The Influence of Interproximal Reduction on Enamel Roughness and Bacterial Adhesion

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Introduction: Interproximal reduction (IPR), also known as enamel stripping, leaves many grooves and furrows on the enamel surface, which may increase the risk of caries. In this thesis, the influence of IPR on the morphology and roughness (Ra) of enamel surfaces and the bacterial adhesion to these surfaces were investigated. The specific aims of this thesis were to assess the roughness of enamel surfaces (both qualitatively and quantitatively) produced by the most commonly used IPR instruments, to investigate the adhesion of bacteria to these surfaces, and to evaluate the effect of polishing after IPR on the amount of bacterial adhesion.

Materials and methods: Sixty-four human premolar teeth that were extracted for orthodontic treatment were collected. Enamel blocks were cut from the interproximal surfaces. Seven different IPR instruments were used for interproximal reduction (n = 8 in each group) and there was a control group (n = 8) consisting of untreated enamel blocks. The morphology and roughness of the sixty-four enamel surfaces were investigated qualitatively and quantitatively using atomic force microscopy. From the seven IPR-treated groups, the samples from the three instruments that yielded significantly different roughnesses, as well as the control group, were used for the adhesion experiments. Adhesion of *Streptococcus sanguinis* ATCC10556 to the enamel surfaces was assessed by counting the colony forming units that adhered to the roughened surfaces after 30 min exposure.

Results: Generally, the larger grit IPR instruments created rougher enamel surfaces (Ra values for medium bur: 702.4 ± 134.4 nm; medium strip: 501.0 ± 115.3 nm; mesh disc: 307.1 ± 106.9 nm) and smoother surfaces were formed by use of the smaller grit instruments (Ra values for fine bur: 407.4 ± 94.8 nm; fine strip: 317.6 ± 49.6 nm; curved disc: 223.9 ± 64.7 nm). The differences in mean roughness within the groups of larger or smaller grit were significant ($p < 0.001$ and $p < 0.05$, respectively), and the differences in mean roughness between instruments of the same type but different grit (*e.g.* large grit bur compared to small grit bur) were all significant with p-values < 0.001 apart from surfaces prepared with different

discs ($p = 0.122$). The smoothest surfaces were created by use of the entire series of Soflex polishing discs after the enamel reduction (Ra = 36.7 ± 13.7 nm), and these surfaces were significantly smoother than the control surfaces (Ra = 148.6 ± 38.5 nm)($p = 0.017$).

The rougher surfaces showed increased streptococcal adhesion. Greatest adherence was to the surface prepared with a medium diamond bur (Ra = 702.4 ± 134.4 nm); the CFUs bound were $12.3 \times 10^5 \pm 0.5 \times 10^5$, followed by the surface prepared by mesh disc (Ra = 307.1 ± 106.9 nm, CFU = $4.0 \times 10^5 \pm 0.5 \times 10^5$), followed by the control surface (Ra = 148.6 ± 38.5 nm, CFU = $1.2 \times 10^5 \pm 0.1 \times 10^5$)($p < 0.001$). The least bacterial adhesion was to the smoothest surface - when Soflex polishing discs had been used following enamel reduction (Ra = 36.7 ± 13.7 nm, CFU = $0.3 \pm 0.05 \times 10^5$).

Conclusions: 1) Larger grit diamond instruments created rougher surfaces than did their smaller grit counterparts; 2) Diamond burs created the roughest enamel surfaces, followed by diamond strips, followed by diamond discs; 3) The Soflex polishing discs created the smoothest surfaces, even smoother than that of the untreated enamel; 4) There was a positive relationship between enamel surface roughness and the number of bacteria that adhered.

Clinical significance: It is recommended that practitioners polish interproximal enamel after IPR to leave the enamel as smooth as possible to reduce possible bacterial adhesion.

Chapter 1

General Introduction – Interproximal Reduction in Orthodontics

Abstract

Interproximal reduction (IPR) is the deliberate removal of part of the dental enamel from the interproximal contact area, decreasing the mesio-distal width of a tooth. This enamel may be removed for various reasons, but most commonly to create space during orthodontic treatment or to correct tooth size discrepancies. Some authors have also encouraged its use as a method by which to enhance post orthodontic stability, particularly in the lower anterior region. With the increased use of removable aligners for orthodontic treatment, where non-extraction therapy is often advocated, the use of IPR becomes a valuable tool to relieve crowding without over-expanding the dental arches.

Removal of this outer enamel has been shown to leave a roughened surface, which may increase the force of bacterial adhesion to the enamel. The aetiology of dental caries and periodontal disease are both related to bacterial adhesion, and the risk of these diseases may be increased if more bacteria bind to roughened enamel. In the past, IPR was only used occasionally in orthodontics, but today it is much more common. Long-term disadvantages of IPR are still poorly understood.

Introduction

Interproximal Reduction (IPR) was first mentioned in the literature when it was recommended to correct a lack of balance in the anterior segments (Ballard, 1944). These tooth size discrepancies were described for the first time in a landmark paper (Bolton, 1958), in which the need for IPR in some cases to accomplish a well interdigitated occlusion was emphasized. Following measurement of the mesio-distal widths of all teeth, the cases in which IPR may be required for ideal occlusion were termed as having a "Bolton's discrepancy."

It is recommended that IPR only be carried out after alignment since IPR on rotated teeth would result in an inaccurate reduction of the contact points (Kelsten, 1969). It has also been demonstrated that an acceptable occlusion can be created even when a Bolton tooth size discrepancy exists, suggesting that no pre-emptive IPR should be undertaken (Heusdens et al., 2000), and instead be carried out after alignment and evaluation of the final occlusion.

There was a theory put forward by Raymond Begg in 1954 surrounding *natural* interproximal attrition (enamel reduction) (Begg, 1954). He observed that in Stone Age man there was natural attrition interdentally that he believed allowed for the reduction in arch length with time, which prevented crowding. However, with our modern processed diets, the occlusal and interproximal wear did not occur and the result was long term crowding of the dental arches. This theory provided part of the justification for his extraction philosophy, where extractions were used to gain the space that would have been achieved through natural means if interdental attrition still occurred.

The use of IPR for gaining space in the lower anterior region was described for the first time in a Class II Division 2 malocclusion case (Lusterman, 1954). At that time IPR was not common and the author stated that most clinicians too readily overlooked the use of enamel reduction in orthodontics.

The thickness of the enamel in mandibular anterior teeth has been investigated (Hudson, 1956) and no definite correlation between tooth size

and enamel thickness at the contact points could be found, but generally, larger teeth had more enamel. It was recommended that up to half of the tooth's enamel could be safely removed from the interproximal region, and it was stated that up to 3 mm of space could be gained from the mandibular anterior teeth alone (Hudson, 1956). The technique of IPR was first described as involving hand-held metallic strips followed by polishing and application of fluoride to the reduced surfaces (Hudson, 1956).

Mechanical means to carry out IPR (rather than the previously described hand-held abrasive strips) have been developed (Paskow, 1970), and a detailed description of IPR using contra-angle handpieces with abrasive discs has also been published (Tuverson, 1980b). Two articles published by the same author on air-rotor stripping were revolutionary in their approach to IPR since they recommended using IPR in the buccal segments (Sheridan, 1985; 1987), and advised that stripping could be used to alleviate up to 8 mm of crowding in this region.

The same author stated that up to 6.4 mm of space could be gained from IPR on the premolars and molars alone by removing 0.4 mm of enamel from each proximal surface (Sheridan and Ledoux, 1989). However, it was later argued that the previously suggested 6.4 mm reduction was an underestimation and that a substantial 9.8 mm of space could be gained in the posterior teeth using this technique (Stroud et al., 1998).

Historically, the use of IPR on the mandibular incisors has been advocated to increase post-treatment stability (Peck and Peck, 1972a; Peck and Peck, 1972b). It was stated that if the mesio-distal dimensions of the incisors fell outside the "Peck index" that the teeth would be predisposed to future crowding. These claims were later disputed by a number of authors (Gilmore and Little, 1984; Blake and Bibby, 1998).

The use of IPR has also been encouraged in adult patients with crowding where removal of teeth is not an option (Sheridan, 1997). A review on IPR was published in which use of elastic or coil spring separators prior to IPR was advised to improve the accuracy and ease of the enamel reduction (Chudasama and Sheridan, 2007). In this paper, it was stated that a maximum of 0.5 mm of enamel be removed from any buccal proximal surface

and that a protective wire be placed to protect the interdental tissues during the procedure.

It has been found that teeth treated with fluoride after stripping have increased resistance to acid attack 48-96 h after the procedure (Rogers and Wagner, 1969) and it is beneficial to apply fluoride immediately after stripping to promote remineralisation (Joseph et al., 1992). It has been recommended that stripping be used in combination with 37% phosphoric acid to promote the remineralisation, and was stated that if carefully polished, the stripped surfaces could be made as smooth, or even smoother, than untouched enamel (Joseph et al., 1992). This statement regarding the smoothness of enamel following polishing was further verified in a number of scanning electron microscopy studies (Piacentini and Sfondrini, 1996; Zhong et al., 1999; Zhong et al., 2000). However, one study concluded that IPR, even with polishing, produced grooves and furrows with significantly rougher surfaces when compared with untreated enamel surfaces (Arman et al., 2006).

Historically there have been concerns over the possible long-term consequences of IPR. The common concern that increased root proximity (caused by reduction of the mesio-distal crown width with subsequent space closure) would increase the risk of periodontal tissue loss has been disputed (Årtun et al., 1987). From the observed sample of anterior teeth, there was no increase in periodontal breakdown with increased proximity of the roots.

Another study investigated the long-term effects of IPR, and no caries was reported due to reduction in tooth width, but it was stated that in all cases the surfaces must be highly polished (Ward, 1955). Later, the correlation between IPR and dental caries was investigated again, but as in the previous study, there was no correlation found between IPR and dental caries after 2 to 5 years following the procedure (Crain and Sheridan, 1990). More recently, there was no increase in caries on surfaces that had received IPR 1 to 6 years prior (Jarjoura et al., 2006). Similar conclusions were drawn, once again, from another study: that there is no increase in caries risk following IPR, stating that it could be safely used in orthodontic patients if the usual guidelines are followed (Zachrisson et al., 2007; Zachrisson et al.,

2011). The results of a recent systematic review suggested no increased caries risk on surfaces treated with IPR, however, it was noted that due to the diversity in methodology of the studies that no reliable conclusions could be drawn (Koretsi et al., 2014).

Indications for IPR

Tooth size discrepancy

There are now many recognised indications for IPR. The first reported use of IPR was to correct tooth size discrepancies when aligning the anterior teeth (Ballard, 1944). An analysis based on mesio-distal widths of the lower teeth in relation to the upper teeth was created (Bolton, 1958). The ratio of the upper and lower teeth widths dictated how well they would interdigitate in the buccal segments ('overall' Bolton's analysis) and whether the size of the anterior teeth would allow class I canines with acceptable overbite and overjet ('anterior' Bolton's analysis). Using the Bolton's ratio, it is possible to calculate the predicted fit of the teeth following alignment. After a detailed space analysis, in cases where there are discrepancies between the upper and lower dentitions, the teeth that are oversized in relation to the others, may then be slenderized by performing IPR. This reduction in tooth width will eliminate the discrepancy and allow a better fitting occlusion at the completion of orthodontic treatment. Cases in which a Bolton's discrepancy is more likely, include: when the patient has diminutive upper laterals; when there are missing teeth; or when there are particularly large, small or unusually shaped teeth in one of the arches.

A recent review on IPR stated that a Bolton's tooth size discrepancy is still one of the main reasons that IPR is used in orthodontic patients (Lapenaite and Lopatiene, 2014). There are various methods used to calculate whether or not a Bolton's tooth size discrepancy exists, since it is sometimes hard to visualise without some form of measurement. It was recently concluded that the use of Vernier calipers on plaster models is still considered the 'gold standard', but that contemporary methods such as the

use of digital photographs, laser scanning and stereophotogrammetry may in fact be more clinically accurate (Naidu and Freer, 2013).

Relief of crowding

IPR was described as a method to gain space in a Class II division 2 malocclusion (Lusterman, 1954). With increasing demand from patients to align teeth without extractions, it's use has become more common and IPR can be used to relieve mild to moderate crowding (Chudasama and Sheridan, 2007) particularly in non-growing patients where excessive expansion or extractions are not possible (Lapenaite and Lopatiene, 2014; Sheridan, 1997). There have been various reports over the last 50-60 years about how much space can be gained from the use of IPR. Earlier studies tended to be more conservative with their recommendations and specified gains of up to 3 mm of space in the mandibular anterior region (Hudson, 1956; Lusterman, 1954). However, later studies have reported amounts as high as almost 10 mm when IPR is performed on premolars and molars alone (Stroud et al., 1998). If one were to add the reported amounts of reduction possible from both the anterior and buccal segments, theoretically almost 13 mm of space can be gained in the mandible from second molar to second molar. However, common sense suggests that arch length discrepancies of 13 mm would most likely be treated with extractions.

Increased stability

In 1972, Peck and Peck suggested that IPR be used to increase post-treatment stability of the lower incisors (Peck and Peck, 1972a). The rationale for this approach was the observation that naturally well-aligned mandibular incisors had specific mesio-distal (M-D) and labio-lingual (L-L) dimensions (Peck and Peck, 1972b). The well-aligned incisors had significantly larger labio-lingual dimension (*i.e.* broad contact points) and a smaller mesio-distal dimension, suggesting that the shape of the incisors may be a factor determining whether or not lower incisor crowding will occur. An index was constructed which uses the M-D/L-L ratio to determine

whether or not an incisor is favourably shaped (Peck and Peck, 1972a). If a particular incisor falls outside of this range, then IPR can be performed to change it's dimensions and place it in the favourably shaped group, which would presumably assist in its long-term alignment.

This work was later criticized, however, since the recommendations were based on a sample of untreated cases in relatively young patients, who may well have gained lower incisor crowding in the future had they been followed long-term (Blake and Bibby, 1998). The Peck and Peck ratio was then investigated over a longer period, in a sample of treated cases, a minimum of 10 years post-retention (Gilmore and Little, 1984). The findings from this study showed only a weak association between the ratio and long-term alignment, suggesting that the shape and dimensions of the teeth may play only a limited role in the long-term stability of lower incisors.

A two-part study from 1980, looked at the post-treatment stability of lower incisors 4 to 9 years after treatment without retention where circumferential supracrestal fiberotomy (CSF) and IPR had been performed (Boese, 1980a; b). All cases had either first or second premolars removed and CSF was performed on teeth where the supragingival fibres had been markedly displaced (which was not clearly defined). The IPR was performed on all cases over three phases: The first phase being early in treatment, as soon as there was alignment of the lower anterior teeth; the second shortly after band removal (usually over a period of 4 to 6 months); and the third phase (not often needed) occured anytime after this whenever contact points became tight or any malalignment was noted. In the second part of the study, the Peck Irregularity Index (Peck and Peck, 1972a) before treatment was, on average, 9.2 mm (Boese, 1980b). During the post-treatment period the Irregularity Index was 0.6 mm, which is still considered within the limits of "perfect alignment." The average amount of total IPR from the lower incisors was 1.7 mm (based on measurements of pre- and post-treatment models) and the inter-canine widths increased by only 0.9 mm. There was no measurable alveolar bone loss. It was concluded that reproximation of the lower incisors used in combination with CSF (where indicated), may increase long-term stability of the lower incisors even

without the use of retention. This conclusion was not supported by a later study investigating the relationship of mandibular incisor dimensions and long-term stability in orthodontically treated cases, where only weak associations were found (Gilmore and Little, 1984).

