ORIGINAL ARTICLE

Biofilm formation on stainless steel and gold wires for bonded retainers in vitro and in vivo and their susceptibility to oral antimicrobials

Marije A. Jongsma · Floris D. H. Pelser · Henny C. van der Mei · Jelly Atema-Smit · Betsy van de Belt-Gritter · Henk J. Busscher · Yijin Ren

Abstract

Objective Bonded retainers are used in orthodontics to maintain treatment result. Retention wires are prone to biofilm formation and cause gingival recession, bleeding on probing and increased pocket depths near bonded retainers. In this study, we compare in vitro and in vivo biofilm formation on different wires used for bonded retainers and the susceptibility of in vitro biofilms to oral antimicrobials.
Materials and methods Orthodontic wires were exposed to saliva, and in vitro biofilm formation was evaluated using plate counting and live/dead staining, together with effects of exposure to toothpaste slurry alone or followed by antimicrobial mouthrinse application. Wires were also placed intra-orally for 72 h in human volunteers and undisturbed biofilm formation was compared by plate counting and live/ dead staining, as well as by denaturing gradient gel electrophoresis for compositional differences in biofilms.
Results Single-strand wires attracted only slightly less biofilm in vitro than multi-strand wires. Biofilms on stainless steel single-strand wires however, were much more susceptible to antimicrobials from toothpaste slurries and mouthrinses than on single-strand gold wires and biofilms on multi-strand wires. Also, in vivo significantly less biofilm was found on single-strand than on multi-strand wires. Microbial composition of biofilms was more dependent on the volunteer involved than on wire type.
Conclusions Biofilms on single-strand stainless steel wires attract less biofilm in vitro and are more susceptible to antimicrobials than on multi-strand wires. Also in vivo, single-strand wires attract less biofilm than multi-strand ones.
Clinical significance Use of single-strand wires is preferred over multi-strand wires, not because they attract less biofilm, but because biofilms on single-strand wires are not protected against antimicrobials as in crevices and niches as on multi-strand wires.

Keywords Orthodontics · Bonded retainer · Biofilm · Antimicrobials

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Introduction

In the last decades, an increasing number of patients are being treated with orthodontic appliances. After an active orthodontic treatment, patients are often given a fixed retainer to prevent teeth from relapsing to their pre-treatment positions. Before the 1970s, fixed retainers were normally banded to the lower canines, but in the early 1970s, the first report was published on the use of an acid-etching technique to bond retainers to the lingual surfaces of the lower canines [1]. Since then, plain stainless steel round or rectangular retention wires have been used as bonded fixed retainers [1, 2]. In the early 1980s, the use of multi-strand wires was described. First, these retention wires were bonded only to the canines [3], while later multi-strand wires were bonded to all six front teeth [4]. The twist in the multi-strand wires provided additional flexibility which allowed physiologic

movement of the bonded teeth instead of fixing them all as one unit and also provided undercut areas for mechanical retention for the composite bonding material [3–5].

Despite the advantage of retainers in preventing teeth from relapsing to their pre-treatment position, the general drawback of retainers is that biofilm and calculus accumulate along the wires of lingually bonded retainers [6], yielding a greater incidence of gingival recession, increased pocket depth and bleeding on probing [7, 8]. Commonly used preventive measures, including toothbrushing, the use of antibacterial toothpastes, possibly supplemented with the use of antibacterial mouthrinses are generally not enough to adequately clean retainer sites, which is despite the generally favourable effects of antibacterial toothpastes and mouthrinses on plaque inhibition in vivo [9–12].

Oral biofilm formation depends not only on the surface characteristics of the substratum surfaces but also on the amount of surface area exposed to the oral environment. Multi-strand retention wires have crevices and therewith possess a larger surface area than single-strand wires, which can be expected to yield increased biofilm formation. Thick oral biofilms have been found on gold surfaces in vivo, but these were barely viable [13]. Therefore, the use of gold-coated wires for fixed bonded retainers has been advocated over the use of stainless steel wires [14]. However, controversial results exist in the literature with respect to biofilm formation on different types of bonded retainers [6, 15–17]. This may be related to the fact that in previously published in vivo studies, biofilm formation was not evaluated on the retention wires themselves but on the tooth surface surrounding the wires. However, a standardised in vitro study on biofilm formation on wires themselves should clarify this controversy.

