical approval: Project 5/98 CSAHS Human ethics review committee UDH). All subjects completed a written informed consent. The subjects included in the study met following selection criterion:

1. Completed apexogenesis of the first premolars confirmed on periapical radiographs.
2. No previous reported or observed dental treatment in the first premolars including orthodontic treatment.
3. No history of trauma and negative history of bruxism.
4. No past and present signs and symptoms of any periodontal disease.
5. No significant medical history that would adversely affect the development or structure of the teeth and jaws.
6. Knowledge of child's residence since birth and history on fluoride consumption.

Each patient received an active sectional appliance in the maxillary arch on one side and a passive appliance on the contralateral side, which served as control. Speed brackets (Strite Industries, Ontario, Canada) were bonded on the first premolars and first molars. β-Titanium–molybdenum alloy (TMA, Ormco, CA, USA) springs were activated to the desired force levels. The right or left first premolars were randomly selected with one time initial buccally directed light force of 25 g (Group 1), and a heavy force of 225 g (Group 2) applied (Fig. 4A–D). The experimental and control first premolar teeth were extracted 28 days after the initial force application. There was no force re-activation during this period. Extractions were performed by either of the two oral surgeons assigned the task; requested to avoid any forceps contact on the cervical cementum. Immediately after extraction, the teeth were stored in sterilized deionized water (Milli Q®) at 23 ± 1°C until due for testing according to the protocol described earlier (31, 32).

Changes in physical properties of human premolar cementum after the application of controlled orthodontic forces (33)

The review of literature does indicate that the hardness of cementum that is influenced by the mineral contents and composition of the cementum could alter its response to the orthodontic force and therefore the process of root resorption. There is a likelihood that the harder the cementum, the less likelihood there is of its resorption (22). Studies from University of Sydney have reported that cementum at the cervical and middle thirds of the root has greater hardness and elastic modulus than that of the apical third (31, 37); apparently because of the variable mineral content of cellular and acellular cementum. It was also found that hardness was positively correlated to the amount of mineralization (63, 64).

The study was conducted to first establish a normative data of hardness and elastic modulus of cementum of young human unprepared teeth on a large sample (Core sample described above) using previously described methodology. The study was also aimed to find out if there were any alterations in the hardness and elastic modulus following application of light and heavy orthodontic force. This sample was further explored for the mapping of the mineral contents in normal and orthodontically treated teeth (Fig. 4E).

In the control groups, the mean hardness on cervical, middle and apical thirds on the buccal surface was 0.25 ± 0.09, 0.24 ± 0.07 and 0.18 ± 0.06 GPa respectively, and on the lingual surface was 0.24 ± 0.08, 0.24 ± 0.06 and 0.21 ± 0.06 GPa. Mean elastic modulus on cervical, middle and apical thirds on the buccal surface was 4.4 ± 2.4, 3.4 ± 2.0 and 2.4 ± 1.8 GPa respectively, on the lingual surface was 3.8 ± 2.1, 3.2 ± 1.4 and 2.4 ± 1.5 GPa (Table 1).

Light force vs. heavy force group

There were no significant differences for hardness and elastic modulus between Group 1 (light force) and

