

Early biofilm formation and the effects of antimicrobial agents on orthodontic bonding materials in a parallel plate flow chamber

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SUMMARY Decalcification is a commonly recognized complication of orthodontic treatment with fixed appliances. A technology, based on a parallel plate flow chamber, was developed to investigate early biofilm formation of a strain of *Streptococcus sanguis* on the surface of four orthodontic bonding materials: glass ionomer cement (Ketac Cem), resin-modified glass ionomer cement (Fuji Ortho LC), chemically-cured composite resin (Concise) and light-cured composite resin (Transbond XT). *S. sanguis* was used as it is one of the primary colonizers of dental hard surfaces. Artificial saliva was supplied as a source of nutrients for the biofilms. The effects of two commercially available mouthrinses (i.e. a fluoride containing rinse and chlorhexidine) were evaluated. Initial colonization of the bacterium was assessed after 6 hours of growth by the percentage surface coverage (PSC) of the biofilm on the disc surfaces.

There were statistically significant differences in bacterial accumulation between different bonding materials ($P < 0.05$), Concise being the least colonized and Transbond XT being the most colonized by *S. sanguis* biofilms. All materials pre-treated with 0.05 per cent sodium fluoride mouthrinse showed more than 50 per cent reduction in biofilm formation. The 0.2 per cent chlorhexidine gluconate mouthrinse caused significant reduction of biofilm formation on all materials except Ketac Cem.

This *in vitro* study showed that the use of a chemically-cured composite resin (Concise) reduced early *S. sanguis* biofilm formation. Also, fluoride had a greater effect in reducing the PSC by *S. sanguis* biofilms than chlorhexidine. Rinsing with 0.05 per cent sodium fluoride prior to placement of orthodontic appliances is effective in reducing early biofilm formation.

Introduction

Dental plaque is present in both healthy and diseased individuals and is the aetiological agent of the two most prevalent diseases affecting industrialized societies: dental caries and periodontal disease (Theilade, 1989). Dental plaque is an example of a biofilm which is defined as a community of bacteria and their extracellular polymers that are attached to a surface (Wilson, 2001).

The build-up of dental plaque on teeth and dental materials is a problem in orthodontics (Øgaard *et al.*, 1988a). Despite advances in orthodontic materials and techniques in recent years, the development of enamel decalcification and dental caries around orthodontic appliances continues to be a problem. Nearly 50 per cent of orthodontic patients exhibit clinically visible white spot lesions during treatment that last approximately 2 years (Basdra *et al.*, 1996). Because it is known that white spot lesions form early (O'Reilly and Featherstone, 1987), it is important to understand the effects of biofilm build-up around orthodontic bonding materials following placement of fixed orthodontic appliances.

Composite resins and glass ionomer cements (GICs) are widely used in securing fixed orthodontic devices. During the 1990s, a major development of hybridization technology underlying composite resins and GICs popularized the use of resin-modified GICs (RMGICs) and polyacid-modified composites (compomers) in orthodontics.

Composites used as orthodontic direct bonding adhesives have a polymeric matrix that can host and nurture a variety of aerobic and anaerobic micro-organisms acting alone or in combination (consortia). Their accumulation can lead to premature debonding and eventually enamel decalcification and periodontal diseases (Matasa, 1995). The use of fluoride-containing orthodontic bonding materials both *in vitro* (Fox, 1990; Badawi *et al.*, 2003) and *in vivo* (Banks *et al.*, 1997) has shown variable results in helping to prevent or reduce decalcification. Significantly elevated levels of fluoride in plaque have been found adjacent to GIC-retained brackets compared with those adjacent to composite resin-retained brackets up to 6 months after the onset of treatment (Hallgren *et al.*, 1993). However, there are no reports in the literature

describing how the plaque biofilm is affected by fluoride in the early stages.

Preventive programmes in orthodontic patients using fluoride mouthrinses and topical fluoride in the form of varnishes, solutions or gels have been recommended (Øgaard *et al.*, 1988b; Rølla and Saxegaard, 1990). O'Reilly and Featherstone (1987) found that toothpastes were unable to stop the development of carious lesions. They suggested that the use of a fluoride mouthrinse (0.05 per cent NaF) used daily, in addition to fluoride toothpaste (1100 ppm fluoride) could completely inhibit or even reverse demineralization. In addition, chemical plaque control with chlorhexidine digluconate has been reported (Lundström *et al.*, 1980; Brightman *et al.*, 1991; Morrow *et al.*, 1992). Anderson *et al.* (1997) concluded that the use of 0.12 per cent chlorhexidine mouthrinses could be beneficial to orthodontic patients in achieving improved oral hygiene. Evidence of the efficacy of chlorhexidine in biofilms was reported by Pratten *et al.* (1998b). Their results demonstrated that chlorhexidine kills biofilms *in vitro* after pulsing twice daily over a period of 4 days.

