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Enamel demineralization with two forms of archwire ligation investigated using an *in situ* caries model—a pilot study

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SUMMARY A modified *in situ* model to assess enamel demineralization around orthodontic devices was developed and a pilot study was conducted to evaluate two types of archwire ligation. Enamel blocks were placed in palatal removable appliances where orthodontic brackets were bonded. The brackets on one side of the appliance were ligated with elastomeric rings and those on the other side with stainless steel wires. Four volunteers (two males, two females), mean age 27 years, wore the appliances for 14 days during which time a 20 per cent sucrose solution was dripped eight times a day onto the enamel blocks. The biofilm formed around the brackets was collected for microbiological analyses and the mineral loss around the brackets was determined by cross-sectional microhardness measurement.

The ligatures evaluated did not differ significantly from each other regarding biofilm weight, total bacteria, total streptococci, mutans streptococci, or lactobacilli counts ($P > 0.05$, Wilcoxon paired test). Enamel demineralization was also not different around the brackets for the different ligation methods ($P > 0.05$, split-split-plot analysis of variance). However, a statistical power analysis based on the data showed a trend to higher demineralization around brackets ligated with elastomeric rings. The developed modified *in situ* model may be suitable to assess the caries potential of clinical procedures used in orthodontic treatment.

Introduction

One of the main problems related to orthodontic treatment is to control enamel demineralization around brackets (Mizrahi, 1982; Øgaard *et al.*, 1988), which is reported to occur in up to 50 per cent of patients undergoing treatment (Gorelick *et al.*, 1982; Årtun and Brobakken, 1986). Fixed orthodontic appliances create new retention areas suitable for biofilm formation whose accumulation in the presence of dietary sugars could result in a bacterial shift and an increase in the number and percentage of cariogenic species such as mutans streptococci and lactobacilli (Rosenbloom and Tinanoff, 1991).

The effect of fixed orthodontic appliances on microbial flora and periodontal status has been evaluated previously (Corbett *et al.*, 1981; Rosenbloom and Tinanoff, 1991; Glans *et al.*, 2003), but only a few studies have included the method of ligation as an additional factor (Forsberg *et al.*, 1991; Sukontapatipark *et al.*, 2001; Turkkahraman *et al.*, 2005). Furthermore, demineralization around brackets tied with different archwire ligation techniques has not previously been evaluated.

In situ models have been accepted to study dental caries (Zero, 1995) and they have been used to assess caries around orthodontic appliances (Øgaard and Rolla, 1992; Benson *et al.*, 1999; Doherty *et al.*, 2002), but the archwire was not ligated to the brackets on those models.

Therefore, this study was designed to test an *in situ* model using archwires ligated to the brackets to evaluate the effect of ligatures on enamel demineralization.

Subjects and methods

The study was approved by the local ethics committee of Piracicaba Dental School, State University of Campinas (Protocol no. 173/2006), and informed consent was obtained from all subjects.

For 14 days, four healthy adults (two males, two females), mean age 27 years, participated in this pilot study. The volunteers were supplied with standardized fluoridated dentifrice (1000 μg F/g) and received an adult-sized, soft-bristled toothbrush. They were instructed to brush their teeth for 1 minute three times a day and to refrain from any other oral hygiene procedures, including mouthwashes, throughout the duration of the study. A removable palatal appliance (Øgaard and Rolla, 1992) was constructed with 15 × 5 × 4 mm cavities, into which three blocks of bovine enamel measuring 5 × 5 × 2 mm were placed on each side. The blocks were mounted in such a way that the natural tooth surfaces were recessed 1 mm below the surface of the appliance to allow biofilm accumulation.