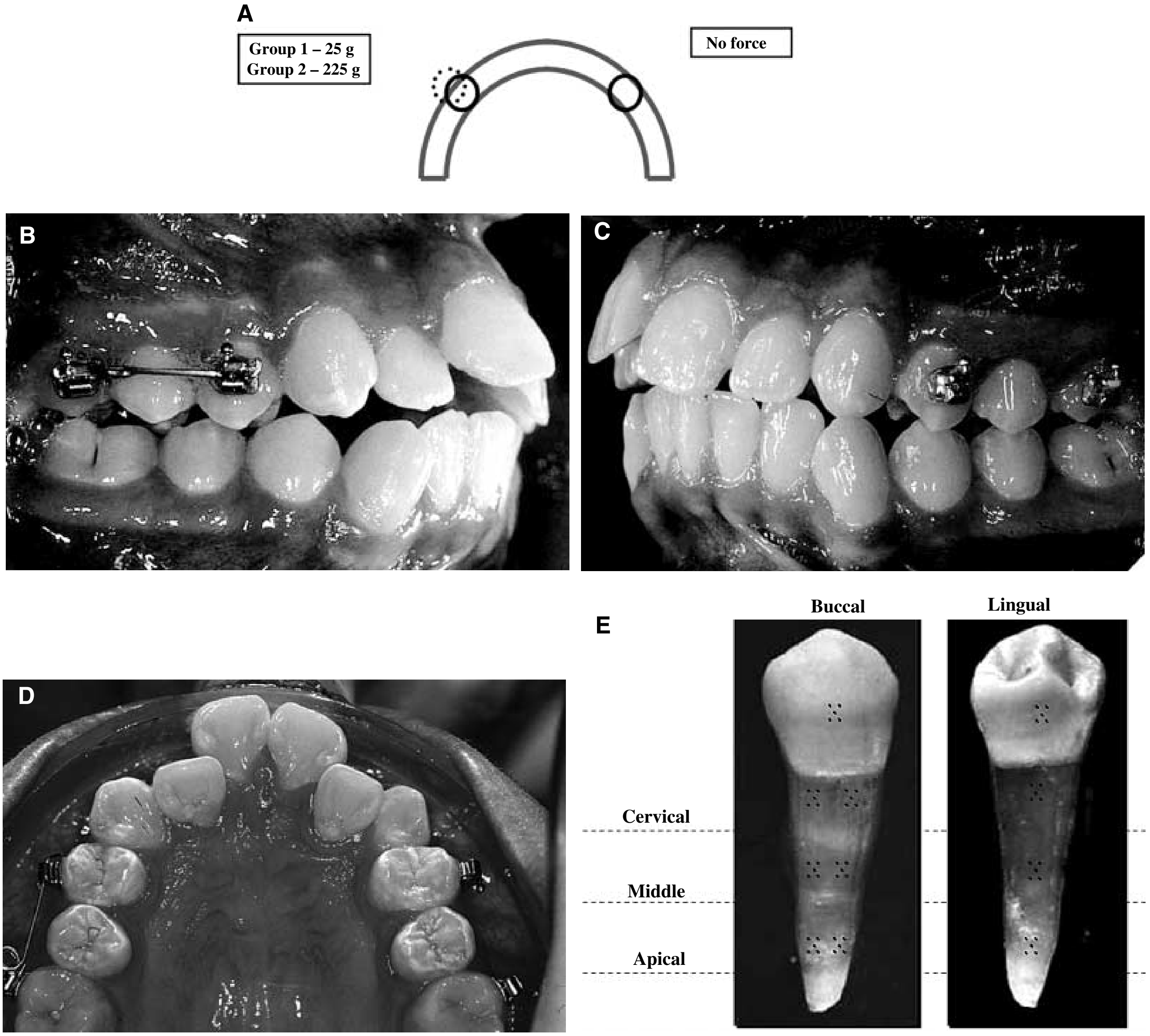


Fig. 4. (A) Diagrammatic representation of light (Group 1) and heavy (Group 2) force groups. Dotted circle on the left side indicates displacement of first premolar. (B) Experimental side with β-titanium–molybdenum alloy wire engaged in speed brackets bonded on the first molar and premolar. (C) Control side without a wire engaged in speed brackets bonded on the first molar and premolar. (D) Occlusal view of the experimental and control sides in the same patient. (E) Location of indentation sites on premolar cementum and enamel.

Group 2 (heavy force) ($p = 0.15$ for hardness, $p = 0.99$ for elastic modulus) and no significant effects for different tooth positions ($p = 0.20$ for hardness, $p = 0.94$ for elastic modulus) (Table 2).

There was a marginally significant interaction between Groups 1 and 2 (light vs. heavy) and position on tooth (cervical, middle and apical thirds) for hardness ($p = 0.05$) and elastic modulus ($p = 0.04$). The changes in hardness and elastic modulus varied in magnitude between Group 1 (light force) and Group 2 (heavy force) at cervical middle and apical thirds but only with marginal significance. Subjects were significantly different in their reaction to the forces as far as elastic modulus was concerned ($p = 0.001$), but not for hardness ($p = 0.16$). There was no obvious trend or pattern in the data consistent with the results of analysis (Fig. 5A–D) (Table 3).

The hardness and elastic modulus of human premolar cementum gradually decreased from cervical to apical areas for buccal as well as lingual surfaces in untreated teeth. A similar trend was also observed in the teeth after application of light and heavy forces.

Table 1. **Mean and SD values for hardness (GPa) and elastic modulas (GPa) in control group (0 force) on buccal and lingual surfaces at three positions**

	Hardness (control)			Elastic modulus (control)		
Position on tooth	N	Mean	SD	N	Mean	SD
11 Buccal-cervical	36	0.2459	0.0896	36	4.405	2.392
12 Buccal-mid	36	0.2353	0.0684	36	3.364	2.004
13 Buccal-apical	36	0.1787	0.0593	36	2.439	1.763
21 Lingual-cervical	18	0.2420	0.0787	18	3.762	2.109
22 Lingual-mid	18	0.2363	0.0629	18	3.196	1.357
23 Lingual-apical	18	0.2062	0.0629	18	2.386	1.506
Total	162	0.2227	0.0755	162	3.307	2.063

Apical cementum has the lowest hardness and elastic modulus values, and cervical cementum has the highest values. The application of light and heavy forces did not show any statistically significant differences in hardness and elastic modulus when compared with untreated teeth.

This study has provided baseline data on hardness and elastic modulus of the untreated human premolar cementum, which can be used as a reference for future studies. The experimental premolars are being further investigated for the changes in the mineral contents and location, types and volumes of resorption craters with the application of the light and heavy forces (Fig. 6).

A quantitative analysis of the mineral composition of human premolar cementum: an EPMA study (34)

The same sample of teeth was used to investigate the changes in the calcium (Ca), phosphorus (P) and fluoride (F) concentrations in cementum after the application of light (25 g) and heavy (225 g) orthodontic forces. This study was based on the premise that the mineral content of cementum may influence resistance or susceptibility to root resorption (22, 30, 67).

Quantitative analysis on the mineral composition of human cementum has shown mean Ca concentrations to vary from 24.0 to 29.24% (68–71) dry wt or 35.6–42.4% ash (72). Mean P concentrations have been found to vary from 11.6 to 13.49% dry wt (68–71) or 16.6–20.0% ash (72). F concentrations in cementum are dependent on the level of systemic and local F exposure and show an increase with age (72–76), stronger F concentrations in the drinking water, and greater duration of exposure to the oral

Table 2. **Mean and SD values for (a) hardness (GPa) and (b) elastic modulus (GPa) in experimental groups (light force and heavy force) on buccal and lingual surfaces at three positions**