To date, no published studies have quantitatively examined the initial attachment of bacteria to orthodontic bonding materials and the effects of chemical plaque control measures on early biofilm formation on these surfaces. This *in vitro* study aimed to quantitatively investigate the characteristics exhibited by one of the initial colonizers of the oral biofilm architecture, *Streptococcus sanguis*, on four commonly used orthodontic bonding materials in a parallel plate flow chamber. The effectiveness of two commercially available mouthrinses on *S. sanguis* biofilm formation was also evaluated.

Materials and methods

Bacterial strain and media

S. sanguis SK36 was used in all the experiments. The organism was grown on Columbia blood agar (Oxoid, Basingstoke, Hants, UK) in order to obtain pure cultures. The cultures were prepared by inoculating a fresh colony into 20 ml tryptone soy broth (Oxoid) with yeast extract (Oxoid) and incubating in a 5 per cent carbon dioxide atmosphere overnight. The broths were centrifuged (10 minutes, 3000 rpm, 21°C) and their final density adjusted to 1×10^8 cells per ml (OD_{560}) with 0.85 per cent saline to

provide a standard inoculum for flow chamber experiments. A mucin-containing artificial saliva with a pH of 6.9 was used as the growth medium for the biofilm (Pratten *et al.*, 1998a), which was based on the artificial saliva formulations by Russell and Coulter (1975) and Shellis (1978). The composition listed is per 500 ml of distilled water: 'lab-lemco' beef extract, 0.5 g (Oxoid), yeast extract, 1 g (Oxoid), proteose peptone, 2.5 g (Oxoid), mucin (hog gastric), 1.25 g (Sigma, Poole, Dorset, UK), sodium chloride, 0.175 g (Sigma), calcium chloride, 0.1 g (Sigma), potassium chloride, 0.1 g (Sigma) and 40 per cent urea, 0.313 ml (Sigma).

Bonding materials

Discs of each of the bonding materials listed in Table 1 were prepared using a polytetrafluoroethylene (PTFE) mould. The mould consisted of five cylindrical holes, which contained PTFE plugs 5 mm in diameter. Discs of the bonding materials were prepared by firstly recessing the PTFE plug to a depth of 1 mm before filling the mould (5×1 mm) with material.

All experimental discs were made following the manufacturers' instructions. Fuji Ortho and Transbond were light activated with a Curing Light XL3000 (3M Unitek, Monrovia, California, USA) for 20 seconds while Concise and Ketac Cem were allowed to bench cure for 5–7 minutes. Bovine enamel discs (Biomaterials Department, Eastman Dental Institute, London, UK) were used as control substrata. The discs were disinfected before each experiment by immersion in 1 ml of 70 per cent ethanol.

Parallel plate flow chamber

Biofilms of pure cultures of *S. sanguis* were cultivated in a flow chamber (FC 71; BioSurface Technologies Corporation, Bozeman, Montana, USA). This flow chamber is specifically designed for insertion of removable discs to evaluate biofilm formation and antimicrobial testing on different surfaces. The flow chamber has a channel 0.2 mm deep \times 11.4 mm wide \times 40.6 mm long with a recess 2 mm deep \times 25 mm long \times 6 mm wide. The polycarbonate channel has a glass viewing port which allows reflected light microscopy of biofilm growth on the discs and in the flow channel. The viewing window consists of a 24 \times 60 mm cover slip and is held in place by an aluminum cover plate. The cover plate also compresses the silicone rubber gasket material to

Table 1 Orthodontic bonding materials used in this study.

Bonding material	Composition	Manufacturer
Concise	Two paste chemically-cured composite resin	3M Dental Products, St Paul, Minnesota, USA
Fuji Ortho™ LC	Light-cured resin-modified glass ionomer cement	Fuji-Ortho L.C., GC Corporation, Tokyo, Japan
Ketac Cem μ	Chemically-cured glass ionomer cement	3M ESPE, Seefeld, Germany
Transbond™ XT	Light-cured composite resin	3M Unitek, Monrovia, California, USA

provide a leak-proof cell. The flow chamber was autoclaved prior to the experiments.

