

The effect of orthodontic bonding materials on dental plaque accumulation and composition in vitro

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Abstract

The aim of this study was to investigate the accumulation and composition of microcosm dental plaque on different orthodontic bonding materials using an in vitro model. Microcosm plaques were grown on discs of a range of bonding materials in a constant depth film fermentor. The biofilms were derived from human saliva and supplied with artificial saliva as a source of nutrients. The number of viable bacteria in the biofilms was determined and the streptococci present were identified to species level. The results showed that there was no significant difference in bacterial accumulation between different bonding materials, however, biofilms grown on materials which were fluoride releasing, did not contain *Streptococcus mutans*. This in vitro study has shown that the use of fluoride-releasing bonding materials may support the growth of supragingival plaque, which does not contain *S. mutans*.

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1. Introduction

Decalcification is a commonly recognised complication of orthodontic treatment with fixed appliances if good oral hygiene is not maintained [1]. These appliances hinder tooth cleaning and favour the retention of dental plaque. Dental plaque is an example of a biofilm which is defined as a community of bacteria (or other microbes) and their extracellular polymers that is attached to a surface [2]. In the mouth, this complex community may contain a variety of species [3], a number of which have been implicated in dental caries. It is now widely recognised that environmental changes (e.g. the placement of an orthodontic appliance) may shift the bacterial community from a healthy one to one that is able to cause disease [4].

The placement of a fixed orthodontic appliance leads to an increase in the volume and number of bacteria within dental plaque [5,6] and a disproportionate

increase in the numbers of mutans streptococci within the bacterial community [7]. *Streptococcus mutans* and *Streptococcus sobrinus* are closely associated with decalcification and several studies have reported an increase in the number of *S. mutans* following the placement of orthodontic appliances [8,9].

Assessing the extent and prevalence of decalcification during orthodontic therapy has been difficult due to the wide range of clinical techniques employed to assess the prevalence of decalcification. This has been highlighted by Mitchell [1] who reported on 10 studies in which between 2% and 96% of patients suffered from decalcification around orthodontic brackets.

Fluoride continues to be the cornerstone of any caries-prevention program and is administered, in many regions of the world, by fluoridation of the water supply [10]. However, the use of fluoride containing orthodontic materials both in vitro [11] and in vivo [12] has shown variable results in their ability to reduce decalcification. Therefore, the use of in vitro models to predict the interactions between dental plaque and orthodontic materials is highly desirable.

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The purpose of this *in vitro* study was to determine whether different materials used to bond orthodontic appliances affected plaque accumulation or its bacterial composition.

2. Materials and methods

2.1. Inoculum and medium

Whole human saliva was used as an inoculum to provide multi-species biofilms consisting of organisms found in the oral cavity. The saliva was collected from 10 healthy individuals; an equal volume from each of the subjects was pooled and mixed with 10% glycerol (v/v). Aliquots of 1 ml were stored at -80°C for subsequent use.

The nutrient source used for the experiments was a sterile mucin-containing artificial saliva [13] with a pH of 6.9. The composition listed is per one litre of distilled water: 'lab-lemco' powder, 1 g (Oxoid, Basingstoke, UK), yeast extract, 2 g (Oxoid), proteose peptone, 5 g (Oxoid), mucin Type III: partially purified from porcine stomach, 2.5 g (Sigma, Poole, UK), sodium chloride, 0.35 g (Sigma), calcium chloride, 0.2 g (Sigma) and potassium chloride, 0.2 g (Sigma).

2.2. Bonding materials

Discs (5 mm in diameter) of each of the bonding materials listed in Table 1 were made, according to the manufacturer's instructions, and polished to a surface roughness of 2.5 Ra using silicon carbide grit on a Knuth Rotor (Struers Limited, Glasgow, UK) under water flushing. Fuji LC or Transbond were light cured for 20 s using a dental curing light (3M Unitek, Bradford, UK) while the resin materials were allowed to bench cure for 5–7 min. Polytetrafluoroethylene (PTFE) plugs, with the same surface roughness, were used as a control substratum.