	1 Light			2 Heavy			Total		
Position on tooth	N	Mean	SD	N	Mean	SD	N	Mean	SD
(a) Hardness									
11 Buccal-cervical	18	0.2715	0.0796	18	0.2748	0.0957	36	0.2732	0.0868
12 Buccal-mid	18	0.2526	0.0750	18	0.2164	0.0622	36	0.2345	0.0703
13 Buccal-apical	18	0.2329	0.0642	18	0.1801	0.0553	36	0.2065	0.0649
21 Lingual-cervical	9	0.2474	0.0792	9	0.2111	0.0838	18	0.2293	0.0813
22 Lingual-mid	9	0.2591	0.0641	9	0.1957	0.0623	18	0.2274	0.0694
23 Lingual-apical	9	0.2241	0.0774	8	0.1781	0.0736	17	0.2024	0.0770
Total	81	0.2494	0.0729	80	0.2146	0.0795	161	0.2321	0.0780
(b) Elastic modulus									
11 Buccal-cervical	18	4.087	1.843	18	3.912	2.164	36	4.000	1.983
12 Buccal-mid	18	3.347	1.616	18	2.245	1.211	36	2.796	1.514
13 Buccal-apical	18	2.833	1.431	18	1.558	0.79113	36	2.196	1.310
21 Lingual-cervical	9	4.211	1.946	9	3.150	1.610	18	3.681	1.817
22 Lingual-mid	9	3.730	1.433	9	2.669	1.110	18	3.200	1.358
23 Lingual-apical	9	2.268	1.298	8	1.878	1.205	17	2.085	1.232
Total	81	3.146	1.695	80	2.578	1.652	161	3.000	1.721

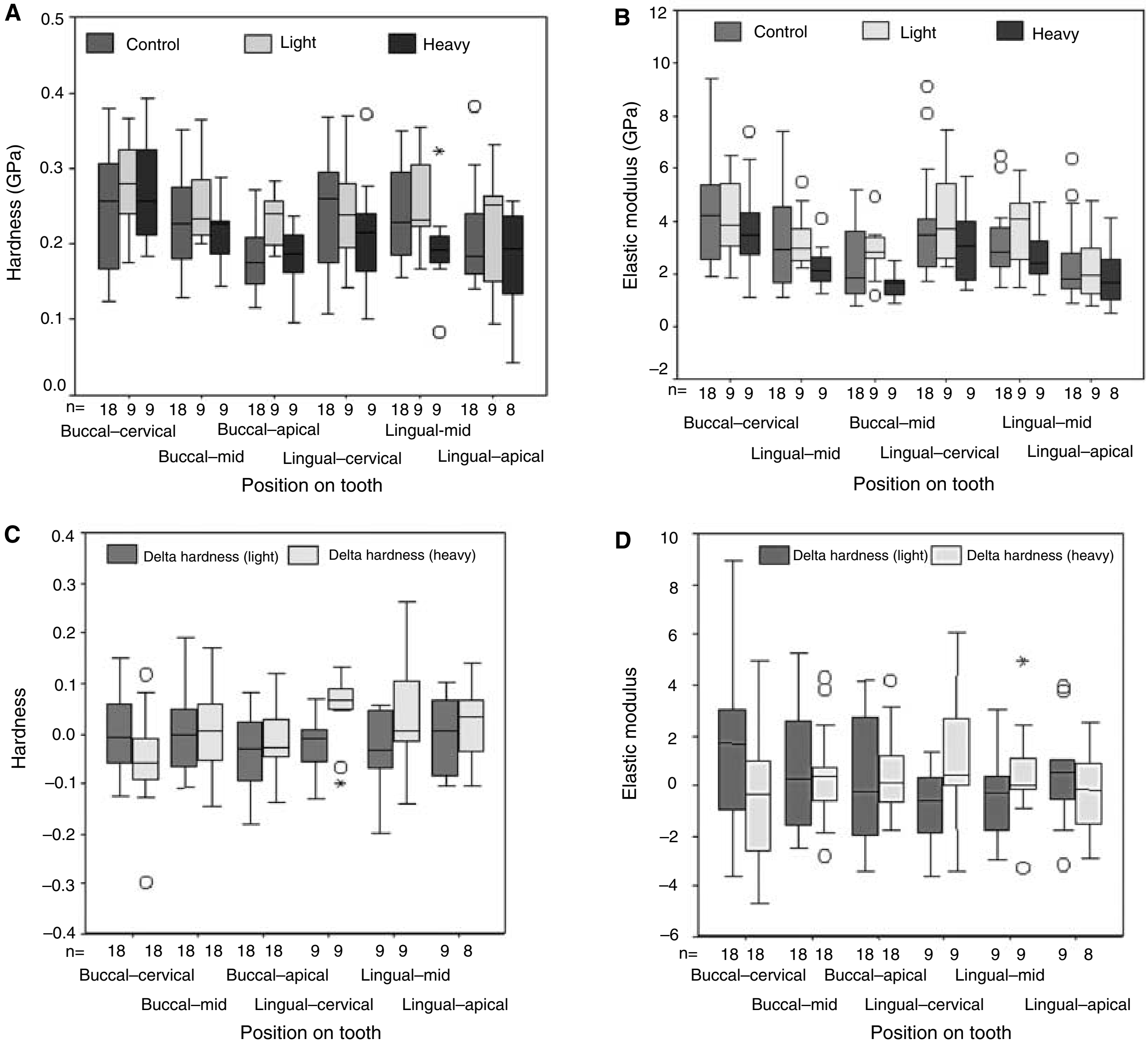


Fig. 5. (A) Box plot of hardness (GPa) in control (0 force), light force and heavy force groups on buccal and lingual surfaces at three positions. (B) Box plot of elastic modulus (GPa) in control (0 force), light force and heavy force groups on buccal and lingual surfaces at three positions. (C) Box plot for change in hardness (delta hardness = control minus experimental) for light and heavy force teeth on buccal and lingual surfaces at three positions. (D) Box plot of change in elastic modulus (delta elastic modulus = elastic modulus control minus experimental) for light and heavy force groups on buccal and lingual surfaces at three positions.

environment (72, 73, 77). In an optimally fluoridated area (0.8–1.3 ppm), concentrations at the cervical, middle and apical third of human cementum have been reported to range from 0.2 to 0.9% wt (72, 73, 77), 0.25–0.79% wt (73), and 0.15–0.8% wt (72, 73, 77), respectively.