Five experimental discs were placed in the flow chamber simultaneously. A peristaltic pump (Watson-Marlow, Falmouth, Cornwall, UK) was used to draw media through the chamber at a rate of 1.14 ml/minute which has been shown to represent the average salivary flow rate in orthodontic patients, corresponding to a shear rate of 11/s (Ulukapi *et al.*, 1997). There was a pulse damper between the peristaltic pump and the flow chamber to help dampen the pulsing effect of the pump. In addition to this a bubble trap for gas bubbles that might accumulate in the tubing before they enter the flow chamber was added to the apparatus.

The flow chamber was placed on the stage of a phase contrast microscope (Olympus BH-2, Tokyo, Japan) equipped with an ultra-long working distance objective ($\times 40$; Olympus ULWD-CD Plan 40 PI; producing a size of field $140 \times 100 \mu\text{m}$). A CCD camera (JVC TK-C1360B, London, UK) was attached to the microscope and images were collected onto a computer using the FlashBus MV-lite frame grabber (Integral Technologies Inc., Indianapolis, Indiana USA).

Production of biofilms

At the start of an experiment, artificial saliva was pumped through the flow chamber for 5 minutes allowing the formation of a conditioning film on the disc surfaces. During this time, the valve of the bubble trap was opened to provide a small reservoir of liquid in the syringe barrel (2–3 ml) to act as a reservoir to take the place of the captured gas bubbles. The broth containing *S. sanguis* was then added to the artificial saliva and the experiment was allowed to proceed for 6 hours. Five hundred millilitres of artificial saliva were inoculated with 1 ml of the standardized inoculum. The culture was maintained at 37°C and stirred for the duration of each experiment. As this was a preliminary test with only a small sample size to demonstrate the functionality and applicability of the flow chamber system, this experiment was repeated once to validate the first experiment.

Corsodyl (0.2 per cent chlorhexidine gluconate; GlaxoSmithKline Consumer Healthcare, Brentford, Middlesex, UK) and Ortho SWIRL (0.05 per cent sodium fluoride; Ortho-Care, Bradford, West Yorkshire, UK) were used to test their effect on the early biofilm formation of *S. sanguis* on the five substratum surfaces. Before the start of the experiment, the discs were immersed in 5 ml of each agent for 1 minute before placement in the flow chamber. The production of biofilm on these chemically pre-treated discs was allowed to take place as described above within a time frame of 6 hours.

Image analysis

Vital staining of the *S. sanguis* biofilm was carried out using SYTO® 13 live-cell nucleic acid stain (Molecular Probes

Europe BV, Leiden, The Netherlands) as the biofilm was grown on non-transparent substratum surfaces. Five microlitres of the stain prepared in dimethylsulfoxide (DMSO) was pipetted onto the biofilm/disc surfaces after the 6 hour experiment. After 3 minutes incubation in the dark, at room temperature, the stained specimens were viewed under the microscope. Ultra-violet light at a wavelength of 470 nm (from a mercury-arc lamp) was used to assist the observation of adherent bacteria on the surface of the discs. Five images per disc were captured at random. The ImageJ (National Institutes of Health, Bethesda, Maryland, USA) analysis software program was used to perform the image analysis. Adhering bacteria were discriminated from the background by a single grey-value threshold yielding a binary black and white image defined by the intensity levels of the original image. The percentage of the surface in the field of view covered by the biofilm was calculated by counting the pixels on threshold images to determine the proportion of pixels representing *S. sanguis* cells. An image covered a surface area of 0.012 mm^2 .

Statistical analysis

Data were analysed with the Statistical Package for the Social Sciences (Version 11.0, SPSS, Chicago, Illinois, USA). Descriptive statistics, including the mean and standard deviation of the percentage surface coverage (PSC) by *S. sanguis* biofilms, were calculated for each of the substrata. A Student's *t*-test was used to determine whether significant differences in PSC by *S. sanguis* biofilms existed between different materials. The effects of fluoride and chlorhexidine mouthrinses on biofilm formation on five substratum surfaces were analysed by univariate two-way analysis of variance (ANOVA). Dunnett's T3 test was used for *post hoc* testing. The level of significance was $P < 0.05$.