Preservation of the inter-canine width has been advocated to increase long-term stability in the mandible and, if stripping is used to gain space rather than expanding in the lower anterior region, the intercanine width is more likely to be preserved. A study examining cases 10 years post-retention, investigated the long-term stability of treatment-induced changes in the maxillary and mandibular arch forms (De la Cruz et al., 1995). It was found that the arch forms tended to return to their pre-treatment shape and that pre-treatment arch form is the best guide for future arch form stability. This corroborated earlier findings, which had reported that 70% of orthodontically treated cases would return to their original arch form after treatment (Felton et al., 1987). However, it was also stated that maintenance of the pre-treatment arch form is not a guarantee for future stability either (De la Cruz et al., 1995).

Improved aesthetics of front teeth

The use of IPR has been advocated to improve anterior tooth shape and aesthetics (Zachrisson, 1986; Lapenaite and Lopatiene, 2014). With use of IPR and lengthening the contact area, there is a reduction in the incidence of black triangles (observed black spaces between the papilla up to the contact point when the contact point is further from the alveolar crest than normal). It has been reported that if the distance from the interproximal bony alveolar crest to the contact point is 5 mm or less that there is almost 100% infill from the interdental papilla (Tarnow et al., 1992). If the teeth are triangular in shape then the contact point will be further from the alveolar crest, increasing the likelihood of black triangles. In these teeth, the use of IPR to alter their proximal surface shape can be beneficial, however, one must be careful when IPR is performed in only one arch, as it may be possible to create a Bolton's discrepancy that did not exist previously. Reduction of the

opposing dentition may be necessary in these cases to balance the discrepancy created.

Conversely, it has been reported that an acceptable occlusion can be obtained when a Bolton's discrepancy does exist (Heusdens et al., 2000), suggesting that IPR should not be performed in advance to correct a discrepancy, but rather, its necessity reassessed following alignment and final occlusion.

To avoid extractions

With an increasing number of patients using removable, aesthetic orthodontic appliances, where extraction therapy is often not advocated, the use of IPR as an alternative to gain space is becoming more popular (Lapenaite and Lopatiene, 2014). The benefit of using IPR to gain space over extraction therapy is that it decreases overall treatment time since the amount of stripping corresponds exactly with the amount of crowding (Jarjoura et al., 2006). Performing IPR when treating a case without any extractions also means that excessive advancement of the mandibular incisors can be avoided (Chenin et al., 2003) as well as over expansion of the dental arches, and satisfactory alignment is still achieved (Sheridan, 1985; 1987).

There are well-accepted guidelines regarding orthodontic treatment with or without extractions (Proffit, 2007). Generally, crowding of 5 to 9 mm may be treated with or without extractions depending on the case. However, in cases where there is an arch length discrepancy of 10 mm or more, extractions are almost always indicated, despite the reported amounts of space that can be created with the use of IPR.

Amount of enamel reduction

Enamel thickness

There have been many studies conducted to investigate the thickness of dental enamel (Gillings and Buonocore, 1961; Shillingburg Jr and Grace,

1973; Peck and Peck, 1975; Richardson and Malhotra, 1975; Moss and Moss-Salentijn, 1977; Stroud et al., 1994; Harris and Hicks, 1998; Stroud et al., 1998; Grine et al., 2001; Hall et al., 2007; Sarig et al., 2015). Radiographs have been used to compare the thickness of enamel in males and females (Stroud et al., 1994). Although it was found that the teeth in males were larger than in females, this difference was due to an increase in thickness of dentine rather than enamel. This was supported by another study that found there was thicker dentine in males than in females (Harris and Hicks, 1998).

The relationship between thickness of proximal enamel, tooth type, tooth width, sex and ethnicity has been investigated (Hall et al., 2007). It was found that laterals had thicker enamel than centrals and that distal enamel was thicker than mesial enamel (each supporting previous findings (Gillings and Buonocore, 1961)). Generally, white subjects had less enamel than black subjects and overall, the tooth width corresponded positively with enamel thickness. There was no difference between male and female enamel thickness (as found previously (Stroud et al., 1994; Harris and Hicks, 1998)). Based on the proximal enamel thickness, it was then suggested that 0.20 mm or less on the mandibular incisors could be safely removed (Hall et al., 2007). It was mentioned, however, that there was substantial variability in enamel thickness both between and within the subjects.

A more recent investigation into enamel thickness (where enamel was measured directly histologically, as opposed to via radiographs) provided similar results. Based on their findings it was suggested that up to 0.5mm per anterior contact area (*i.e.* 0.25mm per surface) and up to 1mm per posterior contact (*i.e.* 0.5mm per surface) may be safely removed for IPR (Sarig et al., 2015).

There have been a large number of suggestions as to how much enamel can be removed by IPR. Interestingly, the initial recommendation (of up to 50% enamel reduction) was made with no scientific justification, yet this has been repeatedly quoted in the literature as a recommended rule with regard to IPR (Hudson, 1956). Initially, it was reported that 3 mm might be gained from IPR on the mandibular incisors (Hudson, 1956), and it was later stated that over 6 mm of space could be gained from IPR on the premolars and

molars by reducing each of those contacts by 0.4 mm (Sheridan and Ledoux, 1989). Later, it was reported that an overwhelming 9.8 mm of space might be gained through use of IPR on each proximal surface of the premolars and molars alone (Stroud et al., 1998). Despite the variation in reported amounts of space that can be gained, it has been repeatedly stated in the literature that up to 50% reduction in proximal enamel is acceptable (Hudson, 1956; Boese, 1980a; b; Tuverson, 1980a; b; Betteridge, 1981; Sheridan, 1985).

An investigation of enamel thickness in mandibular anteriors found that, on average, the thickness of enamel at the contact points of the central incisors was 0.54 mm, at the laterals was 0.65 mm and at the canines was 0.76 mm (Hudson, 1956) (Table 1).

Table 1 Enamel thickness of lower anterior teeth (adapted from Hudson, 1956)

Tooth	*Mesial enamel (mm)*			*Distal enamel (mm)*		
	Min	*Max*	*Average*	*Min*	*Max*	*Average*
Central incisor	0.37	0.88	0.54	0.36	0.70	0.52
Lateral incisor	0.47	1.05	0.65	0.50	0.98	0.68
Canine	0.38	1.11	0.76	0.55	1.80	0.90

From this it was suggested that approximately 0.20 mm of enamel could be removed from the proximal surfaces of each of the central incisors, 0.25 mm from each of the laterals and 0.30 mm from the canines (Table 2). There was no significant correlation between enamel thickness and tooth size. However, it was stated that usually the larger teeth had thicker enamel. Similarly, in 2007, it was recommended that only 0.25 mm per surface be removed from the upper laterals and the lower incisors, since they have thinner enamel (Chudasama and Sheridan, 2007).

Table 2 Suggested maximum enamel reduction of lower incisors (adapted from Hudson, 1956)

Tooth	*Maximum enamel reduction (mm)*
Central incisor	0.2
Lateral incisor	0.25
Canine	0.3

IPR in the buccal segments

The move from initially restricting IPR to the anterior region alone to involving the buccal segments has meant that much larger amounts of space may be gained from using this technique. There is substantially more proximal enamel found in the buccal segments (Shillingburg Jr and Grace, 1973; Stroud et al., 1998) making them a good prospect for IPR. The difficulty of IPR in the buccal segments, of course, is gaining clear access to the contact areas so that careful, accurate reduction can be performed. One must question the benefit of potentially inaccurate IPR and damage to several posterior teeth, versus the removal of only one or two teeth to gain the required space.

A recent study has investigated the actual amount of enamel removed compared to the intended amount (Johner et al., 2013). It was found that generally, slightly less enamel is removed than intended, which is probably reassuring for most clinicians.

Consequences of IPR

Clinicians shall firstly do no harm; it is therefore prudent that safe practices based on scientific evidence are established. It is logical to assume that the roughening of the enamel surface caused by IPR may increase plaque retention and therefore increase the risk of caries or periodontal disease (in susceptible patients) around those surfaces; however, to date no study has determined any significant negative long-term effects from IPR.

References have been made to a number of theoretical risks: increased caries due to surface roughening; gingival recession; alveolar bone loss associated with root proximity; increased susceptibility to demineralization; and increased temperature sensitivity of the slenderized teeth (Twesme et al., 1994; Bishara, 2004; Zachrisson, 2004; Jarjoura et al., 2006). However, from the available literature, it seems there is no evidence to suggest there are any long-term negative effects from properly conducted IPR (Årtun et al., 1987; Crain and Sheridan, 1990; El-Mangoury et al., 1991; Pinheiro, 2002; Jarjoura et al., 2006 Zachrisson, 2007 #159).

Periodontal issues

In 1987, Årtun and colleagues investigated root proximity and long-term periodontal health at least 16 years after orthodontic treatment (Årtun et al., 1987), which may or may not have included any IPR. It was concluded that there was no increased risk of loss of periodontal attachment in the anterior teeth when their roots were in close proximity to one another, usually due to the roots not being parallel. “Close proximity” was defined as less than 0.8 mm between adjacent cemento-enamel junctions. Although the initial sample of 400 patients was impressive, the number of molars with roots in close proximity was fairly small and therefore no conclusions were drawn for the molars.

An earlier study examined the periodontium of mandibular anterior teeth subjected to circumferential supracrestal fiberotomy (CSF) and IPR (Boese, 1980b). Similarly, it was concluded that in the cases seen between 4 and 9 years after treatment, there was no measurable alveolar bone loss or increase in either gingival recession or periodontal pocketing.

Surface roughness

It has long been debated as to whether performing IPR (either by hand held strips, discs, or diamond burs) leaves the enamel surface rougher than untreated teeth. This is of interest since, logically; a rougher surface might increase plaque retention and therefore increase the risk of caries at that

site. There are conflicting statements in the literature; several of the earlier studies stated that the furrows and scratches produced by IPR cannot be removed by polishing (Radlanski et al., 1988; Joseph et al., 1992; Lundgren et al., 1993; Piacentini and Sfondrini, 1996; Arman et al., 2006), but more recent publications suggested that the enamel can be polished to become even smoother than untreated surfaces (Zhong et al., 1999; Zhong et al., 2000; Danesh et al., 2007). This is probably because most studies now recommend the use of thorough polishing following IPR (Piacentini and Sfondrini, 1996; Zhong et al., 1999; Zhong et al., 2000; Arman et al., 2006; Danesh et al., 2007) and because of the development of improved polishing equipment.

A scanning electron microscopy (SEM) study evaluated the roughness of enamel following IPR and compared it with IPR used in combination with acid etching (Joseph et al., 1992). The teeth were first subjected to regular IPR through the use of burs or discs, and then a finishing strip was lightly coated with 37% phosphoric acid and passed over the surface 20 times. Interestingly, the teeth with the combined stripping and etching showed smoother surfaces with a distinct flattening of the grooves and furrows compared to the other groups. As well as showing a smoother surface, the authors suggested that this surface was capable of "self-healing" and had increased potential to remineralise (Joseph et al., 1992). A few years later, another SEM study showed that in most cases the enamel grooves and furrows left from IPR cannot be removed; however, it was also stated that one particular method, using an 8-straight blade tungsten carbide bur and Soflex discs for polishing, could produce a surface smoother than untreated enamel (Piacentini and Sfondrini, 1996).

Further SEM studies concluded that the surface roughness produced by IPR could be minimised to a degree such that the enamel is smoother than an untreated tooth (Zhong et al., 1999; Zhong et al., 2000). However, this was disputed in 2006 in a study involving not only SEM, but also profilometry, to evaluate the surface roughness following IPR (Arman et al., 2006). It was concluded that all methods resulted in a roughened enamel surface;

however, smoother surfaces were obtained when fine Soflex discs were used to polish following stripping.

In another study, the surface roughness of enamel following IPR was investigated using profilometry and digital subtraction radiography, to assess the amount of enamel that was removed using this technique (Danesh et al., 2007). All groups showed a significantly smoother surface following polishing and the digital subtraction radiography showed that an insignificant amount of enamel was removed by polishing with the fine Solfex discs (0 to 0.02 mm). It was strongly recommended that all stripped surfaces be polished to minimize the possible risk of plaque accumulation.

Caries risk

As previously mentioned, one of the main issues of concern with IPR is the possible increase in caries risk due to the increased plaque accumulation on the roughened enamel surfaces. To date, several studies have shown no increase in caries susceptibility following IPR (Jarjoura et al., 2006; Zachrisson et al., 2011), and some cases were followed as long as 10 years after the procedure (Zachrisson et al., 2007).

In an evaluation of patients who had IPR performed 1 to 6 years previously, no significant difference between the treated and untreated surfaces was found (Jarjoura et al., 2006). Interestingly, there was an increase in DMFT and DMFS scores over the study period, implying that the group was at higher risk for caries overall; however, of the carious lesions observed, only three out of the nine were on treated surfaces, the other six were on untreated surfaces. The conclusion was that there was no increased caries risk following IPR and this was in agreement with previous studies (Radlanski et al., 1989; Crain and Sheridan, 1990; El-Mangoury et al., 1991).

One retrospective study looked at a sample of sixty-one cases who had received IPR on all six mandibular anterior teeth at least 10 years prior (Zachrisson et al., 2007). The findings confirmed those of previous studies, stating that there was no increased susceptibility to caries on the treated enamel surfaces. This was investigated again, but with a shorter follow up

period, in patients having received IPR only 4 to 6 years previous (Zachrisson et al., 2011). Out of the 278 surfaces that were reduced in this study, only seven had new carious lesions (2.5%), and of the 84 untreated (control) surfaces, two had new carious lesions (2.4%). The patients were not divided by way of their caries risk, and the seven new carious lesions had come from 3 patients, indicating that these patients may have had a higher initial caries risk. Of the 43 patients examined, none of them reported any increase in tooth sensitivity. However, in the earlier study two out of the 59 patients reported an increase in sensitivity; one who had sensitive teeth in general, and the other reporting sensitivity in the lower anterior region only (Zachrisson et al., 2007). The conclusions drawn, once again, were that there was no increase in caries risk following IPR, and that it could be carried out safely if the correct technique was used within recognized limits.

A recent systematic review stated that no reliable conclusions could be drawn from the studies completed due to the diversity of their methodology (Koretsi et al., 2014). However, statistically, the incidence of caries on surfaces that had had IPR, was the same as the untreated surfaces, indicating no increased risk after the procedure.