Brackets, 3 × 4 mm (Dental Morelli, São Paulo, Brazil) were bonded to the centre of the enamel blocks with Concise

composite resin (3M do Brasil Ltda, São Paulo, Brazil). Before composite application, the blocks were pumice polished, the surfaces were etched with 37 per cent phosphoric acid for 15 seconds, washed with water for 15 seconds, dried for 10 seconds, and the adhesive system was applied over the etched area according to the manufacturers' instructions. A 0.016 inch stainless steel archwire was inserted in the three bracket slots on each side; the brackets on the right side of each appliance were ligated with elastomeric rings and those on the left side with stainless steel ligatures, in a split-mouth design (Figure 1). The volunteers were instructed to remove the appliances and drip a 20 per cent sucrose solution onto each enamel block eight times per day (Cury *et al.*, 2000). After 5 minutes, the appliances were replaced in the mouth. The appliances were brushed three times a day, except for the enamel blocks and archwires. The subjects consumed optimally fluoridated water (0.6–0.8 mg F/L) and were instructed to wear the appliances at all times, removing them only during meal times (Cury *et al.*, 2000).

The total biofilm formed on the enamel blocks, under the ligatures and around the brackets, was collected, weighed, and assessed microbiologically (Tenuta *et al.*, 2006). The enamel blocks were removed from the appliances and enamel demineralization around the brackets (Figure 2) was evaluated by cross-sectional microhardness as previously described (Moura *et al.*, 2006). However, hardness values were converted to express the mineral content change (Kielbassa *et al.*, 1999).

Statistical analyses

Biofilm weight and microbiological counts were analysed using the Wilcoxon paired test. Data concerning enamel demineralization were analysed in a split-split-plot analysis of variance design. An analysis to determine the sample size required to obtain a significant result with adequate probability (power of 80%) was also made (GLMPOWER). The SAS software (version 9.1, 2004; SAS Institute Inc., Cary, North Carolina, USA) was used and the significance level set at $P < 0.05$.

Results

The variables, biofilm weight, total bacteria, total streptococci, mutans streptococci, and lactobacilli counts in the biofilm formed, did not differ significantly ($P > 0.05$, Table 1).

The percentage mineral volume (vol %) of the control areas (under the bracket bases) did not differ statistically between the blocks tied with an elastomeric ring or with a steel wire at any depth ($P = 0.16$, Figure 3). The demineralization observed at a distance of 10 μm from the enamel surface at the edge of the bracket base (position 0) was higher than that observed at the control area, both for the enamel blocks