The aim of this study was to compare in vitro and in vivo biofilm formation on different gold or stainless steel wires with different numbers of strands used for orthodontic bonded retainers and the susceptibility of in vitro-formed biofilms on these retainers for chemical plaque control measures, i.e. exposure to toothpaste slurry, possibly followed by exposure to an antimicrobial mouthrinse.

Materials and methods

Retainers, toothpaste and mouthrinse

Five types of orthodontic wires used for bonded retainers were evaluated in this study, as summarised in Table 1. Lengths of 3 cm were cut out of each wire type and sterilised with 70 % ethanol. For plaque control, a NaF-sodium lauryl sulphate containing toothpaste without antibacterial claims was commercially obtained and 25 wt.% slurries were prepared in sterilised distilled water after centrifugation to remove abrasion particles. Cool Mint Listerine® was also commercially purchased for use as an antimicrobial mouthrinse (Johnson and Johnson, New Brunswick, NJ).

Saliva collection and biofilm formation in vitro

Human whole saliva from five healthy volunteers of both sexes was collected into ice-chilled beakers after stimulation by chewing Parafilm. The saliva was pooled and sonicated on ice-chilled water three times for 10 s with 30-s intervals. All volunteers gave their informed consent to saliva donation, in agreement with the rules set out by the Ethics Committee at the University Medical Centre Groningen (6th February 2009).

A schematic protocol of the experiment is shown in Fig. 1. In one experiment, four samples of each wire type were first placed in a sterile plastic tube containing 4 mL fresh pooled human saliva to allow bacterial adhesion to the wire surface. The tubes were incubated for 4 h at 37 °C in an aerobic incubator while shaking at 60 rpm. After 4 h, samples were removed from the saliva and rinsed in sterile water while one sample was kept for bacterial enumeration. Three samples were individually placed in sterile plastic tubes with 6 mL Tryptone Soya Broth (TSB) and left to incubate under shaking for 48 h. After 48 h, the three samples were removed from the TSB and rinsed in sterile water while again retaining one for bacterial enumeration. The two remaining samples were exposed to either a toothpaste slurry (2 min) or a toothpaste slurry followed by exposure to a mouthrinse (30 s) and rinsed once again. For reference, ground and polished enamel samples (surface roughness of 7 nm, as determined by atomic force microscopy) were included as a reference. All in vitro experiments were done in fourfold for each wire type.

Biofilm formation in vivo

Four stainless steel wires (Forestanit®, Wildcat®, Quadcat® and Pentacat®) were bonded on the palatal and buccal side of the first molar and the second premolar (see also Fig. 1) of eight healthy volunteers in agreement with the rules set out by the Ethics Committee at the University Medical Centre Groningen (23rd June 2011). Different types of retention wires were randomly attached to the right and left side of the maxillary arch. Wires were pre-bent on a plaster model of the volunteers dentition and had a length of 1 cm between the points of attachment to the teeth and were sterilised in 70 % ethanol before use. Volunteers were instructed not to brush or touch the wires with an interdental cleaning aid while brushing the remainder of their dentition with a commercially obtained NaF-sodium lauryl sulphate containing toothpaste without antibacterial claims. No additional oral hygiene products were allowed. Wires were