Conclusion

Interproximal reduction (IPR) is a technique that has been used in orthodontics since the 1940s. Its use is common in circumstances when space is required to relieve crowding, especially when extractions are not wanted or indicated. It is useful in these circumstances and can decrease the treatment time compared with extraction therapy since the amount of reduction achieved in one session corresponds exactly to the amount of crowding. It may also be used in cases where there is a tooth size discrepancy and removal of dental hard tissue from one arch may be necessary to gain a well interdigitated occlusion at the completion of orthodontic treatment. It has been shown that IPR of the mandibular incisors (particularly if combined with circumferential supracrestal fiberotomy (CSF)) may enhance long-term stability, even without retention, and can be carried out on patients with

black triangles, or triangular shaped teeth, to lengthen the contact area and encourage infill of the papilla, thereby enhancing aesthetics in the anterior region.

Since the introduction of IPR, there have been many claims as to how much enamel can be safely removed. Generally, it is accepted that up to half the enamel may be reduced from the proximal contact area, however, the thickness of enamel may vary quite substantially both between and within individuals. As a general indication, up to 0.2 mm from each proximal surface of the mandibular central incisors, 0.25 mm from the laterals and 0.3 mm from the canines can be removed safely. Since there is significantly thicker enamel in the buccal segments, up to 0.4 mm or even 0.5 mm could be removed from the proximal surfaces of each premolar and molar. Although it is reported that a significant amount of space could be gained through use of this technique (up to 9.8 mm), generally, where there is crowding of 6 mm or more, extraction therapy should at least be considered as an alternative.

The potentially harmful consequence of IPR have been documented, yet to date there seems to be no evidence suggesting there are any long-term negative effects of this procedure. Many studies have evaluated the characteristics of the enamel surfaces following IPR and state that with careful polishing, a surface as smooth as, or smoother than, untreated enamel may be obtained. Several long-term studies have also evaluated the incidence of new carious lesions in both the anterior and posterior regions where IPR has been performed, however, no increase in caries risk has been identified. There have also been no reports of any increase in periodontal problems, including gingival recession, periodontal pocketing or alveolar bone loss.

It is possible that inaccurate IPR could result in over-reduction of enamel, ledges and notches in the proximal surfaces, increased tooth sensitivity or damage to the surrounding soft tissues as well as a reduction in self-cleansability. However, carefully conducted IPR performed within the recommended guidelines may be used as a safe method to gain space for relief of crowding, to correct tooth size discrepancies and to improve aesthetics and long-term stability in suitable orthodontic patients.

Future research

With regard to research gaps in the current literature and knowledge, it would be useful for clinicians to know which specific instruments produce the roughest or smoothest surfaces after IPR and also what effect these instruments have on the enamel morphology. In the present study, a number of instruments commonly used for IPR were used and their effects on enamel roughness and morphology demonstrated. The effect of polishing after enamel reduction was also assessed. The changes made to the enamel morphology by the IPR instruments was shown to increase the bacterial adhesion and this may increase the future risk of caries. Further research into the long-term effects of the roughened enamel would be of great value to orthodontic clinics.

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Chapter 2

Enamel Roughness Following Interproximal Reduction

Abstract

Introduction: Interproximal reduction (IPR) removes some of the surface layer of enamel and leaves many grooves and furrows on the tooth surface, which may increase the future risk of caries. The aim of this study was to assess the roughness of enamel surfaces (both qualitatively and quantitatively) produced by the most commonly used IPR instruments and to evaluate the effect of polishing after IPR.

Materials and methods: Sixty-four healthy human premolar teeth that had been extracted for orthodontic treatment were collected and prepared for experiments. Enamel slabs were cut from the interproximal surfaces and then treated with diamond burs, strips and discs, and Soflex polishing discs. All samples were cleaned by sonication in distilled water for 2 min. The control group had no IPR performed and was subjected to cleaning by sonication only. The enamel surfaces were assessed using atomic force microscopy (AFM).

Results: The IPR instruments all produced surfaces rougher than the control sample, however, the samples which received polishing with Soflex discs after enamel reduction were smoother than untreated enamel. Generally, the larger grit IPR instruments created rougher enamel surfaces (Ra values for medium bur: 702.4 ± 134.4 nm; medium strip: 501.0 ± 115.3 nm; mesh disc: 307.1 ± 106.9 nm) and the smaller grit instruments resulted in smoother surfaces (Ra values for fine bur: 407.4 ± 94.8 nm; fine strip: 317.6 ± 49.6 nm; curved disc: 223.9 ± 64.7 nm). The differences in mean roughness within the groups of larger or smaller grit were significant ($p < 0.001$ and $p < 0.05$, respectively), and the differences in mean roughness between instruments of the same type but different grit (*e.g.* large grit bur compared to small grit bur) were all significant with p-values < 0.001 apart from surfaces prepared with different discs ($p = 0.122$). The smoothest surfaces were created by use of the entire series of Soflex polishing discs after the enamel reduction (Ra = 36.7 ± 13.7 nm), and these surfaces were significantly smoother than the control surfaces (Ra = 148.6 ± 38.5 nm)($p = 0.017$).

Conclusions: 1) Larger grit diamond instruments created rougher surfaces than the smaller grit counterparts; 2) Diamond burs created the roughest enamel surfaces, followed by diamond strips, followed by diamond discs; 3) The Soflex polishing discs created the smoothest surfaces, even smoother than that of the untreated enamel.

Clinical significance: IPR should be followed by polishing to create the smoothest possible surfaces and to reduce possible bacterial adhesion.

Introduction

Interproximal reduction (IPR), also known as enamel reduction, interdental stripping, air rotor stripping, slenderizing or reproximation, involves removal of enamel from the mesial and/or distal surfaces of the teeth. It is commonly used to create space, or to correct tooth size discrepancies, during orthodontic treatments with fixed and removable appliances (Joseph et al., 1992; Lapenaite and Lopatiene, 2014) and may be used in both the anterior or posterior regions of the mouth. A recent study has found that the majority of orthodontists (66%) routinely performed IPR in their practices (Barcoma et al., 2015). By reducing the width of enamel at the interproximal surfaces, it has been said that IPR may be effective in improving dental alignment and for enhancing post-orthodontic stability, particularly in the lower anterior region (Peck and Peck, 1972a; Peck and Peck, 1972b). In addition, IPR can reshape and improve anterior dental aesthetics, for example by removing the black triangles that may become evident after alignment of crowded segments (Tarnow et al., 1992; Lapenaite and Lopatiene, 2014).

IPR, however, inevitably alters the surface layer of enamel, changing the enamel surface morphology and contour. Numerous qualitative studies have revealed that removal of this outer enamel leaves many grooves and furrows on the surfaces of the teeth (Radlanski et al., 1988; Joseph et al., 1992; Lundgren et al., 1993; Piacentini and Sfondrini, 1996; Arman et al., 2006). Through scanning electron microscopy (SEM) investigations, grooved and roughened enamel surfaces were observed on the interproximal enamel on both deciduous and permanent teeth (Arman et al., 2006). These grooves and furrows formed hills and valleys, regularly or irregularly distributed, over the entire treated area (Piacentini and Sfondrini, 1996).

The SEM studies provide only a subjective measure of surface roughness. There are few quantitative studies on enamel after IPR, and they have mainly measured surface roughness (Ra) (Lundgren et al., 1993; Arman et al., 2006; Danesh et al., 2007). It has been found that IPR increased the surface roughness, regardless of the instruments used (Zachrisson et al., 2011). This

roughness may increase the susceptibility of stripped enamel to bacterial adhesion and biofilm formation, which is then shielded from the mechanical clearance of salivary flow, brushing or flossing, and thereby may promote demineralization or the build up of plaque and calculus. Numerous studies have established that various dental materials with rougher surfaces promote bacterial adhesion, for example composite resin (Carlen et al., 2001; Mei et al., 2011), porcelain (Kawai et al., 2000), Co-Cr alloy (Gao et al., 1998), and dental implants (Chin et al., 2007). However, other studies have found that IPR did not lead to an increased caries risk (Jarjoura et al., 2006; Zachrisson et al., 2007; Zachrisson et al., 2011). Whether IPR actually increases the susceptibility of the stripped enamel to caries is still a matter of debate (Rossouw and Tortorella, 2003; Zachrisson et al., 2011; Gupta et al., 2012). This may be because roughness is only one parameter of surface topography (detailed surface features) that influences bacterial adhesion, or it may be because the changes in the enamel surface are not significant enough to progress to a clinical event. Other topographic features of enamel surface after IPR are still poorly understood. A comprehensive investigation of surface shapes and features of enamel after IPR is essential for understanding the relationship between IPR and bacterial adhesion.

To allow for both qualitative and quantitative measures of surface roughness, the use of AFM was utilised in this study. The use of SEM would not allow any quantitative measurements to be made and would render the samples unusable for the following bacterial experiments; hence their use was contraindicated.

The aims of this study were to investigate the roughness of enamel surfaces produced by the most commonly used IPR instruments, and to evaluate the effect of polishing after IPR.

Materials and Methods

Enamel sample preparation

Sixty-four human premolar teeth, removed from patients at the University of Otago School of Dentistry for orthodontic purposes, were collected using the following exclusion criteria: presence of staining, demineralization, decay, fluorosis, enamel cracks, defects or restorations. Ethical approval for the study was obtained from the University of Otago Ethics Committee (Ethics Committee reference number 13/105).

The extracted teeth were immediately cleaned and disinfected using 70% ethanol and stored at 4°C in sterile distilled water for less than 1 week before being used in the experiments, as per previously published method (Hosoya et al., 2003). Enamel blocks measuring 3.5 mm (height) x 3.5 mm (width) x 2 mm (depth) were cut from the interproximal surfaces of the teeth. The 2 mm measurement of depth was measured from the highest point of the outer enamel towards the dentine. The blocks were cut using a straight, cylindrical, coarse diamond bur (Meisinger FG 842 012, Hager & Meisinger GmbH, Neuss, Germany) with special care taken to not damage the outer enamel in any way and randomly allocated to one of seven IPR instrument groups or the control group (n = 8 per group).

Enamel surface preparation

The seven IPR instruments that are used most commonly in orthodontic clinics were used in the study (Figure 1 and Table 1), including diamond burs, diamond strips, diamond discs, and Soflex polishing discs. There was also a control group that was not subjected to any IPR procedures.

A total of 64 enamel slabs were used in the experiments (n = 8 per group, including the control group). All the enamel stripping was carried out according to the manufacturers' instructions for each instrument and performed by one investigator. For all groups the sample was held along the axial walls in mosquito forceps whilst the IPR instrument was used on the outer enamel surface. For the burs, the hand-pieces were run at 400,000 rpm

with water-cooling and for discs, hand-pieces were run at 5000 rpm. For the strips, the sample was held in the mosquito forceps and pushed back and forth along the strip horizontally.

Each IPR instrument (*i.e.* bur, strip and disc) was used for one enamel sample only and then replaced. To ensure equal reduction of all teeth, an enamel reduction of 0.2 mm, measured by vernier calipers, was performed on each enamel surface. For the polishing group, the coarse Soflex disc was used until enamel reduction of 0.2 mm had been achieved and then the medium, fine and extra fine Soflex discs were used sequentially for 20s each to polish the reduced surface (*i.e.* 1 min polishing in total).

After completion of IPR, the samples were placed individually in 100 mL of distilled water and cleaned by sonication (Elmasonic S-30, Elma Schmidbauer GmbH, Singen, Germany) for 2 min. The enamel samples in the control group were only cleaned with sonication for 2 min without any IPR performed.

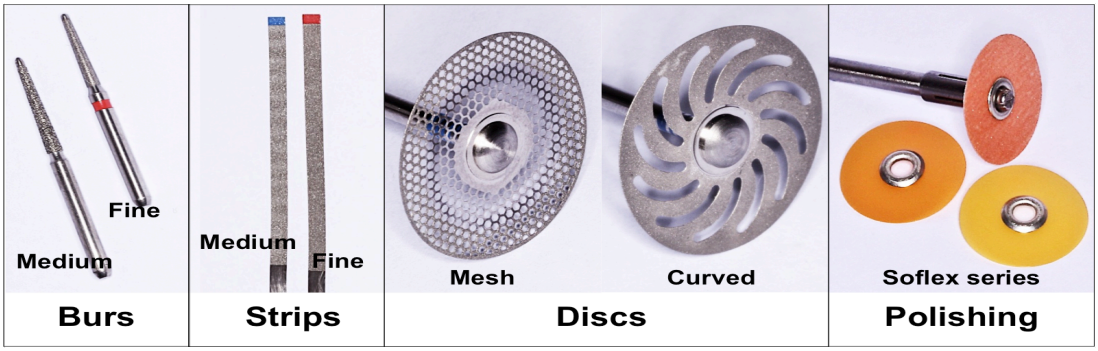

Figure 1 IPR instruments used in the study

Table 3 IPR instruments used in the study

IPR Instruments		*Model*	*Manufacturer*	*Grit*	*Hand-piece*
Burs	***Medium***	Safe-tipped medium diamond	Dentsply, York, USA	Medium (100-120μm)	High speed (400,000rpm) with water cooling
	Fine	Safe-tipped fine diamond	Dentsply, York, USA	Fine (50μm)	High speed (400,000rpm) with water cooling
Strips	***Medium***	SS-Med Interprox strip-W	Dentsply, York, USA	Medium (100-120μm)	N/A
	Fine	SS-Fine Interprox strip-W	Dentsply, York, USA	Fine (50μm)	N/A
Discs	***Mesh disc***	Flexview Mesh disc	Dentsply, York, USA	Medium (100-120μm)	Slow speed (5000rpm)
	Curved disc	Flexview Curved disc	Dentsply, York, USA	Fine (50μm)	Slow speed (5000rpm)
Polishing	***Soflex series***	Soflex system kit	3M ESPE, Irvine, USA	Variable	Slow speed (5000rpm)
None	***(Control)***	N/A	N/A	N/A	N/A

Measurements of surface topography

The surface topography of the prepared enamel samples was assessed using atomic force microscopy (Nanosurf NaioAFM, Liestal, Switzerland), in contact mode with ACLA Probe (Applied NanoStructures Inc., California, USA) at 190 kHz. All enamel slabs from each group were assessed and imaged at three randomly selected areas (50 μm x 50 μm). Surface plots were made to obtain a 3-dimensional perspective of the surface, from which the average surface roughness (Ra), peak height, valley depth, and peak-valley height were calculated for that area (Table 2). Each of the three areas contributed to an overall average calculation, to give an overall surface roughness, peak height, valley depth, and peak-valley height value for that specific enamel sample. A line along the Y-axis of each 50 μm x 50 μm section was randomly selected and measurements plotted to produce a 2-dimensional profile (graph) of the surface through that section.

Table 4 Surface topography measurements*

Parameter	***Meaning***
Roughness (Ra)	The average distance from the roughness profile
Root mean square	Quadratic mean, the square root of the mean of the squares of the samples
Peak height	The maximum z-value where z is a function of x and y coordinates
Valley depth	The minimum z-value where z is a function of x and y coordinates
Peak-valley height	The difference between peak height and valley depth

*All measurements were relative to the centre plane of the profile

Statistical analysis

Statistical analysis was performed using SPSS 19.0 software for Mac (SPSS Inc, Chicago, IL). The data were presented as mean ± SD and compared using a one-way analysis of variance (ANOVA). The threshold for type I error was set at 0.05. Bonferroni correction was used for multiple testing.