Results

The *S. sanguis* biofilms were successfully grown in the parallel plate flow chamber consisting of single cells attached to the surfaces of bovine enamel and four orthodontic bonding materials. These cells often grow into short chains and microcolonies with recruited cells and particulate matter from the bulk fluid (Figure 1).

The mean PSC by *S. sanguis* biofilms for the five different substrata are shown in Figure 2. Early biofilm formation on the different bonding materials was considered low in the initial 6 hours of exposure to the medium containing *S. sanguis*. Concise showed the lowest (0.25 per cent) and Transbond the largest PSC (1.20 per cent). The Student's *t*-test revealed that Concise had a significantly lower PSC of *S. sanguis* than the other four substrata ($P < 0.05$). Ketac Cem showed a significantly lower PSC of *S. sanguis* than Fuji Ortho and Transbond.

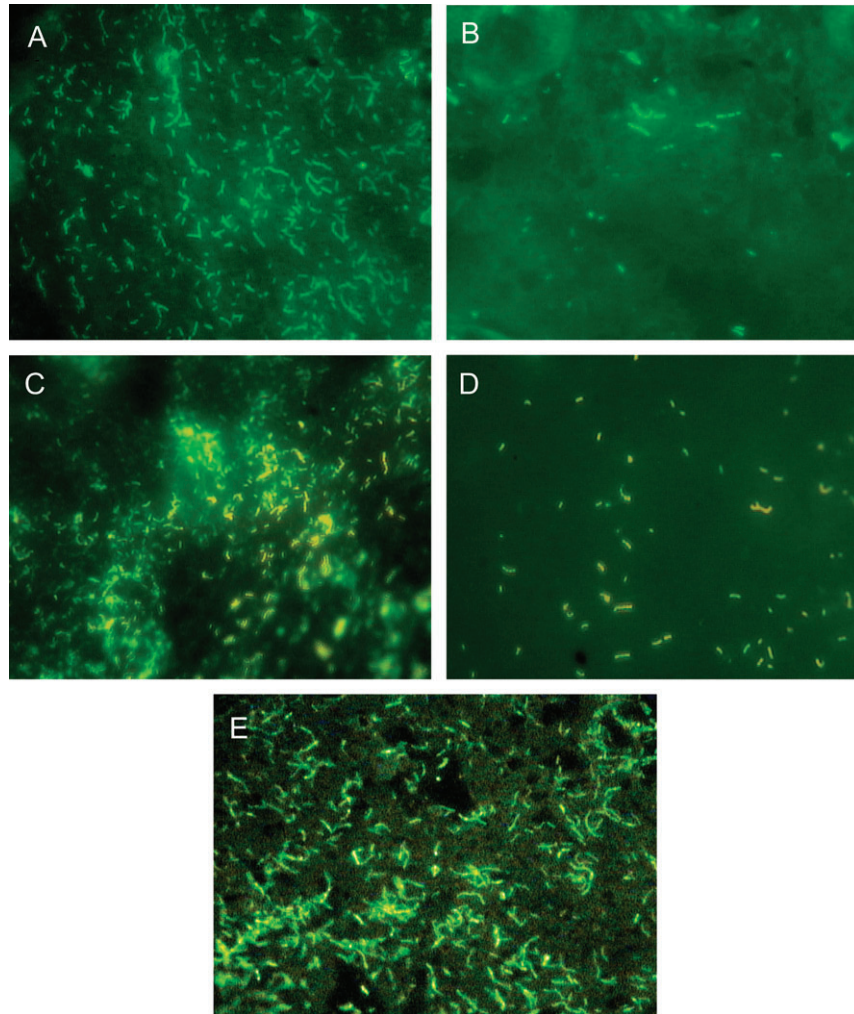


Figure 1 Images of 6-hour *Streptococcus sanguis* SK36 biofilms formed on a disc of (A) bovine enamel, (B) Concise, (C) Fuji Ortho, (D) Ketac Cem and (E) Transbond using SYTO® 13 live-cell nucleic acid stain ($\times 1000$ magnification).