Table 1 Overview of the orthodontic retention wires used in this study

| Wire type	Diameter	Material	Filament	Manufacturer
Forestanit®	0.020 in. (0.5080 mm)	Stainless steel	Single strand	Forestadent, Pforzheim, Germany
RW028	0.028 in. (0.7112 mm)	Gold	Single strand	Gold'n Braces, Inc., Palm Harbor, Florida
Wildcat®	0.0175 in. (0.4445 mm)	Stainless steel	Triple strand	Dentsply GAC Int., Bohemia, New York
Quadcat® (rectangular)	0.016×0.022 in. (0.4064×0.5588 mm)	Stainless steel	Triple strand	PG Supply, Inc., Avon, Connecticut
Pentacat®	0.0175 in. (0.4445 mm)	Stainless steel	Six strands	Dentsply GAC Int., Bohemia, New York

removed after 72 h and oral biofilm was collected from the buccal and palatal enamel, together with a saliva sample. The wires and biofilm collected were stored in an Eppendorf tube containing 1.0 mL filter sterile reduced transport fluid (RTF). Saliva samples were stored on ice.

Evaluation of in vitro and in vivo biofilms

For enumeration, retention wires with adhering biofilm formed in vitro or in vivo and oral biofilm collected from enamel and saliva samples in human volunteers were sonicated three times for 10 s with 30 s intervals in Eppendorf tubes containing 1.5 mL filter sterile RTF on ice-chilled water, to disperse the adhering bacteria. Bacteria were enumerated in a Bürker–Türk counting chamber, and tenfold serial dilutions were prepared in RTF for each wire type and condition and

100 μL was plated onto non-selective blood agar plates. After 7 days of anaerobic incubation at 37 °C, the total numbers of colony forming units (CFUs) were counted and expressed per unit wire length. In addition, the percentage viability of the biofilms was evaluated after live/dead staining (BacLight™, Bacterial Vitality Kit, Molecular Probes Europe BV, Leiden, The Netherlands) of dispersed biofilms. Live/dead stain was prepared by adding 3 μL of SYTO®9/Propidium iodide (1:3) to 1 mL of sterile, demineralised water. Fifteen microlitres of the stain was added to 10 μL of the undiluted biofilm dispersion. After 15 min incubation in the dark, the number of live and dead bacteria were counted using a fluorescence microscope (Leica DM4000B, Leica Microsystems Heidelberg GmbH, Heidelberg, Germany) and expressed as a percentage viability. Scanning electron micrographs of in vitro and in vivo biofilms on wires were taken, as described below.

Fig. 1 **a** Schematic description of the experimental protocol for biofilm growth in vitro and in vivo. All in vitro experiments were carried out in fourfold while in vivo experiments were done in eight human volunteers. **b** Buccal placement of retainer wires for in vivo biofilm growth. **c** Palatal placement of retainer wires for in vivo biofilm growth
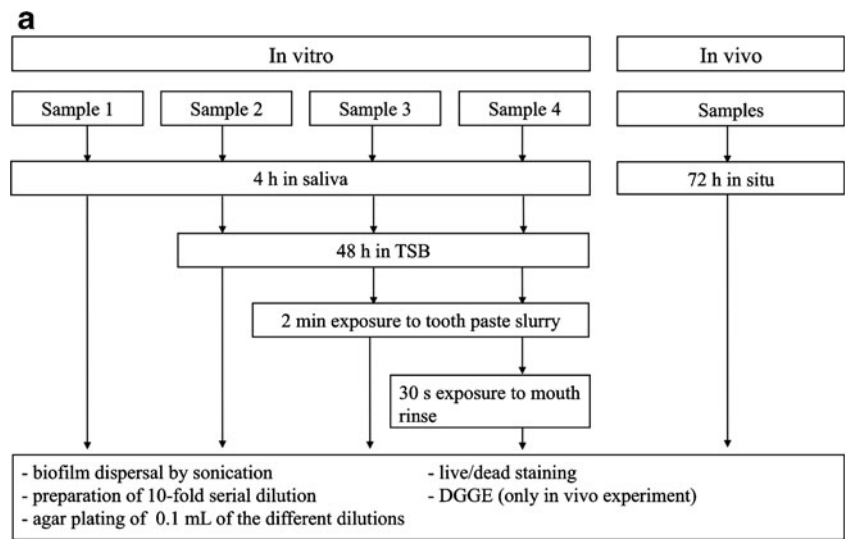
DGGE analysis of in vivo biofilms