Results

Qualitative analysis of enamel surfaces after IPR

The morphologic profiles of the surfaces got progressively smoother when IPR instruments were changed from burs to strips to discs to polishers (Figure 2). The surface roughened with the larger grit diamond bur had the highest and sharpest peaks and troughs (Figure 2). The surfaces prepared with the large grit diamond strips also had sharp peaks and troughs, although the peaks and troughs were smaller. Enamel surfaces prepared with the mesh disc had even smaller peaks and troughs and also had linear scratches evident across the sample. No enamel rod-type structures could be identified in any of the enamel samples. The enamel samples polished by the Soflex series after IPR had a relatively smooth surface with some small linear scratches evident, but the surfaces appeared smoother than the untreated (control) samples, which had soft peaks and troughs across them.

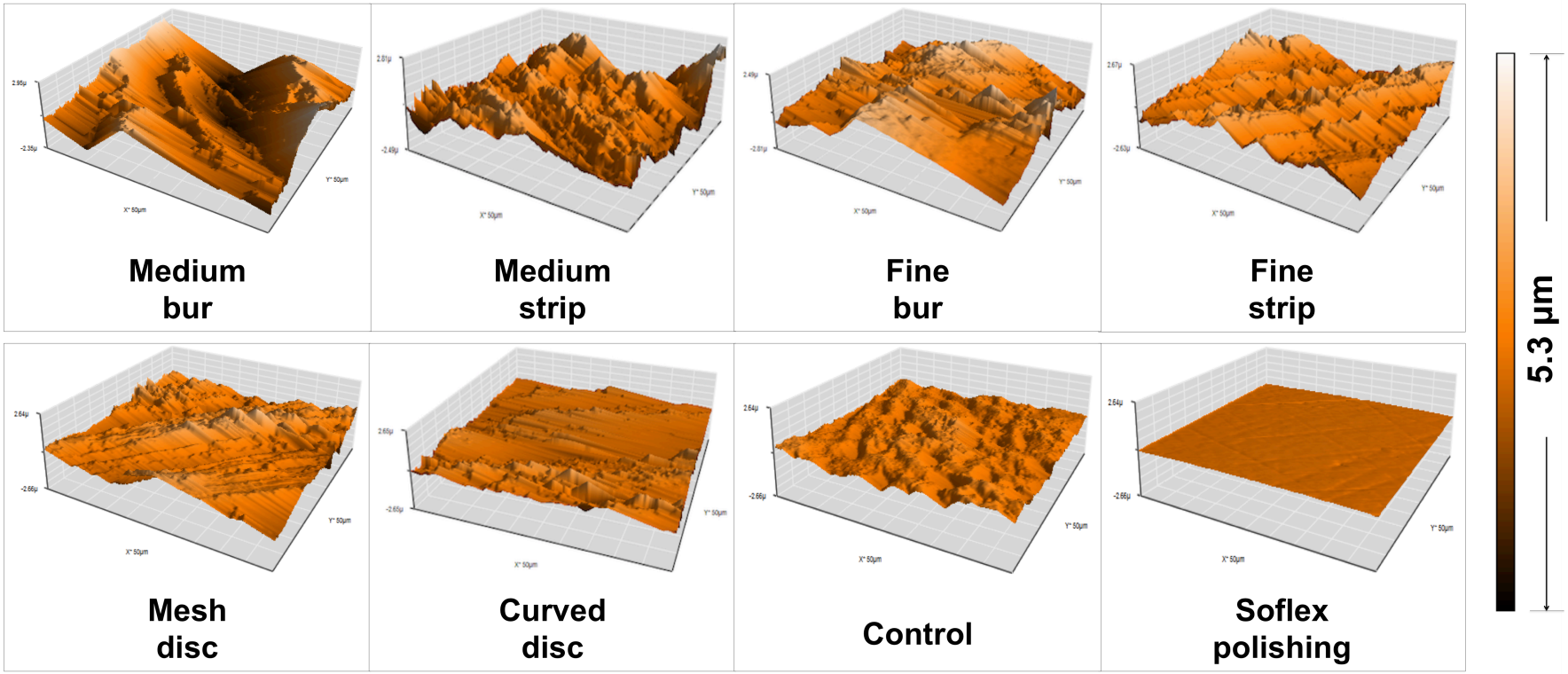

Figure 2 Representative examples of AFM 3D images of enamel surfaces after using different IPR instruments. Height of vertical bar is set at 5.3µm to allow direct comparison of samples. Colour spectrum of samples represents the magnitude of the peaks and valleys of each sample.

Quantitative analysis of enamel surfaces after IPR

The different IPR instruments produced varied enamel surface roughness (Figure 3). Overall, the diamond burs produced the roughest surfaces, followed by diamond strips and discs compared with the control enamel ($p < 0.001$)(Table 3). Use of the Soflex polishing series after IPR created the smoothest surfaces, which were even smoother than the untreated control samples ($p = 0.017$). The larger grit instruments (medium diamond bur, strip and mesh disc) produced rougher surfaces than their smaller grit counterparts (fine diamond bur, strip and curved disc)($p < 0.001$ except for the surfaces prepared by diamond discs where p-value = 0.122)(Figures 2 and 3).

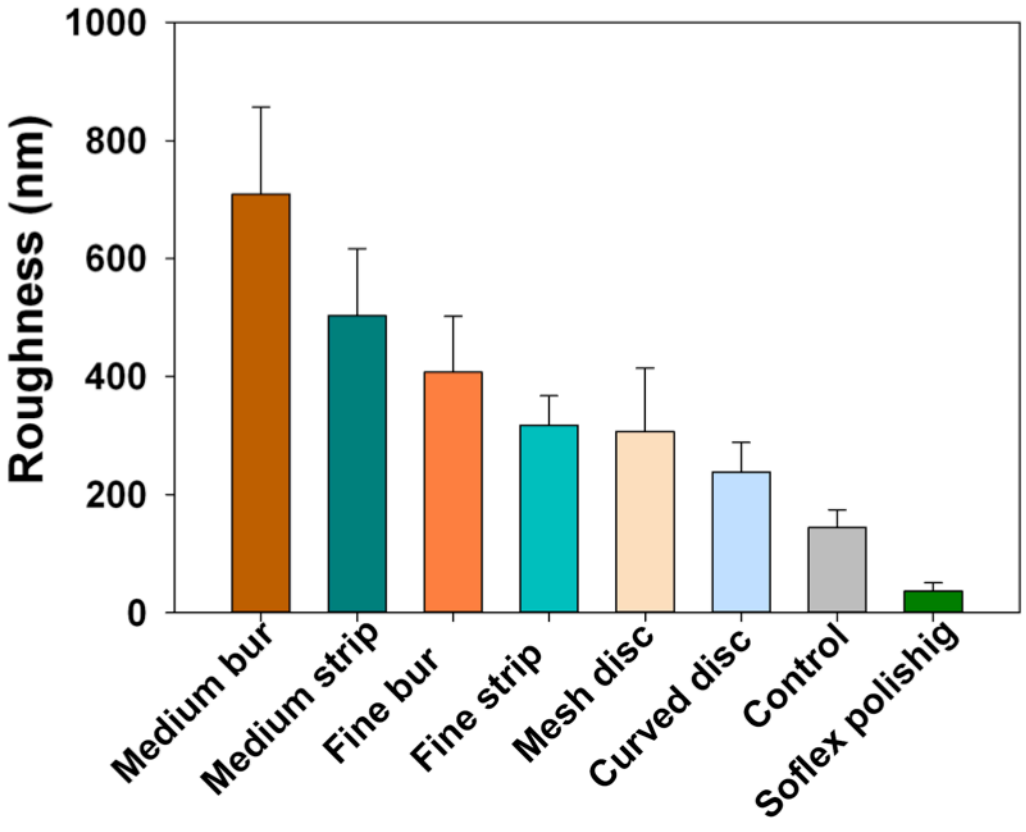

Figure 3 Average surface roughness (Ra) of enamel after using different IPR instruments (nm). Medium bur 702.4 ±134.4, Medium strip 501 ±115.3, Fine bur 407.4 ±94.8, Fine strip 317.6 ±49.6, Mesh disc 307.1 ±106.9, Curved disc 223.9 ±64.7, Control surface 148.6 ±38.5, Soflex polishing discs 36.7 ±13.7.

Table 5 Multiple comparison of roughness created by IPR instruments (p-values)

Instruments		*Burs*		*Strips*		*Discs*		*None*
		Medium	*Fine*	*Medium*	*Fine*	*Mesh*	*Curved*	*Control*
Burs	***Medium***	-	-	-	-	-	-	-
	Fine	<0.001	-	-	-	-	-	-
Strips	***Medium***	<0.001	0.033	-	-	-	-	-
	Fine	<0.001	0.045	<0.001	-	-	-	-
Discs	***Mesh***	<0.001	0.026	<0.001	0.811*	-	-	-
	Curved	<0.001	<0.001	<0.001	0.076*	0.122*	-	-
None	***Control***	<0.001	<0.001	<0.001	<0.001	<0.001	0.036	-
Polishing	***Soflex series***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.017

*Indicates non-significant p-values ($p > 0.05$)

The roughest surface was created by the medium diamond bur (Ra = 702.4 ± 134.4 nm), and was significantly rougher than the surface roughened by the medium diamond strip (Ra = 501.0 ± 115.3 nm), and the mesh disc (Ra = 307.1 ± 106.9 nm)($p < 0.001$). Within the group of smaller grit diamond instruments, the roughest surface was created by the fine diamond bur (Ra = 407.4 ± 94.8 nm), followed by the fine diamond strip (Ra = 317.6 ± 49.6 nm) ($p = 0.045$), and curved disc (Ra = 223.9 ± 64.7 nm)($p < 0.001$). Differences between all groups were statistically significant, except for the differences between the surfaces prepared with discs when compared to each other and to the surfaces prepared with the fine diamond strip (Table 3).

Peak height and valley depth of enamel surfaces after IPR

The 3-dimensional images of the samples revealed the same pattern as described above, whereby the highest peak, deepest valley and largest peak-valley height were recorded on the samples prepared by the diamond burs (Tables 4 and 5), followed by those prepared with the strips and then discs. The lowest readings were recorded on the samples polished after IPR with the Solfex polishing series, which had values even lower than the untreated control samples. A similar pattern can be seen in the 2-dimensional images, which were created from cross-section of the 3-dimensional images (Figure 4).

From the instruments with the larger grit, the medium bur had peak-valley height of 5017.4 ± 763.2 nm, the medium strip was 4737.1 ± 1189.2 nm and mesh disc was 2827.2 ± 742.4 nm (Table 5). Amongst the instruments with smaller grit, the fine bur had peak-valley height of 3856.9 ± 451.9 nm, fine strip was 3510.9 ± 399.3 nm and curved disc was 2646.5 ± 779.3 nm. The lowest value was recorded on the samples polished with Soflex discs, which had a peak-valley height of 580.8 ± 350.3 nm, even lower than the untreated control samples which measured at 2143.8 ± 1397.9 nm.

Table 6 Peak height and valley depth after using different IPR instruments (nm)

Instrument		*N*	*Peak Height*				*Valley Depth*			
			Mean	*Std Dev*	*Min*	*Max*	*Mean*	*Std Dev*	*Min*	*Max*
Burs	***Medium***	8	2717.7	434.6	1943.3	3394.2	-2299.7	392.6	-3050.8	-1891.0
	Fine	8	1902.1	799.8	956.1	2482.9	-1739.2	240.5	-2064.7	-1436.9
Strips	***Medium***	8	2174.3	1200.5	1374.5	4095.6	-2101.0	469.3	-2686.8	-1539.5
	Fine	8	1913.0	382.3	1392.0	2536.4	-1597.9	209.7	-1938.8	-1270.2
Discs	***Mesh***	8	1547.4	401.2	917.0	2085.6	-1279.8	367.8	-1796.9	-706.9
	Curved	8	1568.7	668.1	983.4	2907.6	-1077.9	223.8	-1544.5	-830.7
None	***Control***	8	1233.4	1088.2	521.9	3876.3	-910.4	339.4	-1607.8	-500.6
Polishing	***Soflex series***	8	313.0	246.2	80.9	731.4	-267.8	116.5	-456.5	-95.9

Table 7 Peak-valley height of enamel surface after using different IPR instruments (nm)

Instrument		*N*	*Mean*	*Std Dev*	*Std Error*	*95% Confidence Interval for Mean*		*Min*	*Max*
						Lower Bound	*Upper Bound*		
Burs	***Medium***	8	5017.4	763.2	269.8	4379.4	5655.4	3834.3	6119.1
	Fine	8	3856.9	451.9	159.8	3479.1	4234.6	3162.0	4296.7
Strips	***Medium***	8	4737.1	1189.2	420.4	3743.0	5731.3	2934.6	6166.0
	Fine	8	3510.9	399.3	141.2	3177.1	3844.7	2932.9	4198.3
Discs	***Mesh***	8	2827.2	742.4	262.5	2206.5	3447.9	1623.9	3709.7
	Curved	8	2646.5	779.3	275.5	1995.0	3298.1	1822.6	4050.1
None	***Control***	8	2143.8	1397.9	494.2	975.1	3312.4	1022.5	5484.1
Polishing	***Soflex series***	8	580.8	350.3	123.8	288.0	873.6	176.8	1187.9