2.3. Production of biofilms

The discs of the bonding materials were placed into a constant depth film fermentor (CDFF; Fig. 1) which was operated at 37°C . The CDFF consists of a glass vessel with a pair of stainless steel end-plates, the upper plate has inlets for the addition of gas and medium, as well as a sampling port. A stainless steel turntable, holding 15 PTFE sampling pans fitted flush and distributed equidistantly from its rim, is housed between the two end-plates and rotated by an external motor at 3 rpm. Each PTFE pan has five cylindrical holes 5 mm in

Table 1
Materials used for bonding orthodontic appliances investigated in this study

Bonding material	Composition	Supplier
Concise	Two paste composite	3 M Unitek, Bradford, UK
Concise and Solo	Composite and fluoride-releasing bond	3 M Unitek andOrmco, Orange, CA, USA
Fuji LC	Fluoride-releasing light cure glass ionomer	Fuji-Ortho L.C., GC Corporation, Tokyo, Japan
Sequence	Fluoride-releasing composite resin	Ormco
Transbond	Light cure composite	3 M Unitek

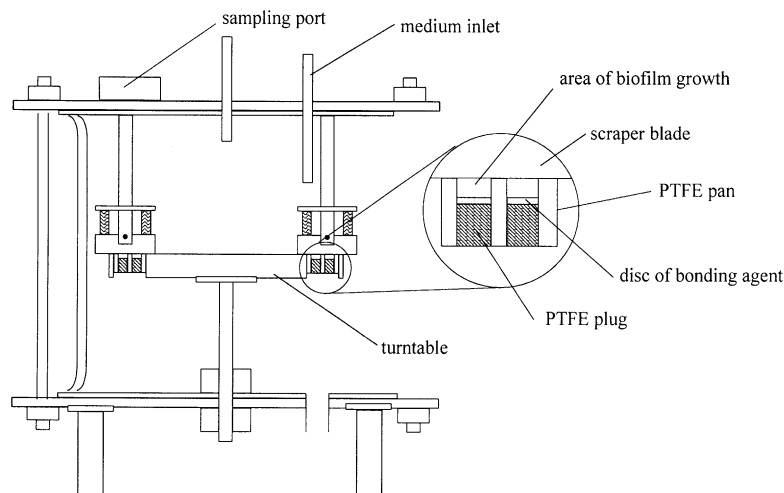


Fig. 1. A schematic of the constant depth film fermentor showing one of the PTFE pans in detail.

diameter, containing PTFE plugs onto which composite discs are placed and recessed to a depth of 300 μm . Spring-loaded scraper blades smear the incoming medium over the surface of the pans maintaining the biofilm, once formed, at a constant depth. The CDFP was autoclaved (121°C, 15 min) and the composite discs inserted, these had previously been sterilised using ethylene oxide (ETO) as they are not all heat stable. ETO sterilisation was achieved in a 100% ethylene oxide atmosphere at 57°C for 2 h. Samples were then exposed to air for 15 h.

The pooled saliva was added to 500 ml of artificial saliva, mixed and pumped into the CDFP over a period of 8 h via a peristaltic pump (Watson-Marlow, Falmouth, UK). After this time the inoculum flask was disconnected and the CDFP fed from a reservoir of sterile artificial saliva using a peristaltic pump at a flow rate of 0.5 ml/min [13]. Sampling pans were aseptically removed from the CDFP at 24, 96, and either 168 or 336 h. For each sample five pans were removed aseptically (each containing 5 discs of one of the test bonding material) for analysis.

2.4. Culture methods

Pans were aseptically removed from the CDFP and the biofilms disrupted by vortexing for 1 min. The total aerobic counts were performed on Columbia blood agar base (CBA; Oxoid, Basingstoke, UK) and anaerobic counts on Fastidious anaerobe agar (FAA; BioConnections, Leeds, UK), each containing 5% defibrinated horse blood (E and O Laboratories, Bonnybridge, UK). For aerobic counts the plates were incubated in atmospheric air for 24 h while for anaerobic counts incubation was for 96 h at 37°C in 10% H₂, 10% CO₂ and 80% N₂.

Colonies from both the aerobic and anaerobic agar plates were selected by their different morphologies and sub-cultured onto CBA and FAA depending on the agar plates they were originally isolated from. The plates were then incubated at 37°C as described previously. A range of carbohydrate-containing peptone agars (Prolab Diagnostics, Cheshire, UK) were used for biochemical differentiation of the isolates. Bacteria were applied to the surface of the agar by the use of a multipoint inoculator (Scan 400; Mast Group, Bootle, UK), which allowed up to 36 different isolates to be simultaneously inoculated onto a series of agar plates. The inoculated carbohydrate plates were incubated at 37°C aerobically for 18–24 h, a change in the colour of the medium from green to yellow around the bacterial isolate indicated a positive result. The streptococci were identified from these isolates by their biochemical profile [14].