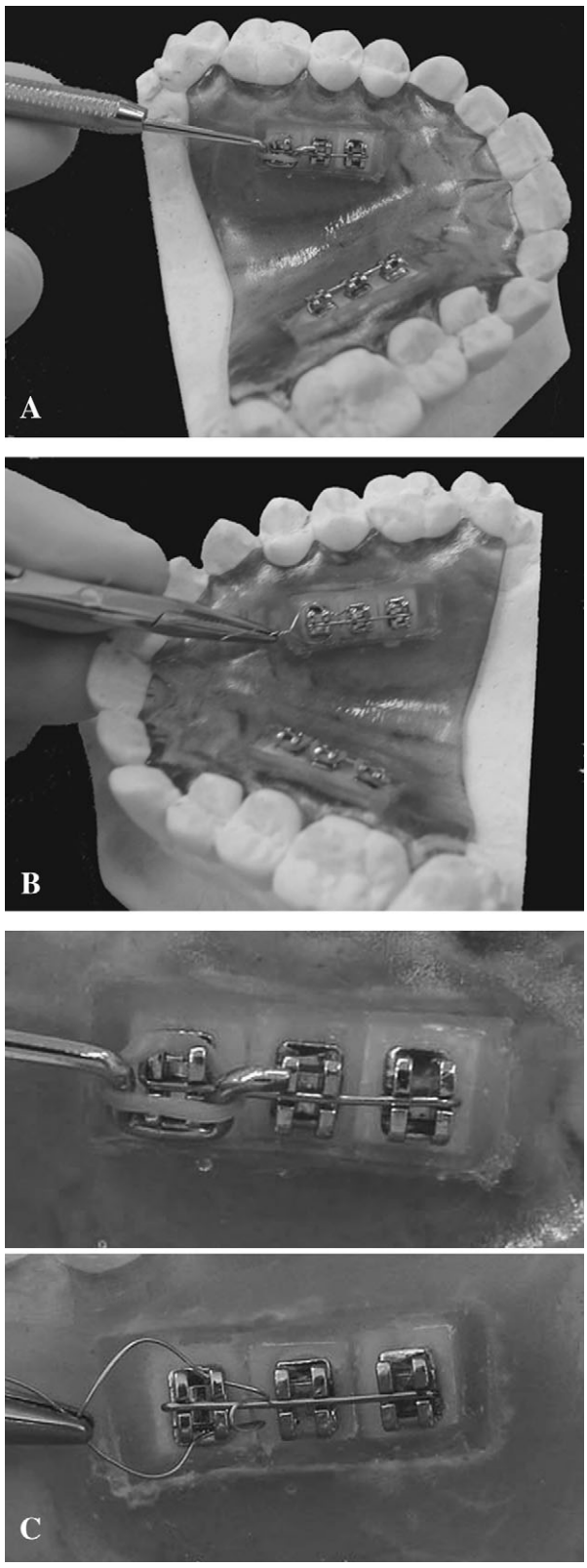

Figure 1 View of the removable palatal appliance. Elastomeric rings were used on the right side of the appliance (A) and steel ligature wires on the opposite side (B). Details are shown in (C).

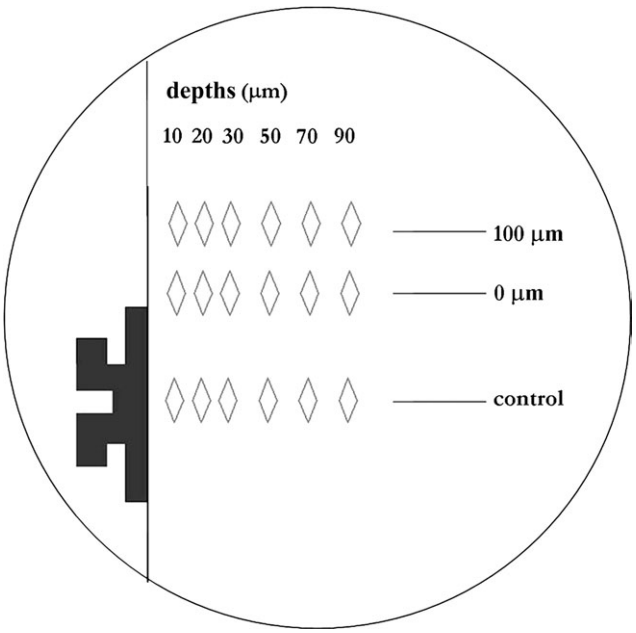

Figure 2 Diagrammatic representation of positions (control, 0, and 100 μm) and depth of indentations (10–90 μm).

ligated with elastomeric rings and steel wires ($P < 0.05$, Figure 3). The differences between the two ligatures were not statistically significant. However, if 10 volunteers had been used, the differences between the ligatures would have been statistically significant (type I error set at 0.05 and power of 80%), based on the data from this pilot study. Thus, a higher demineralization of the enamel around the elastomeric ring compared with the steel wire could have been found either at the edge (distance of 10 μm) or at 100 μm (distances of 10 and 20 μm) from the bracket base.

Discussion

This modified *in situ* model used was able to simulate the clinical condition of plaque accumulation that occurs during orthodontic treatment and caries development. Indeed, due to the sucrose exposure, enamel demineralization was greater around than underneath the brackets (Figure 3). The mineral loss of around 20 per cent is in agreement with *in vivo* studies of enamel caries adjacent to orthodontic brackets (O'Reilly and Featherstone, 1987; Øgaard *et al.*, 1992; Pascotto *et al.*, 2004; Moura *et al.*, 2006). Regarding lesion depth, the value of approximately 30 μm is in agreement with clinical studies conducted for 30 days (Pascotto *et al.*, 2004), showing that using this *in situ* model, the same result can be achieved in a shorter time.

The present model was also used to evaluate the effect of two different archwire ligation techniques on caries adjacent to brackets. It allows simulation of a more clinically related experimental set-up and the study of different aspects related to the caries process around orthodontic devices, i.e. the influence of archwire type, type of ligature, or other materials used during orthodontic treatment. It would also be possible to evaluate the effect of potential prophylactic or therapeutic regimens on dental demineralization in an experimental model that would not damage the natural tooth surfaces.