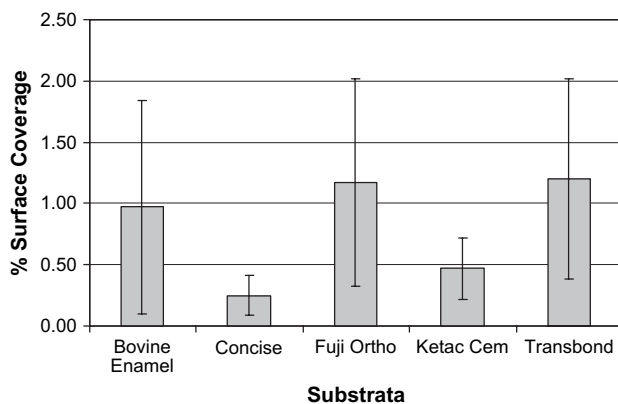


Figure 2 Percentage surface coverage of *Streptococcus sanguis* SK36 on the five experimental substrata. Error bars show standard deviation from two experiments (10 images).

The PSC of biofilms on 0.05 per cent sodium fluoride-treated substrata was very low (Figure 3). The greatest reduction was observed on Transbond (88 per cent) and the least on Concise (52 per cent). Substrata pre-treated with 0.2 per cent chlorhexidine gluconate mouthrinse revealed a reduction in PSC of *S. sanguis* biofilms except for Ketac Cem (Figure 4). Interestingly, chlorhexidine was found to have no effect on the PSC of *S. sanguis* biofilm formed on Ketac Cem. The effect of chlorhexidine on Fuji Ortho was the greatest, with a 64 per cent reduction in PSC, followed by Transbond (58 per cent), bovine enamel (46 per cent) and Concise (32 per cent). Two-way ANOVA revealed a statistically significant difference between the mean values ($P < 0.05$) of PSC on the five substrata before and after treatment with the two antimicrobial mouthrinses. However, the interaction between different substrata and the two

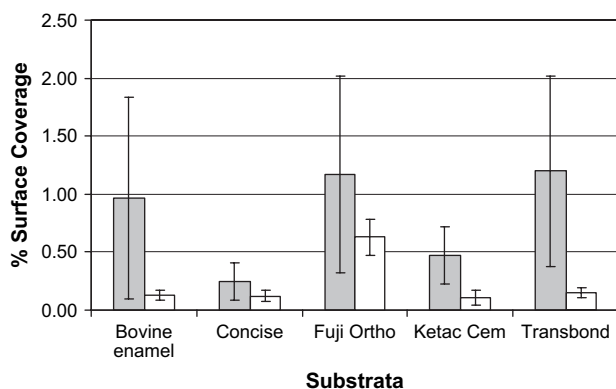


Figure 3 Effect of fluoride on *Streptococcus sanguis* biofilm formation on bovine enamel, Concise, Fuji Ortho, Ketac Cem and Transbond over 6 hours. Error bars represent standard deviation. Shaded bars, control; unshaded bars, fluoride mouthrinse.

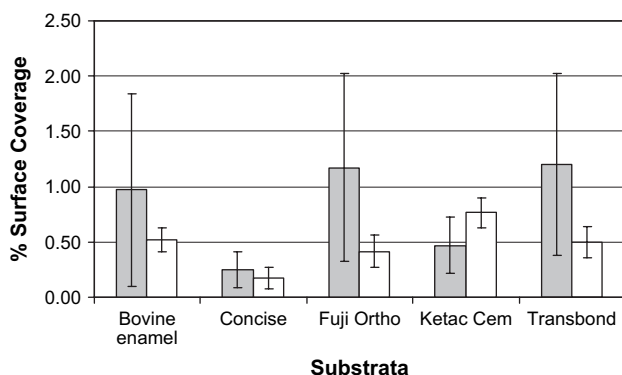


Figure 4 Effect of chlorhexidine on *Streptococcus sanguis* biofilm formation on bovine enamel, Concise, Fuji Ortho, Ketac Cem and Transbond over 6 hours. Error bars represent standard deviation. Shaded bars, control; unshaded bars, fluoride mouthrinse.

mouthrinse treatments was not shown to be statistically significant ($P = 0.056$). For comparison between the effectiveness of the two antimicrobial mouthrinses, the Dunnett's T3 multiple comparisons test indicated that 0.05 per cent sodium fluoride had a greater effect in reducing PSC by *S. sanguis* biofilms than 0.2 per cent chlorhexidine gluconate.

Discussion

Biofilms were successfully grown in a flow chamber system, and image analysis facilitated comparison of the biofilms formed. Although no previous research has employed a flow chamber to study the early primary colonization of a mono-species biofilm on orthodontic bonding materials, the use of a flow chamber to grow biofilms on surfaces of other biomaterials has been demonstrated (Bruinsma *et al.*, 2001; Van der Borden *et al.*, 2004).