All samples of in vivo formed biofilms and saliva were stored at −80 °C until use for PCR-denaturing gradient gel electrophoresis (DGGE) in order to compare the microbial compositions of the biofilms. For extraction of DNA, samples were thawed and centrifuged for 5 min at 13,000×g (Eppendorf Centrifuge 5415D, Hamburg, Germany) and subsequently washed and vortexed with 200 μL TE-buffer (10 mM Tris–HCl and 1 mM EDTA at pH 7.4), again followed by centrifugation for 5 min at 13,000×g. Next, the supernatant was removed and the pellet was subsequently placed in a microwave (500 W, 5 min), after which it was suspended in 50 μL TE-buffer, vortexed and placed on ice. The quality and quantity of DNA samples were measured with a NanoDrop® spectrophotometer (ND-1000, NanoDrop Technologies, Inc, Wilmington, DE) at 230 nm. The final concentration of each DNA sample was adjusted to 100 ng DNA for PCR amplifications.

PCR was performed with a Tgradient thermocycler (Bio-rad I-cycler, GENOtronics BV). For amplification of the 16S rRNA gene, the following bacterial primers were used: F357-GC (forward primer, 5′-GC clamp-TACGGGAGGCAGCAG-3′) [18] containing a GC clamp (5′-CGCCCGCCGCGCCCCGCGCCC GGCCCGCCGCCCCGCCC-3′) [19] to make it suitable for DGGE, and R-518 (reverse primer, 5′-ATTACCGCGGCTGCTGG-3′) [20]. Twenty-five microlitres of each PCR mixture contained 12.5 μL PCR Master Mix (0.05 units/μL Taq DNA polymerase in reaction buffer, 4 mM MgCl$_2$, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP and 0.6 mM dTTP (Fermentas Life Sciences)), 1 μL of both forward and reverse primer (1 μM), and 100 ng DNA (in a volume of 10.5 μL). The temperature profile included an additional denaturing step of 5 min at 94 °C, followed by a denaturing step at 94 °C for 45 s, a primer annealing step at 58 °C for 45 s, an extension step at 72 °C for 1 min and a final extension step of 72 °C for 5 min. PCR products were analysed by electrophoresis on a 2.0 % agarose gel containing 0.5 μg/mL ethidium bromide.

DGGE of PCR products generated with the F357-GC/R-518 primer set was performed as described by Muyzer et al. [21], using system PhorU (INGENY, Goes, The Netherlands). The PCR products were applied on 8 % (w/v) polyacrylamide gel in 0.5×TAE buffer (20 mM Tris acetate, 10 mM sodium acetate and 0.5 mM EDTA at pH 8.3). The denaturing gradient consisted of 30 to 80 % denaturant (100 % denaturant equals 7 M urea and 37 % formamide). Gels were poured using a gradient mixer. A 10-mL stacking gel without denaturant was added on top. Electrophoresis was performed overnight at 120 V and 60 °C. Gels were stained with silver nitrate [19]. Each DGGE gel was normalised according to a marker consisting

of seven reference species comprising common bacterial species associated with oral health and disease [20, 22] and stored at 4 °C. The reference strains were Lactobacillus jenseii ATCC 10449, Streptococcus oralis ATCC 35037, Streptococcus mitis ATCC 9811, Streptococcus sanguinis ATCC 10556, Streptococcus salivarius HB, Streptococcus sobrinus ATCC 33478 and Steptococcus mutans ATCC 10449 [23].