All of the experiments were carried out in duplicate and statistical analysis performed using the student's

t-test (two-sample assuming equal variances) to determine *p*-values.

3. Results

The total aerobic and anaerobic viable counts from the control and test materials are shown in Fig. 2. Each time point represents the viable counts from duplicate experiments with five biofilms sampled from each material (10 total). The control material was quickly colonised, with total aerobic and anaerobic viable counts both over $1 \times 10^7 \pm 1.6 \times 10^6$ colony forming units (CFU) per biofilm specimen at 24 h (Fig. 2a). At 96 h the CFU per biofilm for both the aerobes and anaerobes had increased by approximately 1 log₁₀ with a greater number of organisms growing under aerobic conditions (62%). At 168 and 336 h the total anaerobic count continued to increase as the total aerobic count decreased. At 336 h the biofilms contained 70% of organisms which could grow under anaerobic conditions.

Bacterial accumulation on the five test materials (Figs. 2b–f) showed very similar trends to the control material. Transbond contained the fewest bacteria after 24 h, with an aerobic count of $6.5 \times 10^6 \pm 2 \times 10^6$ CFU per biofilm. All of the materials contained biofilms with total bacterial counts higher than that of the control at 336 h, with Fuji containing the highest number, however, none were significantly higher than the control ($p < 0.05$).

In addition to the total bacterial counts, the proportions of streptococci were also determined (Fig. 3). Indeed, streptococci constituted greater than 80% of all the bacteria isolated from the study. A total of seven streptococci were identified to species level by their ability to utilise a range of carbohydrates. The biofilms grown on the control substratum, PTFE, contained four different species of streptococci with *Streptococcus sobrinus* and *S. mitis* predominating. *S. crista* and *S. mutans* each represented 7% of the streptococcal population. Biofilms grown on Concise contained five *Streptococcus* spp. Again, *S. sobrinus* and *S. mitis* were found to be the predominant species. However, the proportion of *S. mutans* in the biofilms was 14.3%, i.e. double that observed in the control. Interestingly, only *S. sobrinus* was found in the biofilms grown on the Concise and Solo combination material. Biofilms grown on Sequence contained five species, again *S. sobrinus* was the predominant species, however, *S. mutans* was not detected. Biofilms grown on both Transbond and Fuji were predominantly composed of *S. sobrinus* and also contained *S. gordonii* and *S. mitis*. However, unlike Transbond, *S. mutans* was not detected in biofilms grown on Fuji.