Only two previous studies have investigated dental plaque accumulation around orthodontic brackets with

different orthodontic bonding agents. Blunden *et al.* (1994) studied *Streptococcus mutans* adherence to different orthodontic bonding agents, and found that there were statistically significant differences between these agents. In contrast, Badawi *et al.* (2003) concluded that the accumulation of *in vitro* supragingival plaque was not affected by the type of orthodontic bonding material. However, they also found that biofilms grown on fluoride releasing materials did not contain *S. mutans*. Four bonding materials were chosen in this study to represent the different categories of bonding technology used in everyday orthodontic practice (composites, GICs and RMGICs). The results of the early biofilm formation represented by PSC demonstrated significant differences between materials. Concise consistently attracted less *S. sanguis* to its surface compared with Transbond, Fuji Ortho, Ketac Cem and bovine enamel. It is possible that as Concise is a two-paste system and its chemical cure is on-going for a number of hours after initial setting, the release of residual monomers Bis-GMA and TEGDMA from the polymerization reaction exerts an antibacterial effect on the organisms on this material. The potential cytotoxicity of these monomers on bacterial growth has been postulated (Bollen *et al.*, 1997). Eliades *et al.* (1995) quantitatively investigated the release of Bis-GMA and TEGDMA residual monomer from chemically-cured and visible light-cured orthodontic adhesives. The highest monomer release was obtained from chemically-cured composite resin. This may be due to the nature of mixing the base and catalyst pastes of the chemically-cured adhesive, which inevitably increases the bulk porosity (Fan, 1985) and thus contributes to increased bulk inhibition of polymerization and increased potential for monomer leaching. The release of residual monomer from Transbond (light-cured composite) and Concise (chemically-cured composite) in the present study may therefore explain the different level of *S. sanguis* biofilm formed on their surfaces.

Fuji Ortho (an RMGIC) was significantly colonized by a *S. sanguis* biofilm compared with Ketac Cem (a conventional glass ionomer) in the early hours of microbial attachment. Auschill *et al.* (2002) studied, *in vivo*, the effect of dental restorative materials on dental biofilms and showed that resin-ionomer hybrids revealed a very high PSC. The results of the present investigation support their findings.

It is also worth noting that the fluoride release from RMGICs has a relatively constant rate, characterized by a substantially lower initial ion release compared with the other types of GICs (Chadwick and Gordon, 1995). The latter may be associated with the higher resistance to dissolution at the initial setting stage demonstrated by the RMGICs. These two phenomena may contribute to the difference in biofilm formation on Fuji Ortho and Ketac Cem. However, Svanberg *et al.* (1990), Hallgren *et al.* (1992) and Palenik *et al.* (1992) have shown that GICs will inhibit growth of various species of streptococci.

The implication from the present study is therefore that the level of fluoride release from these orthodontic bonding materials may be too low to cause a significant inhibitory effect on early colonization of dental plaque bacteria.

The nature of the conditioning film on the substratum surface is an important factor affecting early biofilm formation. The surface irregularities present on the experimental discs might be masked and levelled by the conditioning film (Hannig, 1999; Elliott *et al.*, 2005) and hence the protective and sheltering role of the rough surface against shear forces present in the flow chamber would be eliminated. In contrast, the conditioning film has been shown to reduce the fluoride transport out of GICs (Rezk-Lega *et al.*, 1991), a possible reason for high bacterial adherence to Fuji Ortho.

In the present study, the differential accumulation of bacteria on the five substrata pre-treated with fluoride and chlorhexidine mouthrinses has been demonstrated and a number of factors identified. The differences in (1) the ability of the antimicrobial agents to bind to the saliva-coated (and possibly uncoated) regions of the substrata during the initial 5 minutes of the experiment when the acquired pellicle was forming; (2) their ability to remain bound to the discs (i.e. their substantivity); (3) their effectiveness, once bound to the discs, at inhibiting adherence of the bacteria; (4) the relative effectiveness of the antimicrobials at killing adherent bacteria; (5) the nature of the substratum (Deng *et al.*, 2004); and (6) the presence of other ingredients in the antibacterial agents (Pratten *et al.*, 1998c), may well all be contributing factors to biofilm formation on the substrata pre-treated with fluoride and chlorhexidine.