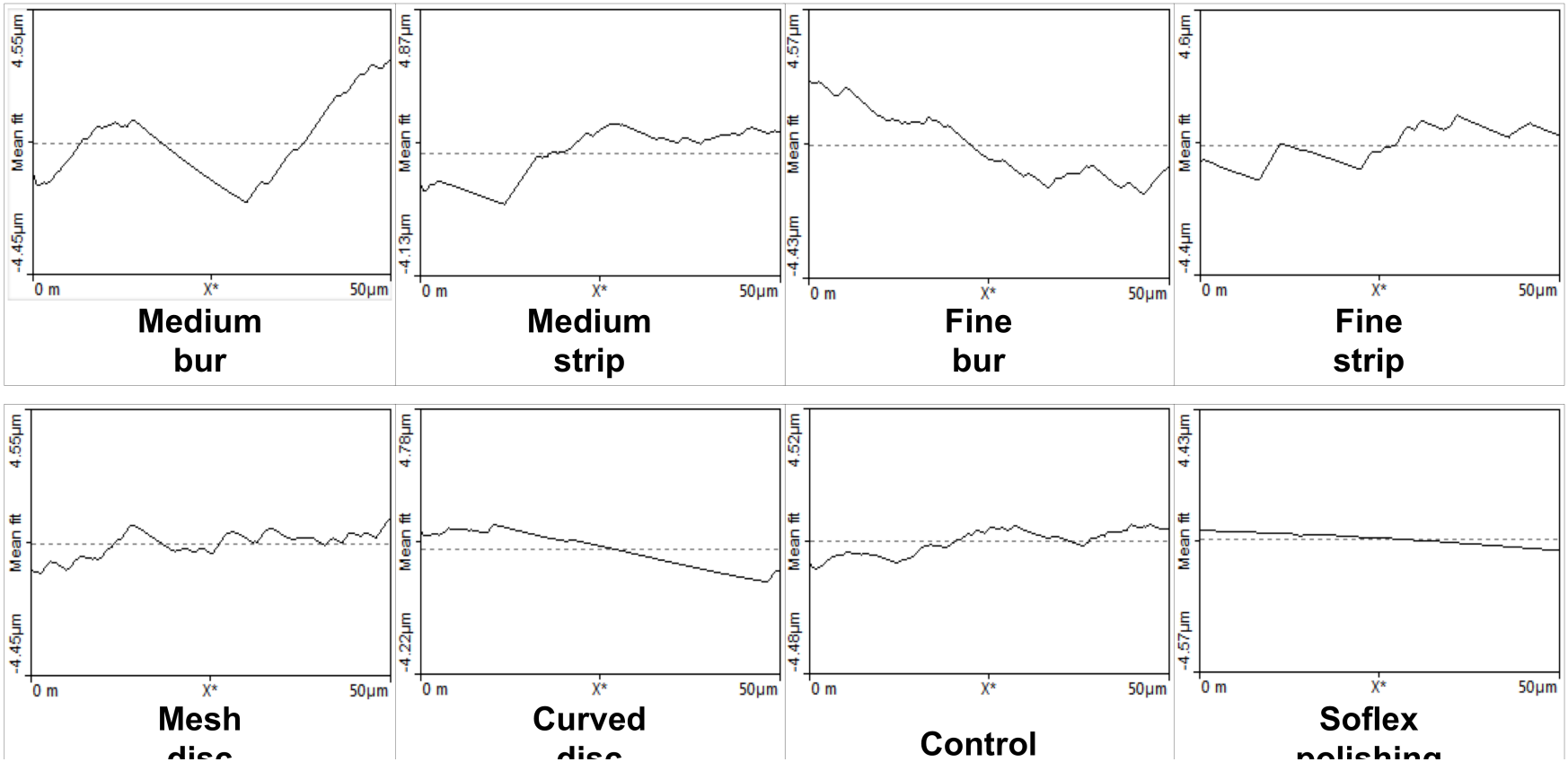

Figure 4 Peak height and valley depth of enamel surfaces after using different IPR instruments. Surface data along the Y-axis from the 3-dimensional images was plotted to give the 2-dimensional data above *(i.e.* cross-section of the 3-dimensional images).

Discussion

Interproximal reduction (IPR), also known as enamel reduction, interdental stripping, air rotor stripping, slenderizing or reproximation, is a routine technique used to reshape teeth and/or obtain space during orthodontic treatments. In IPR, diamond-coated strips or rotating devices are used to remove small amounts of enamel from the sides of the teeth. This procedure is not limited to fixed orthodontic treatment, but is also commonly used in patients wearing removable, transparent plastic aligners, which are becoming increasingly popular due to their low aesthetic impact (Rossouw and Tortorella, 2003; Kravitz et al., 2008). Unfortunately, the IPR procedures have been shown previously to leave grooves and furrows on the enamel leading to a significantly increased surface roughness (Piacentini and Sfondrini, 1996; Danesh et al., 2007), even after polishing (Gupta et al., 2012).

It has long been debated as to whether performing IPR (either by strips, discs, or tungsten carbide or diamond burs) leaves the enamel surface rougher than untreated teeth. This is of interest since, logically, a rougher surface may increase plaque retention and possibly the risk of caries at that site.

The aim of this study was to establish whether the techniques most commonly used for IPR did in fact cause a roughened enamel surface, and to what degree those surfaces could be polished smooth. A secondary aim was to establish techniques to create enamel surfaces with varied roughness for subsequent bacterial adhesion experiments.

The results showed that when diamond coated instruments were used for IPR they produced significant roughening of the enamel surfaces. This was in agreement with previous studies (Radlanski et al., 1988; Joseph et al., 1992; Lundgren et al., 1993; Piacentini and Sfondrini, 1996; Arman et al., 2006) and is important clinically since this may increase the adhesion of bacteria to these surfaces and therefore the caries risk on these teeth in orthodontic patients. Previous research has showed that IPR with the use of

diamond burs or discs, followed by running a finishing strip lightly coated with 37% phosphoric acid over the surface, helped to reduce the roughness created by the diamond instruments (Joseph et al., 1992). It was stated in this particular SEM study, that the demineralised surface was capable of "self-healing" with an increased potential to remineralise. This was contradicted by a later study where the surfaces subjected to the same chemical stripping were actually rougher than if the strips or discs were used alone (Arman et al., 2006). It may be important to note that the earlier study used only quantitative (*i.e.* subjective) methods for assessment of the surface roughness (SEM) (Joseph et al., 1992), whereas the later study that showed the acid etching to leave rougher surfaces, had used qualitative assessment by means of profilometry to measure the surface roughness (Arman et al., 2006). In this study, only mechanical methods of surface polishing (*i.e.* by use of Soflex discs) were investigated.

There has been doubt cast over the accuracy of AFM in the measurement of enamel surface roughness when the taper of the probe is wider than the troughs in the enamel (Vitkov et al., 2008). However, this particular study investigated images produced by *etched* enamel surfaces (not mechanically roughened surfaces) where enamel crystals were exposed as a consequence of dissolution by exposure to phosphoric acid. The authors concluded that the 3-dimensional images produced by AFM were not accurate when compared to the SEM images of the same sample, however, it was not clear whether or not the same area on the samples had been imaged, or whether it was another area on the same sample. To *quantitatively* measure the enamel roughness caused by mechanical means, use of an SEM is of no value. Therefore, to allow measurement of the enamel surface roughness both qualitatively and quantitatively, as well as being able to later use the same samples for bacterial experiments, use of AFM was decided upon for the present study.

Previous research has concluded that up to 0.25mm per anterior tooth surface, and up to 0.5mm per posterior tooth surface is safe to be removed during IPR (Chudasama and Sheridan, 2007; Hall et al., 2007; Sarig et al.,

2015). Therefore, a reduction of only 0.2mm per enamel surface of the premolars in the present study was justified.

The various methods used to perform the IPR in this study showed significant differences in the amount of roughness created. The diamond burs created the roughest surfaces, followed by diamond strips and then diamond discs. This too is in agreement with previous studies where diamond-coated materials as well as other instruments have been used to roughen enamel surfaces (Arman et al., 2006; Danesh et al., 2007). In a previous study, where SEM and profilometry were used to assess the enamel surfaces after IPR, it was found that the diamond-coated instruments all created surfaces that were rougher than the untreated enamel (Arman et al., 2006), and this included when Soflex discs were used for polishing after IPR. The surfaces that were subjected to polishing were not made smoother than the control surfaces, but they did result in surfaces that were smoother than when there was no polishing performed.

In this study it was demonstrated that the use of polishing could create an outer enamel surface even smoother than the untreated (control) surfaces. The polished surfaces were significantly smoother than all other surfaces, with a distinctively flatter appearance under the atomic force microscope (AFM). The previous quantitative study already mentioned, had shown that polishing with the Soflex discs reduced surface roughness, but it did not reduce it to a level that was smoother than the control samples (Arman et al., 2006). One possible explanation for the disagreement between the present results and theirs, may be that for the polishing procedure in this study we used the entire series of Soflex polishing discs (medium, fine, extra fine) for 20 sec each (*i.e.* 1 min of polishing in total), whereas the previous study, used only one Soflex disc (fine grit) and for only 20 sec. The use of the single disc alone for such a short period may not have been adequate to remove the roughness created by the IPR instrument beforehand.

There are conflicting statements in the literature regarding the effect of polishing; several of the earlier studies stating that the furrows and scratches produced by IPR could not be removed by polishing (Radlanski et al., 1988; Joseph et al., 1992; Lundgren et al., 1993; Piacentini and Sfondrini,

1996; Arman et al., 2006), but other more recent publications suggesting that if enamel is polished after stripping it can be made even smoother than untreated enamel (Zhong et al., 1999; Zhong et al., 2000; Danesh et al., 2007). This may be due to the improvement in polishing materials that have only become available more recently. Most studies do now recommend the use of thorough polishing following IPR (Piacentini and Sfondrini, 1996; Zhong et al., 1999; Zhong et al., 2000; Arman et al., 2006; Danesh et al., 2007).

Previously, it has been suggested that composite resin be used to seal the furrows that are left within the reduced enamel (Sheridan and Ledoux, 1989) as this appeared to leave a smooth surface. It was also stated that this might decrease the risk of future caries. Other studies have observed a low incidence of caries on teeth treated with IPR (Jarjoura et al., 2006; Zachrisson et al., 2007; Zachrisson et al., 2011), which raises the question about whether the polishing is really relevant clinically. A smoother surface, logically, seems an advantage, but whether this actually has a significant impact long-term is still unknown.

Without polishing after IPR, the enamel is left significantly rougher than the untreated control surfaces, and since it has been shown that polishing of the surfaces does not significantly increase the amount of enamel removed (Danesh et al., 2007), it is thought that polishing of these surfaces should be a priority.

Conclusion

Different IPR instruments produced different roughnesses and varied topography on the enamel surfaces. Generally, enamel surfaces will have the largest peak-valley height and be roughest with the use of diamond-coated burs, followed by diamond-coated strips and then diamond coated discs. Polishing with Soflex polishing discs reduced the enamel surface roughness after enamel reduction and this surface may be made even smoother than untreated enamel. Despite the shortage of evidence regarding the long-term advantages, or lack of, of polishing, given that it removes an insignificant amount of enamel, and does not seem to increase chair time hugely, in my

opinion I would strongly encourage all clinicians to polish enamel surfaces following IPR procedures.

Clinical implications

IPR should be followed by polishing to create the smoothest possible surfaces and to reduce possible bacterial adhesion. Only a small amount of time is needed to carry out the polishing required to significantly reduce surface roughness of enamel after IPR procedures (1 min in this study).

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Chapter 3

Bacterial Adhesion to Roughened Enamel

Abstract

Introduction: Interproximal reduction (IPR), a procedure often performed to create space or re-shape teeth during orthodontic treatment, leaves a roughened enamel surface. The presence of surface roughness may increase bacterial adhesion and the risk of future dental caries. The aim of this study was to assess the roughness of enamel surfaces (both qualitatively and quantitatively) after IPR, to investigate the adhesion of bacteria to these surfaces, and to evaluate the effect of polishing after IPR on the amount of bacterial adhesion.

Materials and methods: Thirty-two human premolar teeth were collected and cut for the experiments. Enamel surfaces were prepared with diamond bur (n = 8), diamond disc (n = 8), and Soflex polishing discs (n = 8) to create variable surface roughness. The control group (n = 8) had no IPR performed. The enamel surfaces were assessed using atomic force microscopy (AFM). Clarified human saliva was used to create a salivary pellicle on the roughened enamel. *Streptococcus sanguinis* ATCC10556 cells were incubated with the enamel blocks for 30 min at 37°C to allow bacteria to adhere to the samples. Colony forming units (CFUs) were counted to assess the number of bacteria that adhered.

Results: Enamel blocks with significantly different surface roughness were obtained by use of medium bur (702.4 ± 134.4 nm), mesh disc (307.1 ± 106.9 nm), control surface (148.6 ± 38.5 nm), and Soflex polishing discs (36.7 ± 13.7 nm)($p < 0.001$). The number of CFUs was highest on the roughest surface, created by the medium bur (CFUs = $12.3 \pm 0.5 \times 10^5$), followed by the surfaces roughened with the mesh disc (CFUs = $4.0 \pm 0.5 \times 10^5$). The control surface had the next highest count (CFUs = $1.2 \pm 0.1 \times 10^5$) and the smoothest surfaces, created by the Soflex polishing discs, had the lowest count (CFUs = $0.3 \pm 0.05 \times 10^5$)($p < 0.001$). A significant positive relationship was found between the enamel surface roughness and the number of bacteria adhering ($p < 0.001$).

Conclusions: 1) The diamond bur created rougher surfaces than the mesh disc; 2) The Soflex polishing discs created the smoothest surfaces, even

smoother than untreated enamel; 3) There was a positive relationship between enamel surface roughness and the number of bacteria that adhered.

Clinical significance: It is recommended that practitioners polish interproximal enamel after stripping to leave the enamel as smooth as possible in an attempt to reduce streptococcal adhesion.

Introduction

Interproximal Reduction (IPR) is a common clinical procedure used during orthodontic treatment, involving removal of enamel from the mesial and distal surfaces of the teeth. IPR leaves grooves and furrows on enamel leading to significantly increased surface roughness (Piacentini and Sfondrini, 1996; Danesh et al., 2007), even after polishing (Gupta et al., 2012).

A positive relationship between bacterial adhesion and surface roughness has been shown for a variety of dental materials including composite resin (Carlen et al., 2001), porcelain (Kawai et al., 2000), Co-Cr alloy (Gao et al., 1998), and dental implants (Chin et al., 2007). However, some studies have found that IPR (which creates roughened enamel) does not increase caries susceptibility (Radlanski et al., 1989; Crain and Sheridan, 1990; El-Mangoury et al., 1991; Jarjoura et al., 2006; Zachrisson et al., 2011), even during the 10 years following the IPR procedure (Zachrisson et al., 2007). Patients who had IPR in the anterior region showed no significant changes in periodontal health (Boese, 1980), with one report assessing patients as long as 16 years after their orthodontic treatment (Årtun et al., 1987). To date, whether the IPR increases bacterial adhesion to enamel is still a matter of debate (Rossouw and Tortorella, 2003; Zachrisson et al., 2011; Gupta et al., 2012). Previous studies have failed to quantify the bacterial adhesion or measure its strength (Radlanski et al., 1988; Jarjoura et al., 2006; Zachrisson et al., 2011).

Bacterial adhesion to enamel surfaces is an early event in oral biofilm formation and caries development (Peterson et al., 2011; Takahashi and Nyvad, 2011), but the first event is formation of the salivary pellicle, to which the initial bacterial colonizers adhere. This salivary pellicle, the protein rich, organic film covering the tooth surfaces, is detectable on enamel surfaces within 1 min of exposure to the environment within the oral cavity (Hannig, 1999) and permits the adhesion of bacteria and subsequent formation of a dental biofilm. The majority of primary colonisers are oral streptococci, *e.g. Streptococcus sanguinis*, which account for 60-80% of the bacteria in the

dental biofilm within the first 4-8 h (Nyvad and Kilian, 1987; 1990; Diaz et al., 2006; Dige et al., 2009). If patients cannot remove the bacteria adhered to the enamel, the acid produced by this biofilm can eventually cause side effects including gingival inflammation and dental caries.

The aim of this research was to investigate the influence of enamel surface roughness created by IPR on bacterial adhesion, and to evaluate the effect of polishing on reducing bacterial adhesion.

Despite experimental evaluation of several bacteria during pilot studies (*i.e. Streptococcus gordonii*, *Streptococcus oralis, Streptococcus mitis*), in this study, only *S. sanguinis* (strain ATCC10556) was used for the measurement of bacterial adhesion to roughened enamel because of its optimal growth kinetics for the planned experiments.