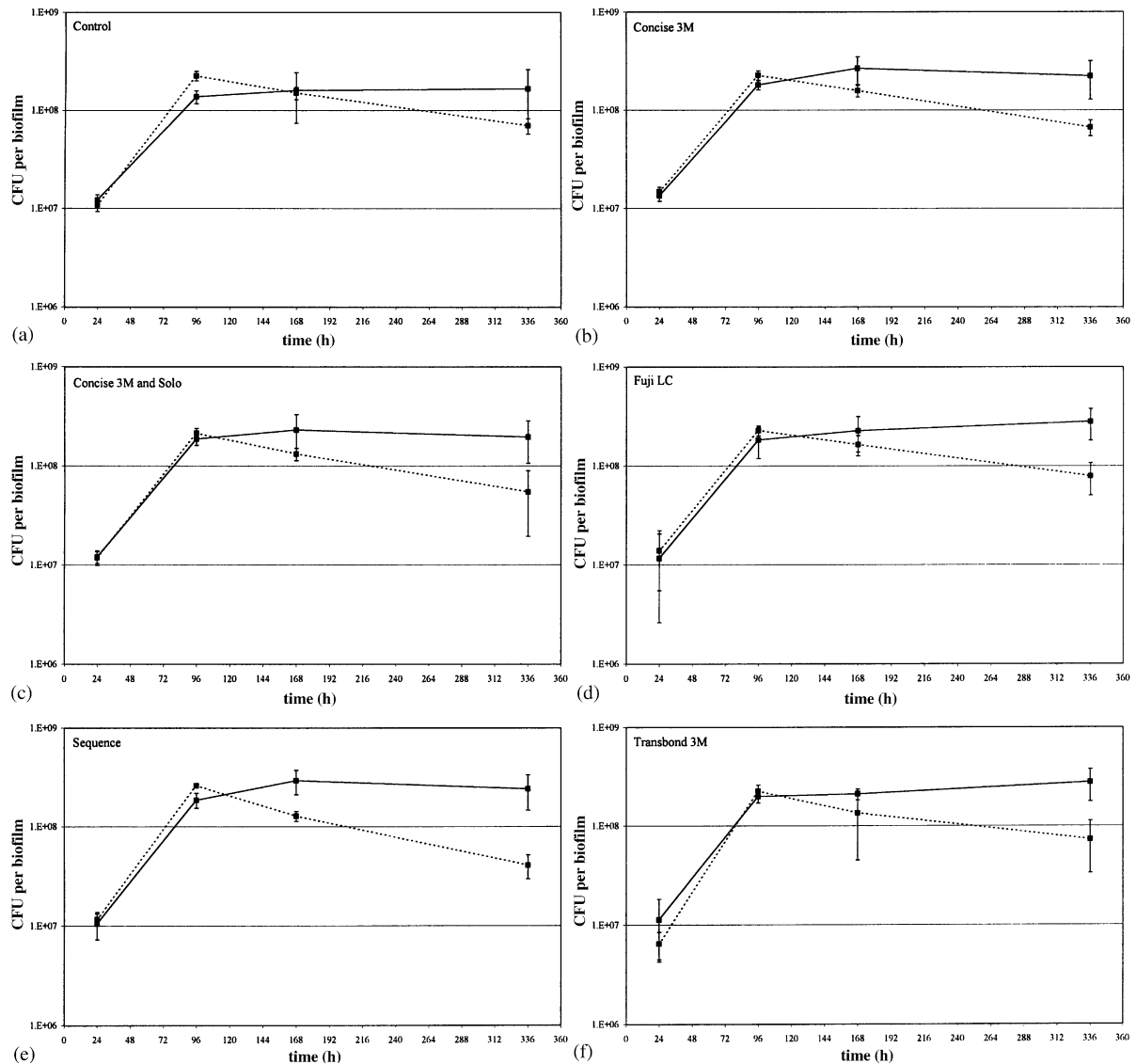


Fig. 2. (a–f) Aerobic (dotted line) and anaerobic (solid line) viable counts from growth on the control, Concise 3 M, Concise 3 M and solo, Fuji LC, Sequence and Transbond 3 M over 336 h. Error bars represent standard deviations from two separate experiments.

4. Discussion

The natural biofilm can often be complex and irreproducible [15] hence it was our aim to grow microcosm plaques under controlled conditions. The model used in this study is particularly suited to studies of biofilms of oral bacteria in that it provides an environment similar to that found in the oral cavity. Biofilms are grown on a solid substratum with nutrients being provided in a flowing, thin-film of liquid, which is continually replenished. The removal of the surfaces of the biofilms by the scraper blade simulates the continuous removal of the outermost layers of supragingival plaque [16].

Identification of streptococcal species by 16S rRNA gene sequencing has been shown to be difficult due to a

high degree of homology in the sequences [17]. It was therefore decided in this study to differentiate these species by biochemical differentiation. Using this technique we identified seven streptococci to species level. This compares with a recent study [18] on the same pooled saliva where eight different streptococci were identified using selective and non-selective media, 16S rRNA sequencing and cloning.

Decalcification can occur around an orthodontic bracket after a course of orthodontic therapy. Dental plaque is a prerequisite for the development of this decalcification, which is due to ecological shifts in the population from environmental changes. One previous study has investigated bacterial accumulation around orthodontic brackets with different orthodontic bonding agents. Blunden et al. [19] investigated *S. mutans*