Scanning electron microscopy

Topography of the wires, in absence and presence of both in vitro- and in vivo-formed biofilms, were visualised using scanning electron microscopy (SEM). Wires were fixed overnight in 2 % glutaraldehyde and post-fixed for 1 h with 1 % osmiumtetroxide. After dehydration through a water–ethanol series, wires were incubated in tetramethylsilane and air dried, the samples that contained biofilm were sputter coated with a gold–palladium alloy, after which they were fixed on SEM stub-holders using double-sided sticky carbon tape and visualised in a field emission scanning electron microscope, type 6301F (JEOL Ltd., Tokyo, Japan) at 2 kV with a working distance of 39 mm and a small spot size.

Statistical analysis

Data were analysed with the Statistical Package for Social Sciences (version 16.0, SPSS Inc., Chicago, IL). A one-way analysis of variance was used to compare the number of CFUs found and the percentage of biofilm viability. A Bonferroni test was used for post hoc multiple comparisons. Statistical significance was set at $p < 0.05$.

DGGE gel images were converted and transferred into a microbial database with GelCompar II, version 6.1 (Applied Maths). The similarities in bacterial composition of the different biofilms were analysed using a band-based similarity coefficient and a non-weighted pair-group method with arithmetic averages was used to generate dendograms indicating similarities in composition [24].

Results

Scanning electron micrographs of the five wires are shown in Fig. 2 and clearly show crevices and niches formed by the multi-strand wires that are absent on the single-strand wires. Furthermore, it can be seen that the roughness of the single-strand gold wire is higher than that of the single-strand stainless steel wire.

The numbers of CFUs on the different wires formed in vitro are summarised in Table 2. There was no significant difference between the number of CFUs adhering to the wires after 4 h incubation in saliva while both single-

Fig. 2 Scanning electron micrographs of the different wire types prior to biofilm formation; magnification, ×75 (bar marker indicates 100 μm). **a** Forestanit® (single strand, stainless steel), **b** RW028 (single strand, gold), **c** Wildcat® (triple strand, stainless steel), **d** Quadcat® (triple strand, stainless steel) and **e** Pentacat® (six strands, stainless steel)
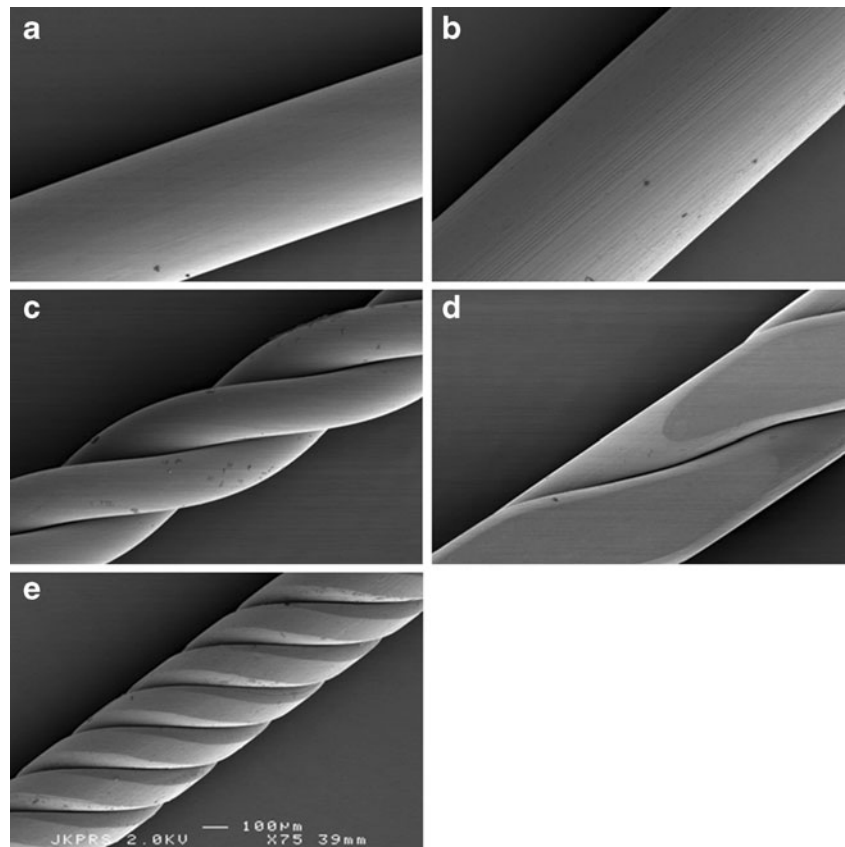
strand stainless steel and gold wires showed less biofilm formation after 48 h compared with the three stainless steel multi-strand wires. For the stainless steel single-strand wire, this difference was significant compared with all three multi-strand stainless steel wires ($p<0.05$), but for the gold single-strand wire, there was only a significant difference compared with the six strands stainless steel wire ($p<0.05$). There was no statistically significant difference in the amount of biofilm formation on the stainless steel versus the gold single-strand wires, neither were there any statistically significant differences between the three multi-strand stainless steel wires. All wires attract highly viable biofilms, with less than 20 % dead bacteria. For comparison, we carried out a similar experiment on enamel surfaces and found a similarly high viability of 74.0±8.5 % (note that the amount of biofilm formed on enamel could not be expressed in units allowing comparison with the amount of biofilm formed per centimetre wire length).