The findings (Figure 3) show that there was a trend to higher demineralization of enamel around brackets tied with elastomers than steel ligatures. This result gives support to the recommendation that the use of elastomeric rings should be avoided in patients with inadequate oral hygiene because these auxiliaries may be more prone to induce dental caries and gingivitis (Forsberg *et al.*, 1991; Sukontapatipark *et al.*, 2001; Turkkahraman *et al.*, 2005). This effect cannot be attributed to microbial change in biofilm formed since no difference was found in microbiota between the two types of ligatures, in agreement with other research (Sukontapatipark *et al.*, 2001; Bretas *et al.*, 2005; Turkkahraman *et al.*, 2005).

Regarding enamel demineralization, it is important to emphasize that the findings observed, even using only four volunteers, are relevant since it was estimated that if 10 volunteers had been used, the model would have had sufficient power to show statistically significant differences between the types of ligatures tested. Thus, the differences between elastomeric rings and steel wires either at the edge (distance of 10 μm) or at 100 μm (distances of 10 and 20 μm) from the bracket base would have been significant at $P < 0.05$. The higher cariogenic potential of the elastomeric

Table 1 Analysis of dental biofilm according to the method of archwire ligation.

Variables	Elastomer		Steel ligature		
	Median	Minimum and maximum values	Median	Minimum and maximum values	*P* value
Biofilm wet weight (mg)	7.9	6.2–42.2	4.1	2.1–44.1	0.1441
Total bacteria (CFU/mg × 10^6)	9.3	1.4–24.5	7.3	1.1–17.5	0.2733
Total streptococci (CFU/mg × 10^5)	15.6	11–27.3	14.6	4.3–18.3	0.4652
Mutans streptococci (CFU/mg × 10^3)	14.5	0.77–28.3	1.95	0.67–13.0	0.2733
Lactobacilli (CFU/mg × 10^6)	0.145	0.102–2.4	0.148	0.130–2.9	0.2850

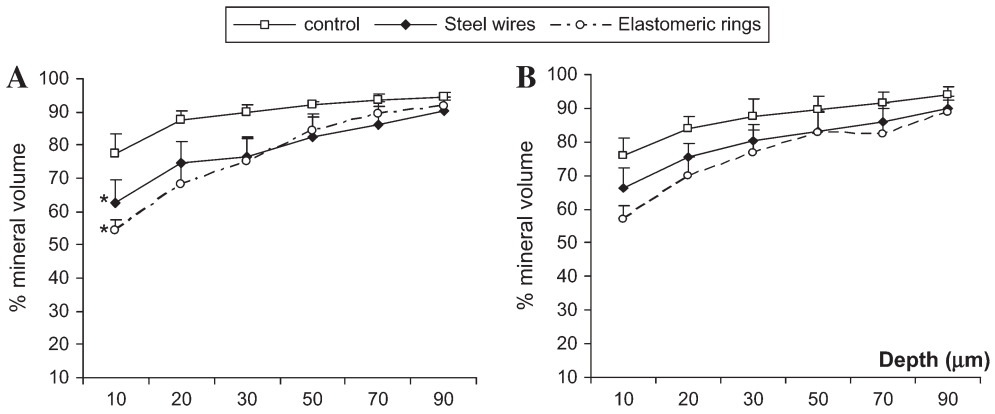

Figure 3 Mean ± standard error of the mean values of percentage mineral volume (vol%) according to the method of ligation and depth, at the edge (A) and at 100 μm from the edge of the bracket base (B). Asterisk indicates statistically significant difference between control and tested areas at each distance ($P<0.05$).

rings could be explained by the differences in thickness and surface characteristics of elastomers and steel wires, which may facilitate biofilm accumulation and protect it against shear forces and saliva action.

Another limitation of this study was the fact that the carious lesions were superficial, requiring microhardness measurements at distance lower than 25 μm from the enamel surface (Arends and ten Bosch, 1992). However, reliable results may be found at any distance from the surface if the area resists the load applied (Meyerowitz *et al.*, 1991; Pascotto *et al.*, 2004; Moura *et al.*, 2006), which occurred in the present study.

Conclusions

The developed modified *in situ* model may be suitable to investigate enamel decalcification adjacent to orthodontic appliances.

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