Differences in the mineral composition of cementum exist among different individuals, from tooth to tooth in the same individual, within the same individual at different ages, (68–72) and between the various cementum types (78, 79).

Existing quantitative studies, however, have been limited by small sample sizes (68, 69, 74), non-homogeneous tooth samples (69, 72, 77, 80–82), and large age ranges of individuals from which the teeth were obtained (69, 73–76, 80–82).

Both chemical analysis and EPMA have been used for quantitative mineral analysis, however, the latter technique is preferred as it is a non-destructive technique with a high level of sensitivity, which can determine the composition of a number of elements at any one time (34).

***Table 3*. Mean and standard errors for change in (a) hardness (control minus experimental) and (b) elastic modulus (control minus experimental) for light and heavy force groups on buccal and lingual surfaces at three positions, based on a model with Subject (hardness: $p = 0.61$; elastic modulus: $p = 0.001$), Position (hardness: $p = 0.20$; elastic modulus: $p = 0.94$), Strength (hardness: $p = 0.15$; elastic modulus: $p = 0.99$) and position-strength interaction (hardness: $p = 0.05$; elastic modulus: $p = 0.04$) as explanatory factors**

	Force			
	Light		Heavy	
Position on tooth	Mean	SE	Mean	SE
(a) Hardness				
Buccal-cervical	−9.02E-04[a]	0.019	−5.35E-02[a]	0.019
Buccal-mid	5.737E-04[a]	0.019	1.178E-03[a]	0.019
Buccal-apical	−3.94E-02[a]	0.019	−1.63E-02[a]	0.019
Lingual-cervical	−1.99E-02[a]	0.027	4.547E-02[a]	0.027
Lingual-mid	−2.94E-02a	0.027	4.717E-02[a]	0.027
Lingual-apical	−5.12E-03[a]	0.027	1.992E-02[a]	0.028
(b) Elastic modulus				
Buccal-cervical	1.379[a]	0.508	−0.569[a]	0.508
Buccal-mid	0.722[a]	0.508	0.365[a]	0.508
Buccal-apical	0.102[a]	0.508	0.384[a]	0.508
Lingual-cervical	−0.831[a]	0.718	0.994[a]	0.718
Lingual-mid	−0.470[a]	0.718	0.462[a]	0.718
Lingual-apical	0.538[a]	0.718	−0.204[a]	0.767

[a] Based on modified population marginal mean.

This study examined the Ca, P and F concentrations in human first premolar cementum using EPMA, and was performed to generate baseline data that was used to examine the changes in the mineral content of cementum subsequent to the application of orthodontic forces.

The sample for normative data comprised 18 maxillary and/or mandibular first premolar teeth, which comprised the control component (0 g) of the core sample of 36 first premolars. These premolars were air-dried, resin-embedded, sectioned, polished and carbon coated (evaporated) in a vacuum at 10^{-4} Torr with a 300 Å layer (Fig. 6A). The Ca, P and F concentrations were measured in intact cementum on the buccal and lingual surface at the midpoint of the cervical, middle and apical third of the root. At each measurement at the cervical, middle and apical third, 15 points were measured, consisting of five lines of 3 points each; each line extending from the outer to middle to inner third of the cementum (Fig. 6B). A total of 90 points were analysed per tooth, making a total of 1620 measurement points in the 18 teeth.

Results have highlighted a significant inter-individual variation in the Ca, P and F concentrations ($p = 0.024$, 0.017 and 0.000, respectively) in human first premolar cementum. There was no significant difference in the Ca, P and F concentrations of cementum between the buccal and lingual surface, except for a significantly higher F content at the cervical region on the buccal surface ($p = 0.000$). There was a decreasing gradient in the Ca, P and F concentrations from the cervical to apical third of the root which was highly significant from the cervical to middle third ($p = 0.000$) and middle to apical third ($p = 0.000$), except for F where there was no significant difference from the cervical to middle third on the lingual surface ($p = 0.966$) (Fig. 7A–C). There was a significant increasing gradient in the Ca and P concentrations from the outer to inner third of cementum at the cervical ($p < 0.01$) and middle ($p < 0.01$) third of the root, but no significant difference at the apical third of the root. For F, there was a significant decreasing gradient from the outer to inner third of cementum at the cervical ($p < 0.01$), middle ($p < 0.01$) and apical ($p < 0.01$) third of the root.

A comparative quantitative analysis of the mineral composition of human premolar cementum after the application of orthodontic forces – an EPMA study (Rex et al, unpublished data)

The baseline data obtained from the sample of control teeth on the mineral composition (Ca, P and F) of cementum (34) was compared with the mineral composition of cementum on the experimental first premolars which were subjected to light (25 g) and heavy (225 g) orthodontic forces.

Results showed that there was little change in the mineral composition of cementum after the application of light forces, however, there was a trend toward an increase in the mineral composition (Ca, P) of cementum at various areas of periodontal ligament compression. The application of heavy forces caused a significant ($p = 0.000$) decrease in the Ca concentration of cementum at certain areas of periodontal ligament tension. The application of both light and heavy orthodontic force did not appear to influence the F concentrations in cementum (Fig. 8A–C). It was not