The single-strand wires attract a differently structured biofilm than the multi-strand wires (Fig. 3). On the multi-strand wires, biofilm is mostly located in the crevices between strands, while on the single-strand wires bacteria are present as a thin film (compare Fig. 3a, b with c). Comparison of the biofilms on single-strand stainless steel versus gold wires gives the impression of a higher degree of clustering of the biofilm on gold, possibly as a result of its larger roughness (compare Fig. 3a with b). In vitro results

show similar viability for gold and stainless steel as well as for enamel after 48 h of biofilm formation.

The number of CFUs cultured from 48-h-old in vitro biofilms on multi-strand wires was not significantly affected by exposure to toothpaste slurries or by exposure to toothpaste slurries followed by exposure to an antimicrobial mouthrinse, although significant drops in viability were observed. Oppositely, 48-h-old biofilms on stainless steel and gold single-strand wires showed significantly reduced numbers of CFUs after exposure to the toothpaste supernatant concurrent with a drop in viability while further reductions in amount of biofilm and viability could be achieved by subsequent exposure to the antimicrobial mouthrinse for single-strand stainless steel wires. Interestingly, biofilms on single-strand stainless steel wires were much more susceptible to chemical plaque control than biofilms formed on gold wires. For comparison, for biofilms formed on enamel surfaces, viability decreased from 74.0±8.5 to 59.5±0.7 % upon exposure to a toothpaste slurry, dropping further down to 19.5±7.7 % upon subsequent exposure to an antimicrobial mouthrinse.

The number of CFUs in biofilms formed in vivo are summarised in Table 3. Buccally placed wires collected more biofilm than palatally placed wires, regardless of the wire type, while no significant differences were found between the different wires placed on the buccal side. Significantly less biofilm had grown on the single-strand

Table 2 The number of CFUs (log units±SD over four experiments with separately cultured bacteria) and the viability found in 4- and 48-h-old biofilms formed in vitro from fresh, human whole saliva on 1 cm lengths of the different wires involved in this study and the effects of exposure to a 25 wt.% toothpaste slurry alone or followed by an additional exposure to a mouthrinse