Materials and Methods

Enamel sample preparation

Thirty-two human premolar teeth, removed for orthodontic purposes, were collected at the University of Otago School of Dentistry using the following exclusion criteria: presence of any staining, demineralization, decay, fluorosis, enamel cracks, defects or restorations. Ethical approval for the study was obtained from the University of Otago Ethics Committee (Ethics Comitttee reference number 13/105).

The extracted teeth were immediately cleaned and disinfected using 70% ethanol and stored at 4°C in sterile distilled water for less than 1 week before being used in the experiments, as per previously published method (Hosoya et al., 2003). Enamel blocks measuring 3.5 mm (height) x 3.5 mm (width) x 2 mm (depth) were cut from the interproximal surfaces of the teeth. The 2 mm measurement of depth was measured from the highest point of the outer enamel towards the dentine. The blocks were cut using a straight, cylindrical, coarse diamond bur (Meisinger FG 842 012, Hager & Meisinger GmbH, Neuss, Germany) and special care was taken to not damage

the outer enamel in any way and allocated to one of three IPR instrument groups or the control group (n = 8 per group).

Enamel surface preparation

Three commonly used IPR instruments (Lapenaite and Lopatiene, 2014) were used in this study (Figure 1 and Table 1), including medium diamond bur, mesh disc and Soflex polishing discs. There was also a control group that was not subjected to any IPR procedures.

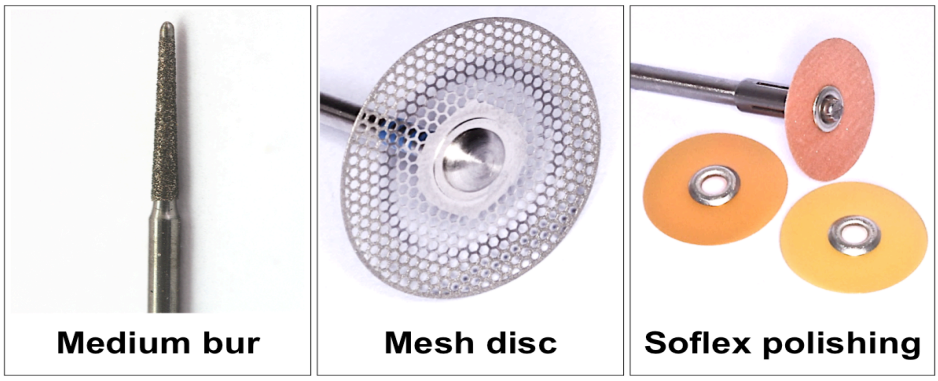

Figure 5 IPR instruments used in the study

A total of 32 enamel blocks were used in the experiments (n = 8 per group). All the enamel stripping was carried out according to the manufacturers' instructions for each instrument and performed by one investigator. For all groups the sample was held along the axial walls in mosquito forceps whilst the IPR instrument was used on the outer enamel surface. For the bur, the hand-piece was run at 400,000 rpm with water-cooling; and for discs, the hand-pieces were run at 5000 rpm. Each IPR instrument (*i.e.* bur or disc) was used for one enamel sample only and then replaced. To ensure equal reduction of all teeth, an enamel reduction of 0.2 mm, measured by vernier calipers, was performed on each enamel surface. For the polishing group, the coarse Soflex disc was used until enamel reduction of 0.2 mm had been reached and then the medium, fine and extra

fine Soflex discs were used sequentially for 20s each to polish the reduced surface (*i.e.* 1 min polishing in total).

After completion of IPR, the samples were cleaned individually in 100 mL of distilled water with sonication for 2 min (Elmasonic S-30, Elma Schmidbauer GmbH, Singen, Germany). The enamel samples in the control group were only cleaned with sonication for 2 min without any IPR procedures.

Table 8 IPR instruments used in the study

IPR Instruments	*Model*	*Manufacturer*	*Grit*	*Hand-piece*
Medium bur	Safe-tipped medium diamond	Dentsply, York, USA	Medium (100-120μm)	High speed (400,000rpm) with water cooling
Mesh disc	Flexview Mesh disc	Dentsply, York, USA	Medium (100-120μm)	Slow speed (5000rpm)
Polishing	Soflex system kit	3M ESPE, Irvine, USA	Variable	Slow speed (5000rpm)
None (control)	N/A	N/A	N/A	N/A

Enamel surface roughness measurements

The surface roughenss of the prepared enamel samples was assessed using atomic force microscopy (Nanosurf NaioAFM, Liestal, Switzerland), in contact mode with ACLA Probe (Applied NanoStructures Inc., Mountain View, California, USA) at 190 kHz. All enamel blocks from each group were assessed and imaged at three randomly selected areas (50 μm x 50 μm), and surface plots were made to obtain average surface roughness (Ra) values.

Bacterial growth

Streptococcus sanguinis ATCC10556 was plated on Columbia Sheep Blood Agar (Cat # 1100: Fort Richard Laboratories, Auckland, New Zealand) and incubated in an anaerobic chamber at 37°C for 24 h. For adhesion experiments, bacteria from the blood agar plates were cultured in 10 mL pre-warmed, sterile Tryptic Soy Broth (30 g of Tryptic Soy Broth (Bacto™) Soybean-Casein Digest Medium powder per L distilled water; TSB) in a glass tube statically, at 37°C, for 14 h. The optical density (OD) of a 1 in 10 dilution of this culture in sterile broth was measured in a spectrophotometer (Ultrospec 6300 Pro Spectrophotometer: Biochrom, Cambridge, UK) at a wavelength of 600 nm (OD_{600}). A portion (0.5 mL) of the remaining bacterial broth was used to inoculate 10 mL sterile, pre-warmed TSB (*i.e.* a 1 in 20 dilution). This culture was incubated at 37°C and the OD_{600} measured every hour to find the mid-log phase (in order to best represent the growth of bacteria within the mouth).

The time taken for *S. sanguinis* ATCC10556 to reach mid-log phase (OD_{600} ~0.8) was found to be 5 h. At this time point, the OD_{600} of a 1 in 10 dilution of the culture was measured and bacteria in the remaining culture were harvested by centrifugation at 8228 x g for 10 min. The bacteria were washed in 1 mL phosphate buffered saline (PBS) and centrifuged again at 8228 x g for 3 min. The supernatant was poured off and bacteria were resuspended in 1 mL of PBS. The bacterial suspension was subjected to sonication at 25% power (Branson Digital Sonifier, Emerson, Danbury, USA) with a probe for 10 s to separate the cells prior to being

used in the adhesion assays. The OD_{600} of a 1 in 20 dilution of the bacterial suspension was measured and the cells were stored on ice until needed.

Measurement of bacterial adhesion

Bacterial adhesion experiments used a modification of a previously published method (Hosoya et al., 2003). Whole saliva was collected on ice on one occasion only from three subjects and an equal volume from each was pooled. Dithiothreitol (125 mM) was added to the saliva to give a final concentration of 2.5 mM. Saliva was clarified as per previously published technique using centrifugation at 40,000 x g for 30 min (Sweet et al., 1990). Following preparation, saliva was transferred into eppendorfs in 1 mL portions and frozen until needed. Once required, it was thawed at room temperature for 30 min before being used. Saliva was not re-frozen if it was not used. Enamel blocks were incubated in a sterile 24-well plate in 1 mL clarified human saliva statically at room temperature (one block per well) for 30 min to allow a salivary pellicle to form. Each block was washed (by dipping in 1 mL sterile PBS) 3 times and placed into an unused well of the sterile 24-well microtitre plate. The sonicated bacteria in PBS (1 mL) at an OD_{600} of 1.0 were added to each well containing an enamel block. The microtitre plate was then gently shaken at 180 rpm for 30 min to mimic the intra-oral flow of saliva across the enamel surfaces.

Whilst being held along the axial walls only, the enamel blocks were washed once (by dipping in 1 mL sterile PBS) to remove any non-adherent bacteria. Extreme care was taken to ensure that the roughened upper surface of the enamel block with attached bacteria was not disturbed. A sterile cotton swab, pre-moistened with PBS, was used to remove bacteria from the roughened surface. Each quarter of the swab was used in a different direction across the sample (*i.e.* vertical, horizontal and each diagonal). The swab was then placed into a sterile eppendorf tube containing 1 mL PBS solution and the tip broken off. The solution was vortexed for 1 min to disperse the bacteria and swab was removed. The bacterial solution was diluted 1 in 100 with PBS then pipetted onto Columbia sheep blood agar plates (Cat # 1100: Fort Richard Laboratories) which had been left to warm to room temperature for 30 min prior to use. Three separate drops of 50 µL of the diluted solution were placed on one agar plate at least 1 cm apart thereby giving three

readings per enamel block. The 32 plates were incubated anaerobically at 37°C for 24 h. Colony forming units (CFUs) from each droplet (three per sample) were counted and averaged to calculate the number of bacteria adhering to each enamel block. To count accurately, the plates were photographed with a macro lens, images printed in colour and blacked out with a marker once counted.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 software for Mac (SPSS Inc, Chicago, IL). The data were presented as mean ± SD and compared using a one-way analysis of variance (ANOVA). The threshold for type I error was set at 0.05. Bonferroni correction was used for multiple testing.

Results

Enamel surface roughness

The different IPR instruments produced significantly different enamel surface roughness ($p < 0.001$)(Tables 2 and 3 and Figure 2).

Table 9 Average surface roughness (Ra) of prepared enamel samples

Instruments	*N*	*Average Ra (nm ± SD)*
Medium bur	8	702.4 ± 134.4
Mesh disc	8	307.1 ± 106.9
None (control)	8	148.6 ± 38.5
Soflex polishing discs	8	36.7 ± 13.7

Table 10 Multiple comparison of enamel surface roughness after using different IPR instruments (p-values)

Instruments	*Medium bur*	*Mesh disc*	*Soflex polishing discs*
Medium bur	-	-	-
Mesh disc	<0.001	-	-
Soflex polishing discs	<0.001	<0.001	-
None (control)	<0.001	<0.001	0.017

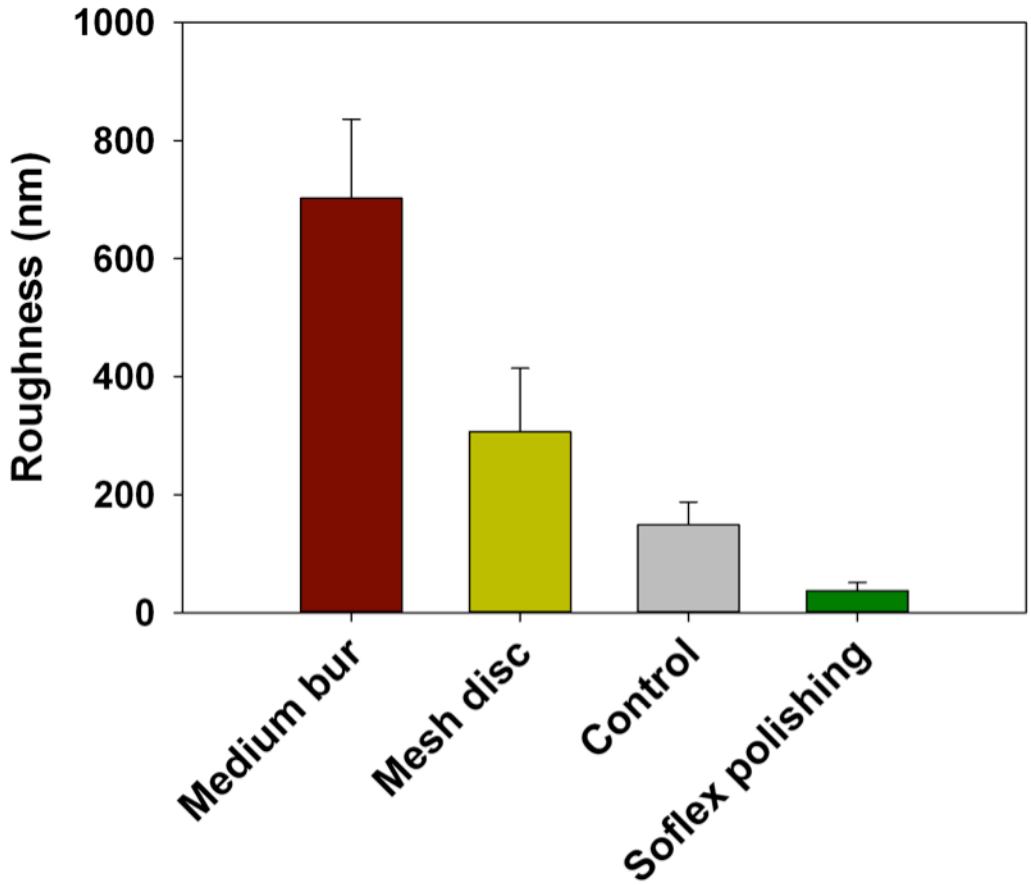

Figure 6 Surface roughness (Ra) of enamel samples prepared using different IPR instruments Medium bur 702.4 ±134.4, Mesh disc 307.1 ±106.9, Control surface 148.6 ±38.5, Soflex polishing discs 36.7 ±13.7.

Bacterial adhesion

Optical Density (OD_{600}) readings were taken following overnight growth of bacteria as well as at hourly intervals for the sub-culture so that the mid-log phase could be identified. For *S. sanguinis* ATCC10556, the OD_{600} of the nine overnight cultures was, on average, 1.64 ± 0.16 (Table 4). The mid-log phase was presumed to occur at an OD_{600} of ~0.8, and this was reached approximately 5 h following sub-culture of the overnight culture (with an average OD_{600} of 0.79 ± 0.10).

Table 11 OD_{600} readings for *S. sanguinis* ATCC10556

Experiment	*Overnight OD_{600}*	*Average ± SD*	*OD_{600} at 5 h*	*Average ± SD*
1	1.75		0.72	
2	1.72		0.73	
3	1.32		0.64	
4	1.73		0.82	
5	1.59	1.64 ± 0.16	0.79	0.79 ± 0.10
6	1.47		0.72	
7	1.68		0.85	
8	1.83		0.99	
9	1.71		0.87	

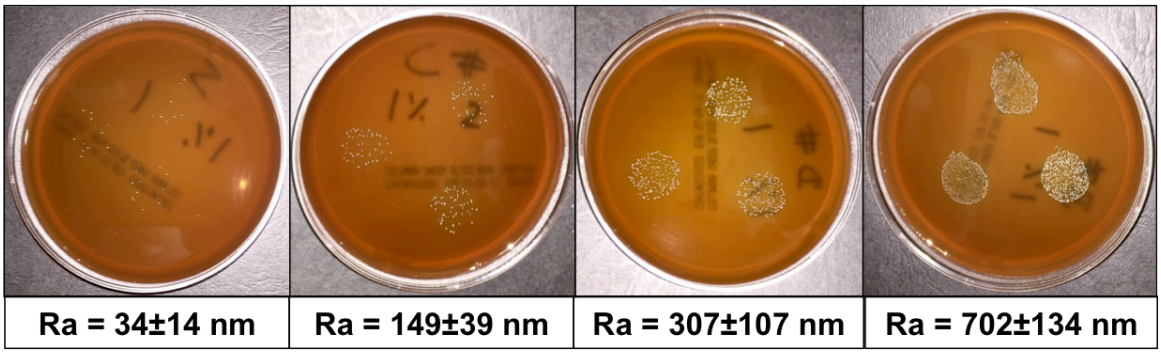

Figure 7 Representative examples of CFUs from enamel blocks with variable surface roughness (Ra) (nm). Ra 34 ±14 = 0.3 ± 0.05 x10^5 CFU, Ra 149 ±39 = 1.2 ± 0.1x10^5 CFU, Ra 307 ±107 = 4.0 ± 0.5 x10^5 CFU, Ra 702 ±134 = 12.3 ±0.5 x10^5 CFU.