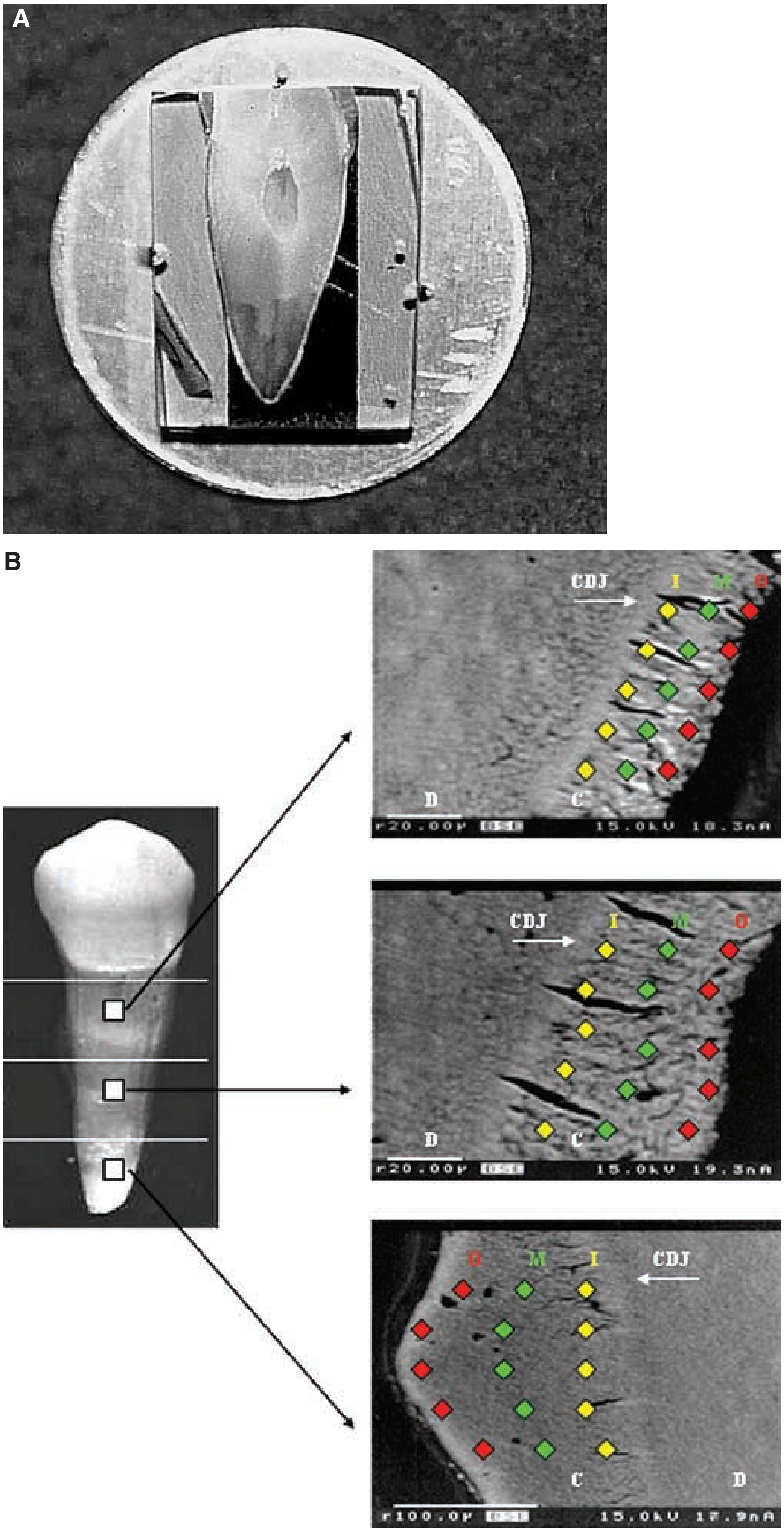


Fig. 6. (A) Completed specimen polished, mounted and carbon coated. (B) Points of analysis on the buccal and lingual surface at the cervical, middle and apical third of the root. D, dentine; C, cementum; CDJ, cemento-dentinal junction (hypermineralized zone); O, points of analysis at the outer third of cementum; M, points of analysis at the middle third of cementum; I, points of analysis at the inner third of cementum.

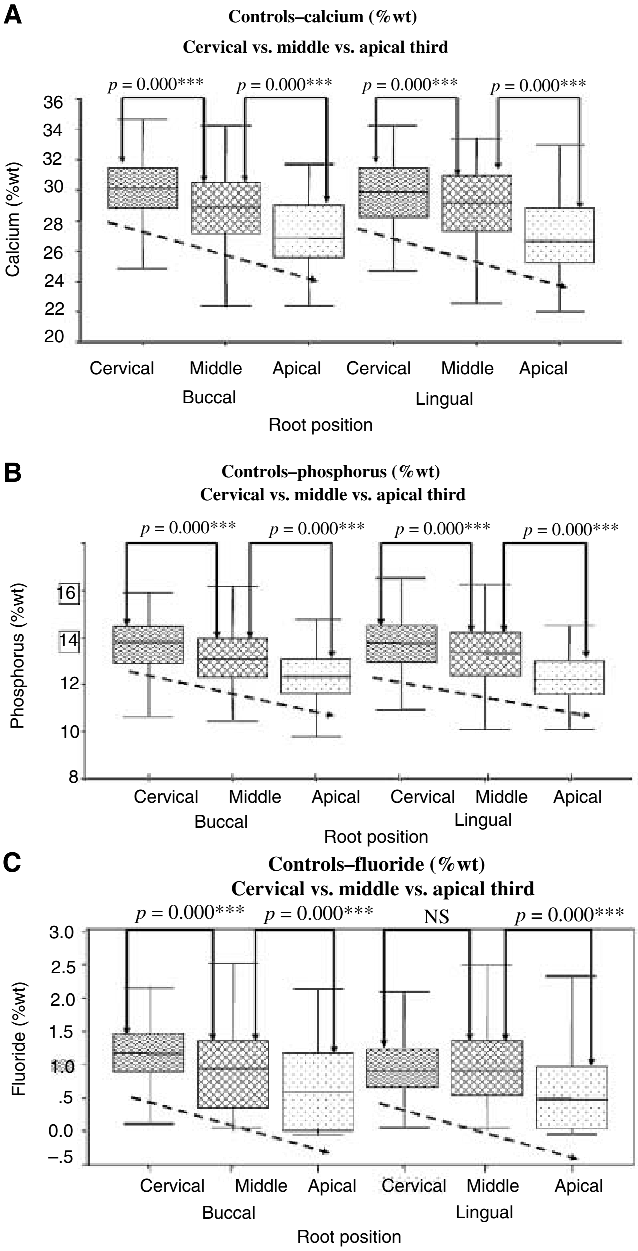


Fig. 7. Variation of the percentage weight of (A) calcium, (B) phosphate and (C) fluoride from the cervical to apical zones on the buccal and lingual sides.