Wire type	4-h-old biofilm		48-h-old biofilm		48-h-old biofilm exposed to toothpaste slurry		48-h-old biofilm exposed to toothpaste slurry and mouthrinse	
	CFUs	Live (%)	CFUs	Live (%)	CFUs	Live (%)	CFUs	Live (%)
Forestanit®	4.1±0.3	>95	5.7±0.3	87.0±8.4	1.6±1.4[c, d]	10.0±4.1[d]	0.3±0.4[c, d]	0.0±0.0[d, e]
RW028	4.5±0.2	>95	6.0±0.4	86.7±7.6	4.5±1.2[a, d]	43.7±8.0[a, d]	4.9±1.2[a]	20.0±11.3[a, d, e]
Wildcat®	4.2±0.3	>95	6.5±0.3[a, c]	90.5±4.9	6.2±0.1[a, b, c]	56.0±1.4[a, d]	6.3±1.2[a, c]	42.0±5.7[a, b, d]
Quadcat®	4.7±0.5	>95	6.6±0.8[a, c]	89.0±1.4	6.2±0.2[a, b, c]	45.5±10.6[a, d]	6.2±0.3[a, b, c]	27.5±3.5[a, d, e]
Pentacat®	4.6±0.2	>95	6.9±0.2[a, b, c]	82.5±7.8	6.5±0.2[a, b, c, d]	56.5±0.7[a, d]	6.6±0.1[a, b, c]	46.5±0.7[a, b, d]

[a] Significantly different from Forestanit® (single strand, stainless steel)

[b] Significantly different from RW028 (single strand, gold)

[c] Significantly different from 4 h

[d] Significantly different from 48 h without exposure to toothpaste slurry or mouthrinse

[e] Significantly different from 48 h with exposure to toothpaste slurry

stainless steel wire placed palatally compared with other palatally placed wires, while all multi-strand wires on the palatal side collected similar amounts of biofilm. All in vivo biofilms formed on the different retention wires contained a similar percentage of live bacteria (see Table 3), while oral biofilm collected from enamel surfaces in vivo was slightly less viable (64.2±6.8 and 64.0±6.4 % for bucally and palatally sampled oral biofilm).

Scanning electron micrographs (Fig. 4) show that also in vivo the single-strand wires attract a differently structured biofilm than the multi-strand wires. On the multi-strand wires, biofilm is mostly located in the crevices between strands, while on the single-strand wires bacteria are present as a thin film. There is also a clear difference between wires placed buccally compared with wires placed palatally. Biofilm on buccally placed wires covers the entire wire surface, whereas smooth surfaces of palatally placed wires are either clean or only covered with a thin organic film. Biofilm on the multi-strand, palatally placed wires is almost entirely located in crevices and niches, while palatally placed single-strand wires collect biofilm mostly on the side of the wire facing the tooth surface and thus out of reach by the tongue.

Microbial composition of the in vivo-formed biofilms on enamel was equally variable among volunteers (Fig. 5) as the variation in the composition of biofilms formed on different wire types. Microbial compositions of saliva from different volunteers had a tendency to cluster, but the


Fig. 3 Scanning electron micrographs of 48-h-old biofilms formed in vitro on selected wire types; magnification, 750× (bar marker indicates 10 μm). **a** Forestanit® (single strand, stainless steel)—biofilm is present as a thin, scattered film. **b** RW028 (single strand, gold)—scattered clusters of biofilm are formed. **c** Quadcat® (triple strand, stainless steel)—biofilm is mostly located in the crevices between strands
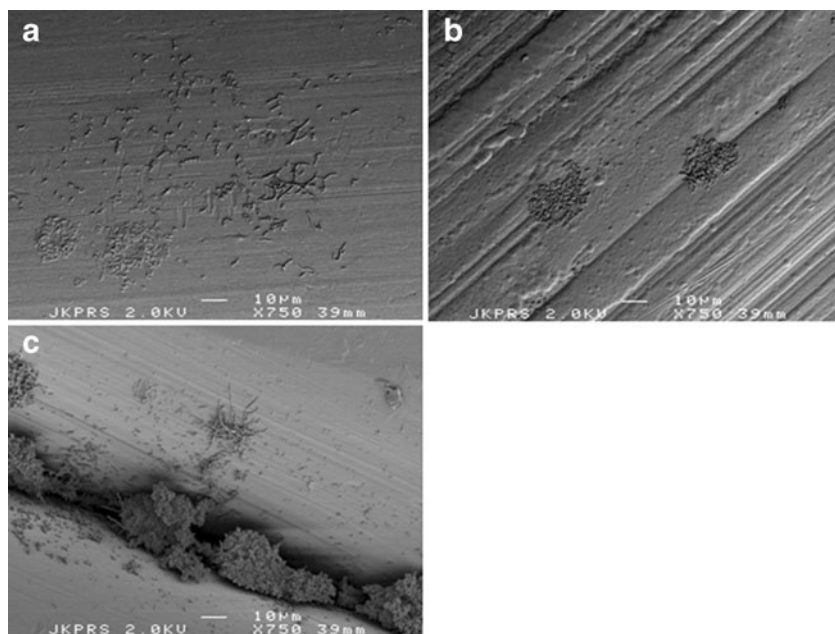
Table 3 The number of CFUs in and the viability of biofilms formed in vivo (log units±SD over eight different volunteers) on 1 cm lengths of the different wires involved in this study