Although there was no significant interaction between different substrata and the two mouthrinse treatments ($P > 0.05$) on PSC by *S. sanguis* biofilms, this does not technically mean there is no interaction between these two effects, rather that the data did not allow a conclusion that the interaction was not due to chance. The P -value found was close to significance ($P = 0.056$), suggesting that there may well be an interactive 'trend' between the nature of the substratum and the effects of antimicrobial treatment. Further studies on the physico-chemical properties of the substratum surfaces both with and without antimicrobial treatment are needed for clarification.

Conclusions

1. The parallel plate flow chamber provided a reproducible and quantitative tool to study early *S. sanguis* biofilm formation *in situ* on different orthodontic bonding adhesives. Using this technique it was also possible to evaluate the ability of antimicrobial agents to prevent biofilm formation under conditions similar to those which would exist *in vivo*. The potential to screen anti-plaque and antimicrobial agents for use in orthodontics using this approach may be of considerable value.

2. There were statistically significant differences in early *S. sanguis* biofilm formation on the various orthodontic bonding materials tested. These differences may be attributable to the physical and chemical characteristics of the materials.
3. Fluoride mouthrinse (0.05 per cent sodium fluoride) appeared to be more effective than 0.2 per cent chlorhexidine gluconate, achieving a mean reduction of PSC of *S. sanguis* biofilm of 72 per cent compared with the control.

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References

- Anderson G B, Bowden J, Morrison E C, Caffesse R G 1997 Clinical effects of chlorhexidine mouthwashes on patients undergoing orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedics* 111: 606–612
- Auschill T M, Arweiler N B, Brex M, Reich E, Sculean A, Netuschil L 2002 The effect of dental restorative materials on dental biofilm. *European Journal of Oral Sciences* 110: 48–53
- Badawi H, Evans R D, Wilson M, Ready D, Noar J H, Pratten J 2003 The effect of orthodontic bonding materials on dental plaque accumulation and composition *in vitro*. *Biomaterials* 24: 3345–3350
- Banks P A, Burn A, O'Brien K 1997 A clinical evaluation of the effectiveness of including fluoride into an orthodontic bonding adhesive. *European Journal of Orthodontics* 19: 391–395
- Basdra E K, Huber H, Komposch G 1996 Fluoride release from orthodontic bonding agents alters the enamel surface and inhibits enamel demineralisation *in vitro*. *American Journal of Orthodontics and Dentofacial Orthopedics* 109: 466–472
- Blunden R E, Oliver R G, O'Kane C O 1994 Microbial growth on the surfaces of various orthodontic bonding cements. *British Journal of Orthodontics* 21: 125–132