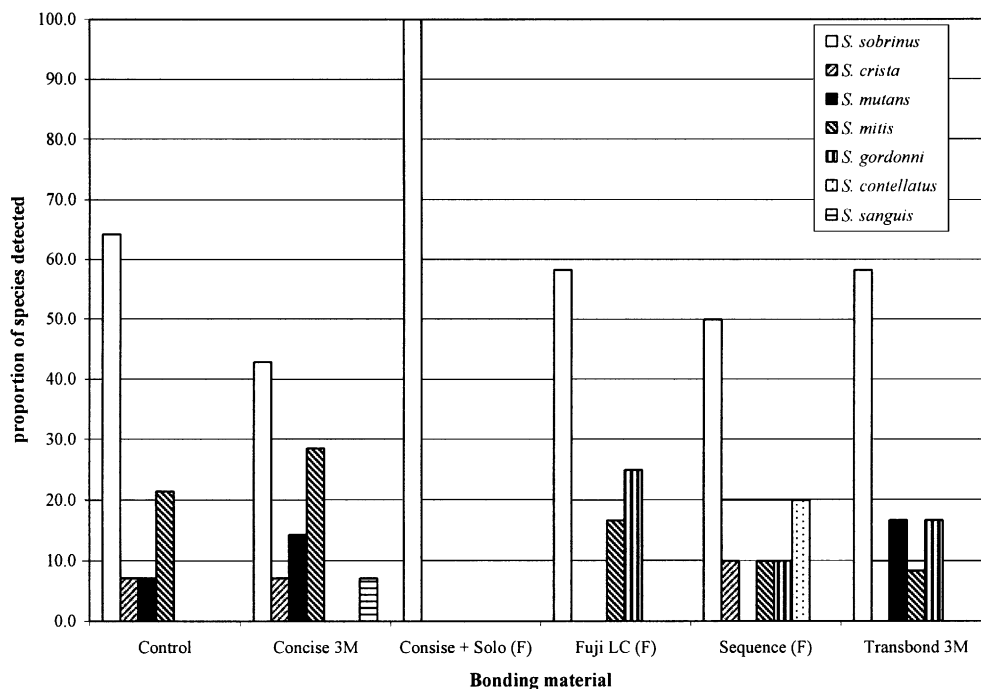


Fig. 3. The proportions of streptococci identified from two separate experiments at 24, 96, 168 and 336 h on the different bonding materials and the control. F denotes a fluoride releasing system.

adherence to different orthodontic bonding agents and they found that there were significant differences between different bonding agents. Studies which have focused on the total numbers of bacteria on bonding materials, have reported that there is no significant difference between bacterial growth on different materials [20,21]. Other workers [22,23], however, have suggested that there are differences in bacterial growth with different materials, due to fluoride release from glass ionomer cements. Interestingly, within our microcosm plaques we also found differences in the prevalence of *S. mutans* on the various materials investigated although the overall numbers of bacteria on each of the materials altered very little. In the present study *S. mutans* was not detected in the biofilms grown on the fluoride-releasing materials of Sequence, Fuji LC and Concise with the fluoride-releasing bond, Solo.

Fluoride release from these materials may inhibit the metabolism of plaque bacterial populations. It has previously been shown that *S. mutans* was the most susceptible *Streptococcus* spp. to sodium fluoride at various pH levels and that the presence of fluoride might influence the relative proportions of the species of streptococci in dental plaque [24]. Chadwick and Gordon [25] examined different orthodontic bonding materials for fluoride release and found that glass ionomer-based materials, such as Fuji LC, showed substantially greater amounts of fluoride release when compared with resin-based materials, such as Sequence. For all the materials tested, however, 70% of fluoride

release occurred within the first month with fluoride release rates highest during the first days of testing, declining to lower but more stable levels. In our 2-week study, both types of material appeared to be successful in preventing growth of *S. mutans* within the plaque population.

Although the prevalence of *S. mutans* is of interest, several epidemiological studies have indicated that *S. sobrinus* (found in the biofilms growing on all materials) is more closely associated with high caries activity [26]. Other studies have suggested that both these mutans streptococci species preferentially colonise the most caries prone sites [27]—a combination we observed on the control, Concise and Transbond materials, which did not contain fluoride. From our study we cannot determine whether local demineralisation will occur, only that the organisms that may be responsible are present in the population.

5. Conclusion

Results obtained suggest that the accumulation of in vitro supragingival plaque is not affected by the type of orthodontic bonding material present. However, biofilms formed over freshly made bonding materials known to release fluoride did not contain *S. mutans*. The clinical significance of this phenomenon is yet to be determined.

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