Table 12 Average number of Colony Forming Units (CFUs) per enamel block

Instruments	*N*	*Roughness (nm) ± SD*	*CFUs (x10^5/enamel block) ± SD*
Medium bur	8	702.4 ± 134.4	12.3 ± 0.5
Mesh disc	8	307.1 ± 106.9	4.0 ± 0.5
None (control)	8	148.6 ± 38.5	1.2 ± 0.1
Soflex polishing discs	8	36.7 ± 13.7	0.3 ± 0.05

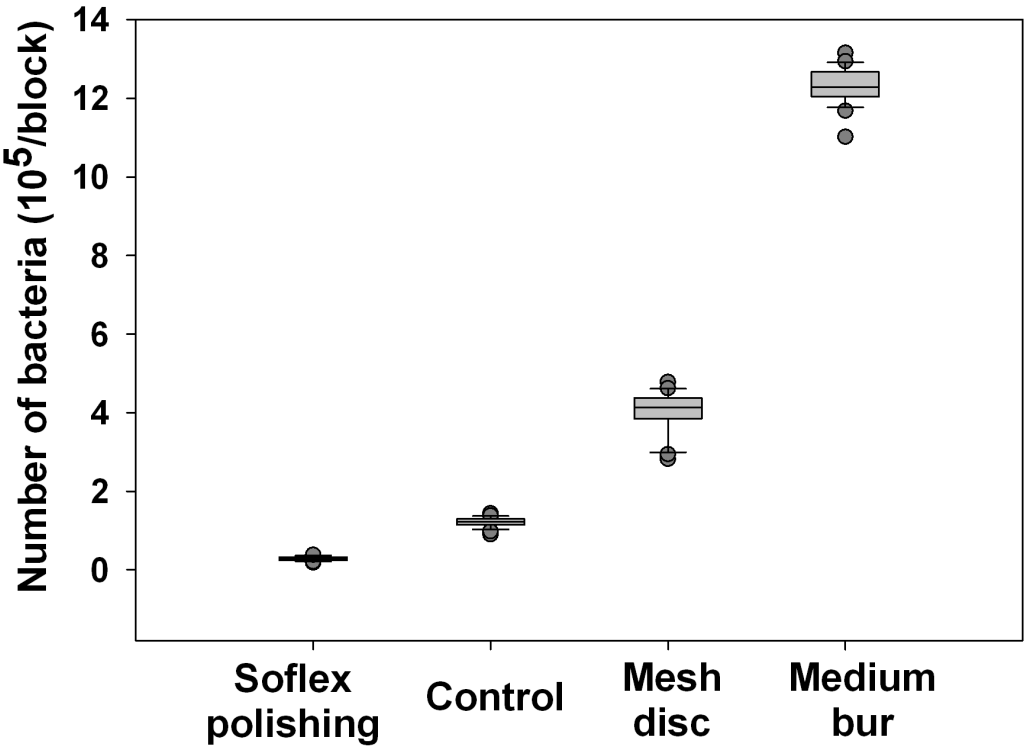

Figure 8 Average number of bacteria adhering to enamel blocks with variable surface roughness (x10^5/enamel block). Soflex polishing discs = 0.3 ± 0.05, Control surface = 1.2 ± 0.1, Mesh disc = 4.0 ± 0.5, Medium bur = 12.3 ± 0.5.

The enamel surfaces that were rougher gave an increased number of bacterial colonies (Figures 3 and 4 and Table 5). The roughest surface, prepared with the medium diamond bur (Ra = 702.4 ± 134.4 nm) gave the highest number of colony forming units (CFUs) per enamel block (12.3 x 10^5 ± 0.5 x 10^5), followed by the next roughest surface, prepared by the mesh disc (Ra = 307.1 ± 106.9 nm) which had a

CFU count of 4.0 x 10^5 ± 0.5 x 10^5 ($p < 0.001$ (Table 6)). The smoothest surface, prepared with the Soflex polishing discs (Ra = 36.7 ± 13.7 nm) had the lowest number of CFUs per enamel block (0.3 x 10^5 ± 0.05 x 10^5)($p < 0.001$). The polished enamel was smoother and had a lower CFU count than the control surface (Ra = 148.6 ± 38.5 nm), which had CFU of 1.2 x 10^5 ± 0.1 x 10^5 per enamel block ($p < 0.001$). More *S. sanguinis* adhered to the rougher enamel surfaces ($p < 0.001$). There was a significant positive relationship between the surface roughness and the number of bacteria adhering to the enamel (Figure 5).

Table 13 Multiple comparison of number of bacteria adhering after using different IPR instruments (p-values)

Instruments	*Medium bur*	*Mesh disc*	*None (control)*
Medium bur	-	-	-
Mesh disc	<0.001	-	-
None (control)	<0.001	<0.001	-
Soflex polishing discs	<0.001	<0.001	<0.001

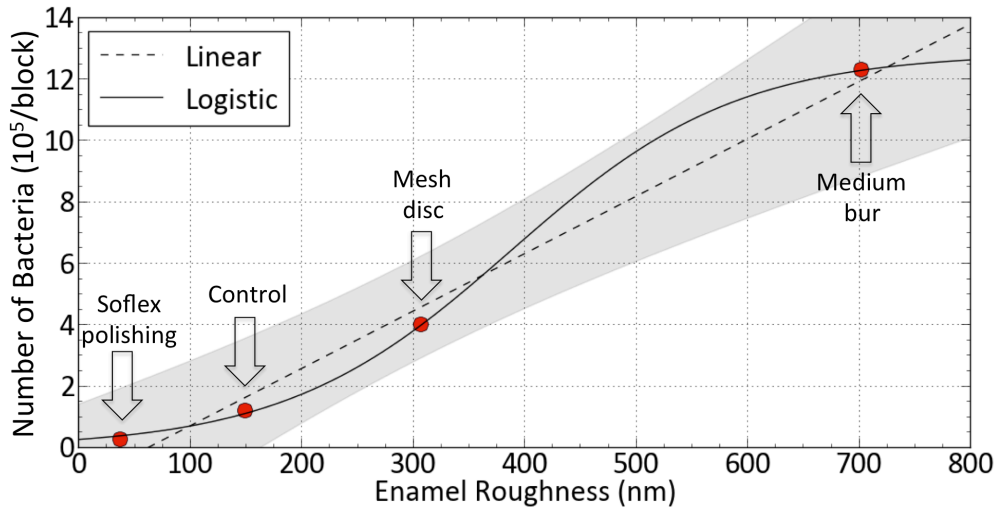

Figure 9 Linear and logistic regression plots for surface roughness and bacterial adhesion*

*For both models the coefficient of determinants were very high (>0.98).

Discussion

The process of bacterial adhesion to enamel and its subsequent effects has long been of considerable interest to dental researchers. The development of tooth decay depends on adhesion of initial colonizers to a salivary pellicle followed by further plaque development and enamel demineralization (Keyes, 1968). Development of a salivary pellicle on enamel occurs within 1 min of exposure to the oral environment (Hannig, 1999) and initial bacterial adhesion (within the first 4-8 h) consists predominantly of oral streptococci (including *Streptococcus sanguinis, Streptococcus gordonii and Streptococcus mitis*) (Nyvad and Kilian, 1987; 1990; Diaz et al., 2006; Dige et al., 2009). Adhesion of these bacteria then allow the early colonisers including *S. mutans* and *S. mitis* to attach along with bridging bacteria such as Fusobacteria spp. which increases the thickness and complexity of the dental biofilm (Peterson et al., 2011). Over time, the balance can shift in favour of the acid-producing bacteria (disease-associated organisms) and dental caries may result (Kleinberg, 2002).

The process of interproximal reduction (IPR), used commonly in orthodontic patients, is one that may result in a roughened enamel surface and may therefore increase the bacterial adhesion to this surface. It is still unknown whether this abraded enamel has increased susceptibility to bacterial adhesion in the mouth (Rossouw and Tortorella, 2003; Zachrisson et al., 2011; Gupta et al., 2012). Previous studies have used subjective methods and have not quantified the bacterial adhesion or measured the strength of adhesion to roughened enamel (Radlanski et al., 1988; Jarjoura et al., 2006; Zachrisson et al., 2011). However, initial colonizing bacteria have previously been shown to firstly adhere to the cracks and pits on the enamel surface (Nyvad and Fejerskov, 1987).

Dental caries is a multifactorial disease in which the host, diet and bacterial flora play a role (Keyes, 1968). In orthodontic patients who wear fixed appliances, there is an increase in the number of plaque retentive sites within the mouth and an increased risk for enamel surface demineralization (Gorelick et al., 1982; Mizrahi, 1982), which is of particular concern when present in combination with a roughened enamel surface after a procedure such as IPR.

Effect of roughness on bacterial adhesion

In this study it was found that a rougher enamel surface increased the number of *S. sanguinis* that adhered to it. There was a positive relationship between the roughness of the enamel surface and the number of bacteria that adhered, which *may* indicate an increased potential for dental caries on these surfaces. However, previous long-term studies investigating roughened enamel, which presumably may encourage the development of caries, have shown that this does not necessarily equate to an increased amount of decay clinically (Radlanski et al., 1989; Crain and Sheridan, 1990). Despite the fact that a *potential* risk clearly exists, an increase in the actual incidence of caries on interproximal surfaces that have undergone IPR versus those that have not, has never been demonstrated (Crain and Sheridan, 1990). This may be due to the multifactorial nature of dental decay, where increased bacterial adhesion in a non-susceptible host may not necessarily have a clinically significant effect. On the other hand, in a patient with an existing high caries risk, an increase in the bacterial adhesion may result in a significant increase in dental decay. It is the multifactorial nature of dental caries that makes it particularly hard to conclude, in *in vivo* conditions (where it is very hard to control for all contributing factors), which *particular* aspect of the patients' history (including previous IPR), has affected the outcome of disease or lack of.

Despite experimental evaluation of several bacteria during pilot studies (*i.e. Streptococcus gordonii*, *Streptococcus oralis, Streptococcus mitis*), in this study, only *S. sanguinis* (strain ATCC10556) was used for the measurement of bacterial adhesion to roughened enamel because of its optimal growth kinetics for the planned experiments. S. *sanguinis* belongs to the indigenous flora and is generally associated with oral health rather than disease (Caufield et al., 2000; Becker et al., 2002). However, as a pioneer species in initial colonisation, it allows subsequent adhesion of disease causing bacteria, which is why it's adhesion is of interest. It is presumed that, *S. sanguinis* ATCC10556 is representative of other strains of the same species, and that increased adherence of these bacteria will lead to an increase in overall plaque levels and potentially increase the risk of dental decay.

Effect of 3-D morphology on bacterial adhesion

The changes in enamel surface morphology following IPR have been thoroughly documented in the past (Radlanski et al., 1988; Joseph et al., 1992; Piacentini and Sfondrini, 1996; Lucchese et al., 2001). In this study, it was shown that the altered enamel surface (whether made rougher or smoother) had a significant effect on the amount of bacteria that would adhere to it. The rougher surfaces had increased numbers of bacteria, which is in agreement with other studies showing increased plaque accumulation at sites with rougher surfaces (Radlanski et al., 1988; Gao et al., 1998; Kawai et al., 2000; Carlen et al., 2001; Chin et al., 2007).

Conclusion

Different IPR instruments produce varying degrees of enamel surface roughness. The roughest surfaces are produced with use of diamond burs followed by diamond discs. The smoothest surfaces are created when polishing follows enamel reduction, and this may result in surfaces even smoother than untreated enamel. Larger numbers of *S. sanguinis* adhered to the rougher enamel, showing that increased enamel surface roughness promoted its adhesion.

Clinical implications

It is recommended to polish the enamel after IPR to reduce streptococcal adhesion, especially given the relatively short time required to create a smooth enamel surface (1 min in this study).

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Chapter 4

General Discussion

Interproximal reduction

Interproximal reduction (IPR) is the deliberate removal of a small amount of dental enamel from the interproximal contact area, which decreases the mesio-distal width of a tooth. This enamel may be removed for various reasons, but most commonly to create space during orthodontic treatment or to correct tooth size discrepancies (Lusterman, 1954; Hudson, 1956; Bolton, 1958; Lapenaite and Lopatiene, 2014). Some authors have encouraged its use as a method by which to enhance post orthodontic stability, particularly in the lower anterior region (Peck and Peck, 1972a; Peck and Peck, 1972b). With the increase in use of aesthetic, removable aligners for orthodontic treatment, where non-extraction therapy is often advocated, the use of IPR has become a hugely valuable tool to aid in relief of crowding without over expanding the dental arches.

Removal of this outer enamel has been shown to leave a roughened surface (Radlanski et al., 1988; Joseph et al., 1992; Lundgren et al., 1993; Piacentini and Sfondrini, 1996; Arman et al., 2006), which in theory may increase bacterial adhesion to it. The aetiology of dental caries and periodontal disease are both related to bacterial plaque accumulations, and the risk of these may be increased if more bacteria are able to bind to roughened enamel. In the past, IPR was only used occasionally in orthodontics, but today is much more common (Barcoma et al., 2015). The long-term disadvantages of IPR are still poorly understood (Årtun et al., 1987; Crain and Sheridan, 1990; El-Mangoury et al., 1991; Pinheiro, 2002; Jarjoura et al., 2006; Zachrisson et al., 2007).

Surface changes to enamel following IPR

To better understand the effects of IPR on enamel surfaces, we evaluated the morphology and measured the roughness that was created on enamel surfaces using the most commonly used instruments for IPR (Chapter 2). Previous research has shown that the enamel surface becomes rougher with the use of IPR instruments (Radlanski et al., 1988; Joseph et al., 1992; Lundgren et al., 1993; Piacentini and Sfondrini, 1996; Arman et al., 2006); and polishing of these surfaces may result in enamel smoother than it was prior to any enamel reduction (Joseph

et al., 1992; Piacentini and Sfondrini, 1996; Zhong et al., 1999; Zhong et al., 2000). However, most studies measured enamel roughness quantitatively (e.g. SEM imaging), and only a limited number of studies have used quantitative methods to assess the enamel surfaces after IPR (via profilometry) (Arman et al., 2006; Danesh et al., 2007). In the present study, the surface changes caused by IPR instruments were evaluated qualitatively and quantitatively using atomic force microscopy (AFM) at the nanometer level (Chapter 2). It was shown that the use of diamond instruments with a larger grit created rougher enamel surfaces than when those with smaller grit were used. The burs created the roughest surfaces, followed by the strips and then the discs. Polishing with Soflex discs after enamel reduction created surfaces even smoother than the untreated enamel.