- Bollen C M L, Lambrechts P, Quirynen M 1997 Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. *Dental Materials* 13: 258–269
- Brightman I J, Terzhalmy G T, Greenwall H, Jacobs M, Enlow D H 1991 The effects of 0.12% chlorhexidine gluconate mouthrinse on orthodontic patients aged 11 through 17 with established gingivitis. *American Journal of Orthodontics and Dentofacial Orthopedics* 100: 324–329
- Bruinsma G M, Van der Mei H C, Busscher H J 2001 Bacterial adhesion to surface hydrophilic and hydrophobic contact lenses. *Biomaterials* 22: 3217–3224
- Chadwick S M, Gordon P H 1995 An investigation into the fluoride release of a variety of orthodontic bonding agents. *British Journal of Orthodontics* 22: 29–33
- Deng D M, Buijs M J, ten Cate J M 2004 The effects of substratum on the pH response of *Streptococcus mutans* biofilms and on the susceptibility to 0.2% chlorhexidine. *European Journal of Oral Sciences* 112: 42–47
- Eliades T, Eliades G, Brantley W A, Johnston W M 1995 Residual monomer leaching from chemically-cured and visible light-cured orthodontic adhesives. *American Journal of Orthodontics and Dentofacial Orthopedics* 108: 316–321
- Elliott D, Pratten J, Edwards M, Crowther J, Pettie A, Wilson M 2005 Bacterial biofilm development on hydroxyapatite-coated glass. *Current Microbiology* 51: 41–45
- Fan P L 1985 Polymerisation defects. In: Vanherle G, Smith D C (eds) *Posterior composite resin dental restorative materials*. Peter Szulc, Amsterdam, pp. 287–296
- Fox N A 1990 Fluoride release from orthodontic bonding materials. An *in vitro* study. *British Journal of Orthodontics* 17: 293–298
- Hallgren A, Oliveby A, Twetman S 1992 Caries associated microflora in plaque from orthodontic appliances retained with glass ionomer cement. *Scandinavian Journal of Dental Research* 100: 140–143
- Hallgren A, Oliveby A, Twetman S 1993 Fluoride concentration in plaque adjacent to orthodontic appliances retained with glass ionomer cement. *Caries Research* 27: 51–54
- Hannig M 1999 Transmission electron microscopy of early plaque formation on dental materials *in vivo*. *European Journal of Oral Sciences* 107: 55–64
- Lundström F, Hamp S E, Nyman S 1980 Systemic plaque control in children undergoing long-term orthodontic treatment. *European Journal of Orthodontics* 2: 27–39
- Matasa C G 1995 Microbial attack of orthodontic adhesives. *American Journal of Orthodontics and Dentofacial Orthopedics* 108: 132–141
- Morrow D, Wood D P, Spechley M 1992 Clinical effects of subgingival chlorhexidine irrigation on gingivitis in adolescent orthodontic patients. *American Journal of Orthodontics and Dentofacial Orthopedics* 101: 408–413
- Øgaard B, Rølla G, Arends J 1988a Orthodontic appliances and enamel demineralisation. Part 1. Lesion development. *American Journal of Orthodontics and Dentofacial Orthopedics* 94: 68–73
- Øgaard B, Rølla G, Arends J, ten Cate J M 1988b Orthodontic appliances and enamel demineralisation. Part 2. Prevention and treatment of lesions. *American Journal of Orthodontics and Dentofacial Orthopedics* 94: 123–128
- O'Reilly M M, Featherstone J D B 1987 Demineralisation and remineralisation around orthodontic appliances – an *in vitro* study. *American Journal of Orthodontics and Dentofacial Orthopedics* 92: 33–40
- Palenik C J, Behnen M J, Setcos J C, Miller C H 1992 Inhibition of microbial adherence and growth by various glass ionomers *in vitro*. *Dental Materials* 8: 16–20
- Pratten J, Wills K, Barnett P, Wilson M 1998a *In vitro* studies of the effect of antiseptic-containing mouthwashes on the formation and viability of *Streptococcus sanguis* biofilms. *Journal of Applied Microbiology* 84: 1149–1155
- Pratten J, Smith A W, Wilson M 1998b Response of single species biofilms and microcosm dental plaques to pulsing with chlorhexidine. *Journal of Antimicrobial Chemotherapy* 42: 453–459
- Pratten J, Barnett P, Wilson M 1998c Composition and susceptibility to chlorhexidine of multispecies biofilms of oral bacteria. *Applied and Environment Microbiology* 64: 3515–3519
- Rezk-Lega F, Øgaard B, Rølla G 1991 Availability of fluoride from glass-ionomer luting cements in human saliva. *Scandinavian Journal of Dental Research* 99: 60–63
- Rølla G, Saxegaard E 1990 Critical evaluation of the composition and use of topical fluorides with emphasis on the role of calcium fluoride in caries inhibition. *Journal of Dental Research* 69 (Special Issue): 780–785
- Russell C, Coulter W A 1975 Continuous monitoring of pH and Eh in bacterial plaque grown on a tooth in an artificial mouth. *Applied Microbiology* 29: 141–144
- Shellis R P 1978 A synthetic saliva for cultural studies of dental plaque. *Archives of Oral Biology* 23: 485–489
- Svanberg M, Mjör I A, Ørstavik D 1990 *Mutans streptococci* in plaque from margins of amalgam, composite and glass ionomer restorations. *Journal of Dental Research* 69: 861–864
- Theilade J 1989 Dental plaque and dental calculus. In: Lindhe J (ed.) *Clinical periodontology*, 2nd edn. Munksgaard, Copenhagen, pp. 92–128
- Ulukapi H, Koray F, Efes B 1997 Monitoring the caries risk of orthodontic patients. *Quintessence International* 28: 27–29
- Van der Borden A J, Van der Mei H C, Busscher H J 2004 Electric-current-induced detachment of *Staphylococcus epidermidis* strains from surgical stainless steel. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 68: 160–164
- Wilson M 2001 Bacterial biofilms and human disease. *Science Progress* 84 (Part 3): 235–254

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