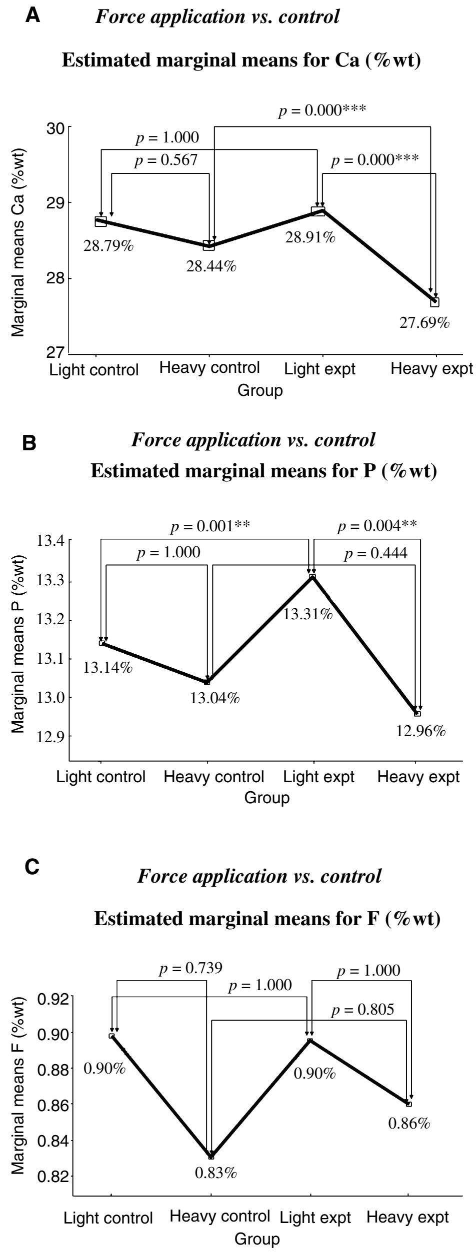


Fig. 8. (A) Percentage weight of calcium in control and force application groups. There is a significant decrease in the heavy force vs. control teeth in the percentage weight of calcium. (B) Percentage weight of phosphate in control and force application groups. There is a significant decrease in the heavy force vs. control teeth in the percentage weight of phosphate. (C) Percentage weight of fluoride in control and force application groups. There is no significant difference between the force application vs. control teeth in the percentage weight of fluoride.

possible to explain the trend in mineral composition changes with the application of orthodontic forces given our current understanding of tooth movement and the root resorption process.

Volumetric analysis of root resorption craters and volumetric analysis of root resorption craters after application of light and heavy orthodontic forces: a scanning electron microscopic (SEM) study (35)

It has been noted that the type of treatment and mechanics largely determines the location of resorption. In palatal expansion, resorption develops mainly in the cervical part of the mesiobuccal surfaces and furcation areas with only limited involvement of other areas (81). However, such cervical resorption generally remains undiagnosed unless it is extensive, whereas apical root resorption is often readily visible on radiographs (17, 19, 82).

The topography of tooth root surfaces has been examined using radiographs (83), light microscope (18) and SEM (24, 25, 84). It has been reported that SEM provides enhanced visual and perspective assessment of root surfaces, and when recorded in stereo pairs, they provide resolution and detail not attainable with histological models reconstructed from serial sections (18). The resorption craters so far have been mainly looked for their quality and their quantitative evaluation has been limited to their size in two dimensions.

Studies by Hellsing and Hammarström (85) on rat molars and Rygh (28) on Wistar rats showed changes in root morphology and resorption process after application of orthodontic forces. Rygh's study on human teeth showed similar findings. There was no attempt at quantitative analysis of resorption in these studies. Earlier, Kvam (86) found resorption craters were initiated as small cavities with diameters measuring about 6 μm on the pressure side of the experimental group after application of 50 g of force for 5, 10, 15, 20, 25, 30, 45 and 76 days. He noted that marginal root resorption began at 10 days and the number of small, round and thin walled craters increased and then merged into extensive but shallow excavations. He also noted that all teeth that had been moved for a period longer than 15–20 days showed marginal root resorption while in those observed for longer than 25 days, the resorption had extended into dentine. Acar et al. (87) compared the effects of continuous and discontinuous forces on root resorption in a human model. Measuring two-dimensional (2D) composite electron micrograph images of the specimen, the authors concluded that there was less resorption and apical blunting was less severe with discontinuous force. However this paper has been put up for debate (88). In previous studies resorption was quantified by making histological cuts or by surface area measurements of resorption craters.

We aimed to examine the craters not only from the perspective of surface area but also their depth and to quantify the craters by measuring their volumes. The first obstacle in this regard was first to establish a methodology, which could enable us to study the microscopic resorption craters in 3D and record the spatial positions of the surfaces of the craters and thereby to determine their volumes. The study of craters volume, their location and qualitative characteristics in relation to type of tooth movement and force levels will pave a way for better understanding of the resorption process.