These results are in agreement with previous studies that show a roughened enamel surface after IPR procedures (Radlanski et al., 1988; Joseph et al., 1992; Lundgren et al., 1993; Piacentini and Sfondrini, 1996; Arman et al., 2006), and that polishing will reduce this to create a smooth surface (Joseph et al., 1992; Piacentini and Sfondrini, 1996; Zhong et al., 1999; Zhong et al., 2000). Previous research has showed that IPR with the use of diamond burs or discs, followed by running a finishing strip lightly coated with 37% phosphoric acid over the surface, helped to reduce the roughness created by the diamond instruments (Joseph et al., 1992). It was stated in this particular SEM study, that the demineralized surface was capable of "self-healing" with an increased potential to remineralize. This was contradicted by a later study where the surfaces subjected to the same chemical stripping were actually rougher than if the strips or discs were used alone (Arman et al., 2006). It may be important to note that the earlier study used only qualitative (i.e. subjective) methods for assessment of the surface roughness (SEM) (Joseph et al., 1992), whereas the later study that showed the acid etching to leave rougher surfaces, had used quantitative assessment by means of a profilometry to measure the surface roughness (Arman et al., 2006). In the present study, only mechanical methods of surface polishing (i.e. by use of Soflex discs) were investigated.

Bacterial adhesion to enamel following IPR

Bacterial adhesion is a key event in the development of dental caries (Keyes, 1968). Although dental caries is multifactorial, it is assumed that a rougher enamel surface may increase bacterial adhesion and therefore, possibly the risk of caries in these patients. Some studies have shown no increase in dental caries following IPR (Jarjoura et al., 2006; Zachrisson et al., 2007; Zachrisson et al., 2011). However, it is not known whether this is because the possible increased risk of caries from this procedure is not clinically significant, or whether the multifactorial nature of the disease made it too difficult to assess the true effect on the long-term outcomes.

A recent systematic review stated that no reliable conclusions could be drawn from the studies completed due to the diversity of their methodology (Koretsi et al., 2014). However, statistically, the incidence of caries on surfaces that had had IPR, was the same as the untreated surfaces, indicating no increased risk after the procedure.

Previously, it has been established that several roughened dental materials have increased bacterial adhesion, for example, composite resin (Carlen et al., 2001), porcelain (Kawai et al., 2000), Co-Cr alloy (Gao et al., 1998) and dental implants (Chin et al., 2007). A recent study also showed that there is a positive correlation between the roughness of composite resin and bacterial adhesion forces (Mei et al., 2011), however, no such studies have been carried out with enamel as yet.

In this study the number of bacteria adhering to the enamel surfaces with different roughnesses after IPR procedures was assessed (Chapter 3). It was shown that rougher enamel surfaces had significantly increased numbers of bacteria adhered to them. Our findings are in agreement with previous studies, which mainly qualitatively assessed the bacterial adhesion (Radlanski et al., 1988; Jarjoura et al., 2006; Zachrisson et al., 2011). The quantitative analysis in this study, furthermore, found a positive relationship between the enamel surface roughness and the amount of bacterial adhesion.

Streptococcus sanguinis, an initial colonizer of the dental biofilm, was used in this study to quantify the amount of bacterial adhesion to the enamel surfaces. In the oral cavity, firstly, a salivary pellicle is formed followed by adherence of the

initial colonizers (including S. sanguinis) and subsequent formation and thickening of the dental biofilm. Initially (within the first 4-8 h), this biofilm is dominated by oral streptococci (Nyvad and Kilian, 1987; 1990; Diaz et al., 2006; Dige et al., 2009), which can progress to become a more cariogenic dental biofilm (Peterson et al., 2011). It is empirical that patients are able to remove the bacteria adhered on the enamel, to avoid the associated side effects caused by the acid producing bacteria within this biofilm including dental caries and gingival inflammation (Kleinberg, 2002).

Limitations within the studies

Although the IPR techniques used in this study mimicked the clinical procedures and followed the manufacturer instructions, they were not performed intra-orally and it is not conclusive as to whether the results can be extrapolated to the clinical environment (i.e. they had reduced external validity). This may affect the clinical application of the results.

Another limitation of the study is that only the most commonly used IPR instruments in clinics were included, and other relatively less commonly used instruments such as coarse diamond instruments were not included in the study. The coarse instruments may reduce the amount of time taken to remove the same amount of enamel tissue compared with the finer grit instruments, and would likely decrease chair time, however, they may create a very rough surface. Based on the results of the study, where instruments with larger grits produced rougher enamel surfaces, it is presumed that diamond instruments with even larger grit (such as coarse diamond burs) would produce even rougher surfaces than those observed in this study. Further study is needed to confirm this.

In this study only four degrees of roughness were used in the adhesion experiments. It is not possible to determine the specific nature of the relationship between the number of bacteria adhering and surface roughness. In future research it would be beneficial to use surfaces with Ra = 500 nm and Ra > 800 nm.

Though it was found difficult to thoroughly remove the grooves and furrows left on the enamel surface after IPR using conventional cleaning and polishing methods (Piacentini and Sfondrini, 1996; Gupta et al., 2012), polishing is usually

recommended after clinical IPR procedures. In this study, polishing with the entire series of Soflex discs significantly reduced surface roughness and produced enamel surfaces even smoother than the untreated control samples.

This study used a single species of bacteria, S. sanguinis, one of the initial colonizers during dental biofilm formation. There is usually large variation amongst different microorganisms, their adherence and pathological behaviour. More bacterial strains and other more cariogenic bacteria such as S. mutans are recommended to be included in future studies to provide a better understanding of this point.

Clinical implications

In this study we investigated the enamel morphology and roughness caused by various IPR instruments and evaluated to what extent this affected the bacterial adhesion to these surfaces.

It was found that the IPR created surfaces rougher than untreated enamel, regardless of the instruments used (burs, strips or discs). The instruments with larger grits created rougher surfaces than their counterparts with smaller grits. The burs created the roughest surfaces, followed by strips and then discs. The smoothest surfaces were created when the series of Soflex series polishing discs were used after reduction of the enamel to polish the enamel back to a state that was even smoother than the untreated enamel samples. Thus, routine polishing with Soflex discs after IPR is recommended to minimize enamel surface roughness.

It is important for clinicians to be aware of the extent of roughness created using various enamel stripping instruments, so that a careful, evidence-based, clinical decision can be made regarding the use of them. The instruments with larger grits, which may take less time in the dental chair to remove the same amount of enamel, create rougher surfaces, compared with instruments that have smaller grits. With the preceding results, it is recommended that clinicians carefully chose which IPR instruments to use based on the amount of time available and the amount of enamel to be removed. Careful and thorough polishing of all roughened surfaces is strongly recommended.

Future research

Future studies evaluating the influence of IPR on enamel in vivo may provide more valuable information for clinical application.

It would also be beneficial to include more strains and other species of bacteria, including later colonisers and more cariogenic bacteria, to see whether the increase observed in the initial colonizers would follow on to the later stages in development of the dental biofilm.

A long-term follow-up study, e.g. with the treated teeth left in situ, would be beneficial. This would help to assess the true clinical relevance of any possible increased risk of caries and other biofilm-related diseases that the IPR procedure may impose during orthodontic treatments.

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Chapter 5

Summary

Summary

Interproximal reduction (IPR) is performed on many orthodontic patients for a variety of reasons. It is important to understand the influence of IPR on the morphology and roughness of enamel and the consequences for bacterial adhesion to the treated teeth. In this thesis, **Chapter 1** reviewed the topic of IPR and it's use in orthodontics. It discussed tooth size discrepancies, relief of crowding, post treatment stability, improved aesthetics and possible avoidance of extractions. It also reported on the thickness of enamel and how much can be safely removed as well as some of the possible long-term consequences of this treatment. From studies carried out to date, properly conducted IPR appears to not increase the risk of caries in orthodontic patients.

The influence of IPR on the enamel surfaces is still poorly understood. In **Chapter 2** the most commonly used IPR instruments were used to remove 2 mm of the outer enamel on blocks cut from human premolars to mimic the clinical procedure of IPR. The effect of the different IPR techniques on enamel surfaces was evaluated qualitatively and quantitatively . In this chapter, groups of enamel blocks were treated with diamond coated IPR instruments (burs, strips and discs, of both larger and smaller grits), one group was polished (Soflex polishing discs), and one control group was untreated. It was found that the diamond instruments with larger grits created rougher surfaces than their smaller grit counterparts. The roughest surfaces were created by the burs, followed by the strips and then the discs. The smoothest surfaces were created when enamel reduction was followed by polishing with the Soflex discs, and these surfaces were even smoother than the untreated enamel samples.

The concern with creating rough enamel is that it may increase bacterial adhesion. It is well known that dental plaque accumulation predisposes to dental caries and this is especially relevant in orthodontic patients who are already at risk of increased plaque with a larger number of plaque retentive sites in the mouth whilst wearing fixed appliances. **Chapter 3** investigated whether the rougher surfaces created by the IPR instruments increased bacterial adherence. Enamel surfaces with four significantly different roughnesses were prepared. Adhesion of *Streptococcus sanguinis* was assessed by counting the colony forming units

adhering to these surfaces. A positive relationship was found between the enamel surface roughness and the number of bacterial colony forming units bound to the surface.

Chapter 4 summarized the main results and conclusions of this thesis, and discussed the clinical implications of the research findings. In this chapter, the direction for future research was also suggested.

Appendix A

Maori Consultation

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
TE KOMITI RAKAHAU KI KĀI TAHU

Tuesday, 23 April 2013.

Dr Li Mei,
Sir John Walsh Research Institute,
DUNEDIN.

Tēnā Koe Dr Li Mei,

The influence of surface roughness of enamel on bacterial adhesion.

The Ngāi Tahu Research Consultation Committee (The Committee) met on Tuesday, 23 April 2013 to discuss your research proposition.

By way of introduction, this response from The Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum it states ″Ngāi Tahu acknowledges that the consultation process outline in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago″. As such, this response is not ″approval″ or ″mandate″ for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee base consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of interest and importance.

The Committee suggests researchers consider the Southern District Health Board's Tikaka Best Practice document. The document covers the collection, storage and disposal of blood and tissue samples. This document is available on the Southern District Health Board website.

We wish you every success in your research and The Committee also requests a copy of the research findings.

This letter of suggestion, recommendation and advice is current for an 18 month period from Tuesday, 23 April 2013 to 8 October 2014.

Nāhaku noa, nā

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Research Manager Māori
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Te Whare Wānanga o Otāgo
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Appendix B

Ethical Approval

13/105

Academic Services
Manager, Academic Committees, Mr Gary Witte

18 April 2013

Dr L Mei
Department of Oral Sciences
Faculty of Dentistry

Dear Dr Mei,

I am again writing to you concerning your proposal entitled "**The influence of surface roughness of enamel on bacterial adhesion**", Ethics Committee reference number **13/105**.

Thank you for the email of 15 April 2013 responding to the Committee, and for providing your amended information sheet and consent forms. Thank you for clarifying how patients will be recruited. You have also advised that you will not include any patients that are supervised by or are treated by the researcher, therefore eliminating the risk of biased treatment planning. We confirm that the amendments made to the Information Sheet and Consent Form are approved.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

Yours sincerely,

Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Professor R D Cannon Head Department of Oral Sciences

Appendix C

Participant Information Sheet

UNIVERSITY of OTAGO

SAPERE AUDE

The influence of surface roughness of enamel on bacterial adhesion

INFORMATION SHEET FOR
PARTICIPANTS / PARENTS / GUARDIANS.

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you and we thank you for considering our request.

What is the Aim of the Project?
We aim to find out if the bugs that cause dental decay stick better to a rough tooth or a smooth tooth. Previous research cannot give us an answer to this question.

What Type of Participants are being sought?
We are looking for teeth with strong enamel, removed for orthodontic treatment. We do not want teeth with any white spots, decay, fillings, or other defects.

What will Participants be Asked to Do?
Participants will not be asked to do anything other than donate their teeth for the purpose of this research. We will only use the teeth if we have had your full consent to do so.

What Data or Information will be Collected and What Use will be Made of it?
We will only collect the teeth that have been removed. We will not gather any personal information from you so there will be no connection to you personally in the research.

What if Participants have any Questions?

If you have any questions about our project, please feel free to contact us anytime:

- Lydia Meredith: 0274246053; lyd.meredith@gmail.com
- Dr. Li Mei: 03479 7480; li.mei@otago.ac.nz

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Appendix D

Participant Consent Form

The influence of surface roughness of enamel on bacterial adhesion

CONSENT FORM ***FOR PARTICIPANTS***

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

1. My participation in the project is entirely voluntary.
2. No personal identifying information will be included in the study.
3. My participation in the study will be kept entirely confidential.
4. The results of the project may be published and will be available from the University of Otago Library (Dunedin, New Zealand).

I agree to take part in this project.

Name: .....

Signature: .....

Date: .....

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (Ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Acknowledgments

Li – Thank you for ALL of your efforts. We finally got there!! Your advice for writing has been absolutely invaluable. Thank you for being so helpful, patient and persistent with me. I so appreciate your wider appreciation of this topic and your tips on how to tackle things. I couldn't have done this without you.

Mauro – Thank you for all of your help and support, not only towards this thesis but also throughout my entire specialist degree. Your clinical skills and knowledge are outstanding – I look up to you with such admiration. Your kind, supportive and hugely complimentary words will never be forgotten.

Richard – Thank you!! Your efficient and thorough critique of my initial experimental methods, the final write up and everything in between has been utterly invaluable. I so appreciate the time you put into evaluating any work I ever gave you – thank you again.

Jules – Oh Jules. I can't believe we had to complete this journey without you. Thank you for your help in the early stages of this thesis, but thank you more for everything you had done before that. I thank you for your memorable teaching in the days here as an undergrad student, and more recently for your support and encouragement during the primary examinations in Sydney. You had such a special style, Jules. I still can't believe that you're gone.

Geoff Thompkins – Thank you, Geoff!! Your help in the lab was just priceless – you made sure I had valid methods for the research and then stood at my side in the lab to carry them out properly since I had no idea what I was doing! You were always so helpful and generous with your time – I can't thank you enough.

Sam Lowrey – Thank you for your help with the AFM analysis – this was all as new to you as it was to me, so thank you for your help with figuring it all out!

To all my colleagues who have either been through this course before me or were going through it at the same time – we have all leant on each other at various sticky

moments and have all come (or will come) through it okay in the end. Thanks for all the memories!!

Finally, I would like to express my deepest appreciation to my parents and my dearest Shane. Your continued support and friendship throughout the toughest times along this journey has been what's kept me going. Your love and the sacrifices you have made are so appreciated - thank you. I love you all so much. This thesis is dedicated to you!!