Thirty-six human first premolars of the core sample previously described were prepared for SEM imaging. Pairs of stereo images (Fig. 9A) were taken and 3D quantitative volumetric analysis was performed using *AnalySIS Pro 3.1* (SIStereo Imaging, Münich, Germany) (Fig. 9B). It was found that mean volume of the resorption crater in light-force group was 3.49-fold greater than the control group, and the heavy-force group 11.59-fold more than control group ($p < 0.001$). The heavy force group had 3.31-fold greater total resorption volume than the light force group ($p < 0.001$) (Fig. 9C).

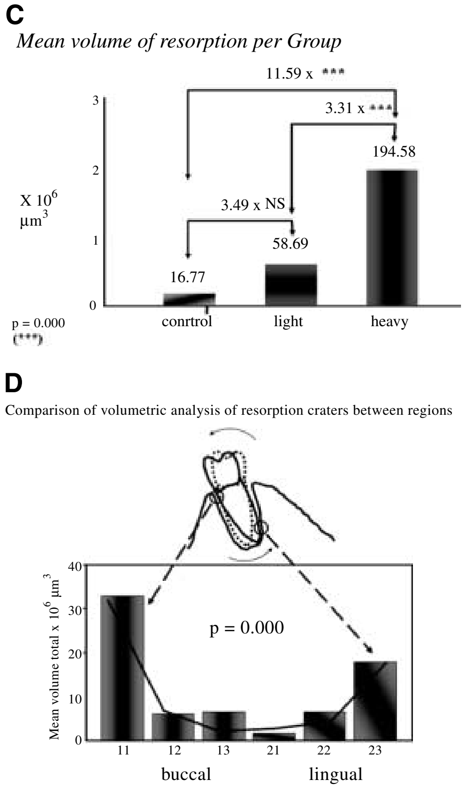
Buccal cervical and lingual apical regions demonstrated significantly more resorption craters than the other regions ($p < 0.001$) (Fig. 9D). There was more resorption by volume in the heavy force group as compared to the light group and controls. Although there was more resorption recorded in the light group, the difference in amount of resorption between the light and control groups was not of significant statistical difference. There was significantly more resorption on the buccal cervical and lingual apical regions of the root surfaces than other regions suggesting that high-pressure zones may be more susceptible to resorption after 28 days of force application.

This has been the first successful attempt to quantify volume of resorption craters which has been developed, tested and executed in the measurement of the volume of the craters (89, 90). A further attempt was made to correlate findings of the 2D measurements with the 3D measurements obtained through a newly developed technique.

Validation of 2D vs. 3D measurements of root resorption craters on human premolars after application of light and heavy orthodontic forces (Chan et al, unpublished data)

Previous findings by the authors have demonstrated that accurate 3D volumetric measurements of resorption craters could be obtained. A mathematical analysis of the 2D/3D relationship enabled us to determine an appropriate digital model for the shape, type and dimensions of resorption craters. It also helped to resolve the issue of 'hemispheric' model vs. a 'layered' model of craters.

The 2D measurements were strongly correlated to 3D measurements ($r = 0.991^{**}$). Within the light and heavy force groups, the measurements were strongly



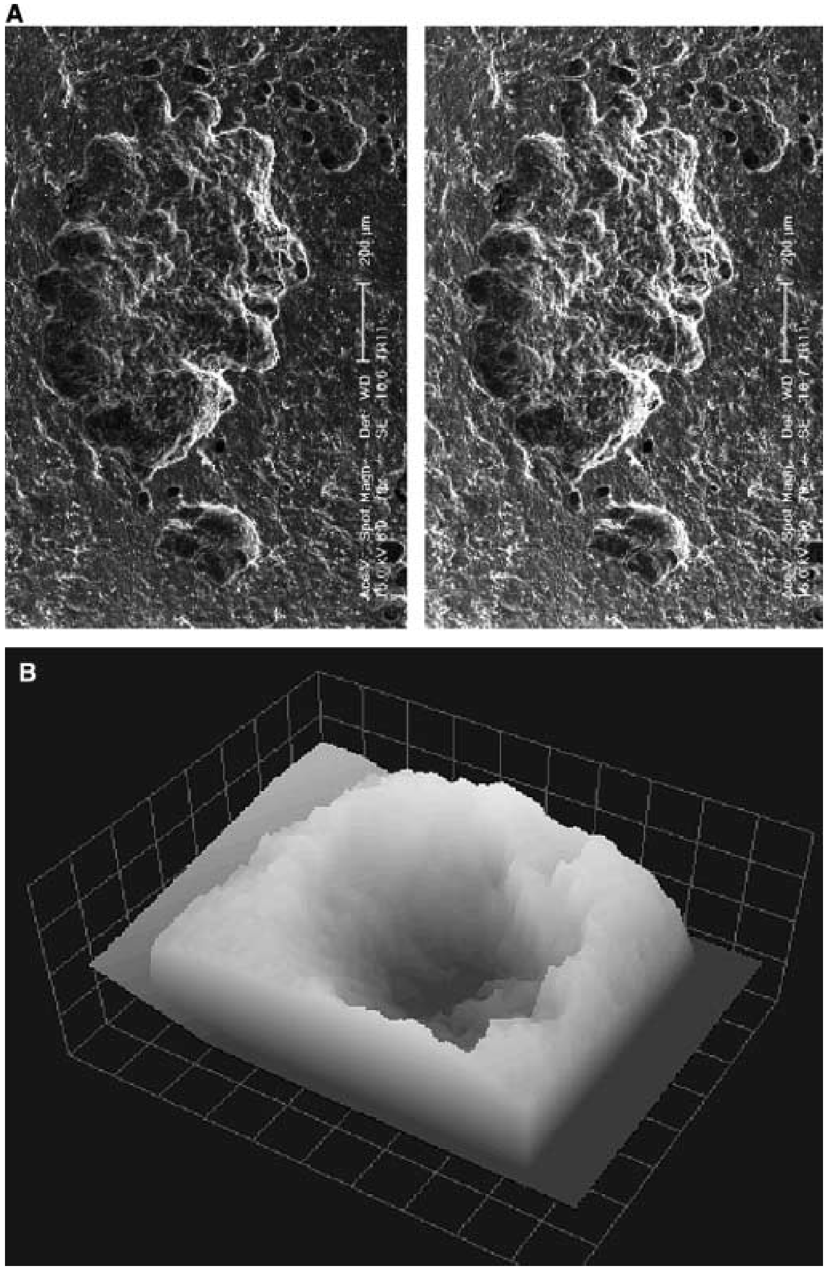


Fig. 9. (A) A pair of image taken at ±3° to obtain the stereo image of the crater. (B) Three-dimensional image of the root resorption crater. (C) The mean volume of the resorption crater in light force group was 3.49-fold greater than control, and the heavy force group 11.59-fold more than control group. The heavy force group had 3.31-fold greater total resorption volume then light force group. (D) Comparison of volumetric analysis of resorption craters between regions. Buccal cervical and lingual apical regions demonstrated significantly more resorption than the other regions.

correlated as well ($r = 0.978^{**}$ and $r = 0.994^{**}$ respectively). In a 4-week experimental period, 2D measurements of root resorption craters are as reliable as 3D measurements if they are conducted adequately.

It was concluded that 2D measurements of root resorption craters are as reliable as 3D measurements in terms of quantifying the severity and for correlating with the force levels. A typical crater (area < $2 \times 10^6 \mu m^2$ and volume < 300×10^6 μm^3) in this study conformed more to a hemispheric model; larger craters tend to be layered. Extended duration (8 weeks) of force applications needs to be investigated to determine whether deeper resorption craters occur in relation to duration.

Summary and future directions

New methods to evaluate the physical properties and mineral composition of human root cementum have been devised. Two main issues of research related to the determination of the physical properties of dental tissues were lacking in information: 1; on the appropriate storage method and duration protocol, and 2; necessity of tooth embedding and sectioning required for the material to be tested with industrial indenters.

A storage protocol for the tooth material to be tested for physical properties was formalized and it was found that Milli Q® was the most appropriate storage media if the teeth need to be stored for a duration of a few weeks. The use of tooth embedding in sample preparation and sectioning was eliminated with the advantages of UMIS-2000 that allowed elastic modulus and hardness to be tested directly on a cementum surface of the unprepared tooth. For the testing to be done on all the surfaces a repositioning device was developed and using an initial sample of teeth, a complete map of hardness and elastic modulus of all cementum surfaces of control teeth was developed. This standardized methodology was repeated on a larger sample whereby normative data on hardness and elastic modulus was generated.

The hardness and elastic modulus of human premolar cementum gradually decreased from cervical to apical regions for buccal as well as lingual surfaces. A large intra-individual variation for both hardness and elastic modulus was found.

A statistical analysis of data of physical properties on control teeth with those who had been subjected to orthodontic forces did not reveal significant variations probably because of large inter-individual variations. Current investigations are in progress to look into these properties in a more homogeneous sample whereby light and heavy forces are applied within the same individual on their contra lateral sides. The initial findings are interesting and show significant alterations in hardness and elastic modulus among light and heavy force group of teeth. The findings will be published in due course.

Exhaustive baseline data were generated for Ca, P and F in cementum using quantitative analysis by EPMA. When compared to experimental teeth there was no significant difference in Ca, P and F levels between the light and control groups. There was also no significant difference in F levels between heavy and control groups. There were significant inter-individual variations in the present sample. The inconsistent increase or decrease of Ca, P and F contents between control and experimental teeth at sites of compression and tension were difficult to explain based on current knowledge. This change in mineral composition of cementum under different levels of mechanical stress needs to be studied further with a closer scrutiny of geographical area of origin of the subjects providing the samples.

The newly developed 3D volumetric quantitative analysis software was the first of its kind that has allowed a deeper understanding of the resorption craters. This phenomenon can be studied in detail in relation to various types of tooth movement, force vectors, and force levels generated by a variety of appliances including newer generation 'invisible' appliance.

The marriage of highly evolved computer software with well designed and executed experiments will allow us to understand the true effects of environmental factors such as force magnitude on biology of tooth movement and the periodontium. Although a host of newer concepts have been added to the process of root resorption there is still much to be learned from this complex process.

An experimental study investigating the cellular, molecular and molecular control of the resorption process has been completed (37). The findings will soon be available for publication. A study conducted in twins to look at the inheritance of root resorption has also been recently completed (Ngan et al, unpublished data). Further work is in progress to investigate the resorption process more closely using intrusion orthodontic forces. The protocol using 3D micro CT scanner to quantify resorption is under significant progress. The results of these studies are likely to add more to the quantitative and qualitative evaluation of the changes in tooth structures subsequent to different levels of orthodontic forces. Study is also in progress to look at the role of trace elements in cementum in humans. The role of systemic fluoride during orthodontic tooth movement is currently being investigated in rats. This might shed further light into the prevention of root resorption in orthodontics.

A clinician may now wish to know: 'can we tell which tooth is more susceptible to resorption?' or, 'who would have more chances of root resorption when braces are placed?' These questions may not be far from being answered. With the present understanding of resorption, it appears that the possible answer to these questions may well lie in isolating the gene that makes teeth more susceptible to resorption or one that delays its repair. We are also looking at the possible correlation of enamel hardness with cementum. A device used to quantify resorption *in vivo* in a clinical setting may one day materialize.

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