Wire type	Buccally placed		Palatally placed	
	CFUs	Live (%)	CFUs	Live (%)
Forestanit®	7.4±0.3[a]	73.6±6.9	6.7±0.5	73.0±13.1
Wildcat®	7.5±0.2[a]	70.9±14.5	7.2±0.4[a]	75.0±6.7
Quadcat®	7.6±0.3[a]	73.5±8.4	7.3±0.5[a]	74.9±9.9
Pentacat®	7.6±0.1[a]	75.6±6.5	7.3±0.4[a]	74.2±10.2

RW028 became unavailable during the course of the study, and no in vivo data are available

[a] Significantly different from Forestanit® placed palatally

composition of biofilms formed on the retainer wires, including enamel surfaces could not be related with a specific material or wire type.

Discussion

Although the use of the bonded retainers to prevent teeth from relapsing back to their original, pre-treatment position is generally accepted in orthodontics, the tendency of these retainers to collect oral biofilm and calculus is considered a disadvantage. It has long been suggested that the increased numbers of retention areas in crevices and niches of multi-strand wires do not yield higher biofilm attraction than on single-strand wires [6]. The present study for the first time confirms that there is indeed little difference in biofilm formation in vitro on single- versus multi-strand wires, and differences were only statistically significant after 48 h of biofilm formation. Highly interesting, biofilms formed in vitro on multi-strand wires appear less susceptible to oral antimicrobials than biofilms on single-strand wires, probably because of their protected growth in crevices and niches on multi-strand wires. Likely, the protected growth in crevices and niches is the reason why in vivo more biofilm accumulated at the undercut areas of the multi-strand wires and on the surrounding lingual tooth surfaces than with single-strand wire retainers [17]. On tooth surfaces, minute irregularities have been demonstrated to protect microorganisms and stimulate biofilm accumulation [25]. Biofilm formation on surgical meshes and suture materials also demonstrate more biofilm formation on multi- than on mono-filament structures [26–28], with aerobic and anaerobic bacteria being isolated in nearly equal numbers of viable bacteria from monofilament sutures made of different materials used in intra-oral dentoalveolar surgery [29].

The lack of a significant difference in in vitro numbers of viable bacteria on single-strand retention stainless steel and gold wires in the current study indicates that the influence of the material on initial biofilm formation is low, which is in agreement with literature, stating that roughness is the dominant factor in biofilm adhesion [30]. Five-day-old oral biofilms on gold surfaces in vivo are known to be thick

Fig. 4 Scanning electron micrographs of 48-h-old biofilms formed in vivo on selected wire types; magnification, 75× (bar marker indicates 100 μm) and 750× (bar marker indicates 10 μm). **a** Forestanit® (single strand, stainless steel) buccally placed—biofilm is present as a thick fully covering film. **b** Forestanit® (single strand, stainless steel) palatally placed —biofilm is present as a thin, scattered film. **c** Quadcat® (multi-strand, stainless steel) buccally placed—biofilm is present in the crevices between strands as well as on the smooth surfaces. **d** Quadcat® (multi-strand, stainless steel) palatally placed—biofilm is mostly located in the crevices between strands
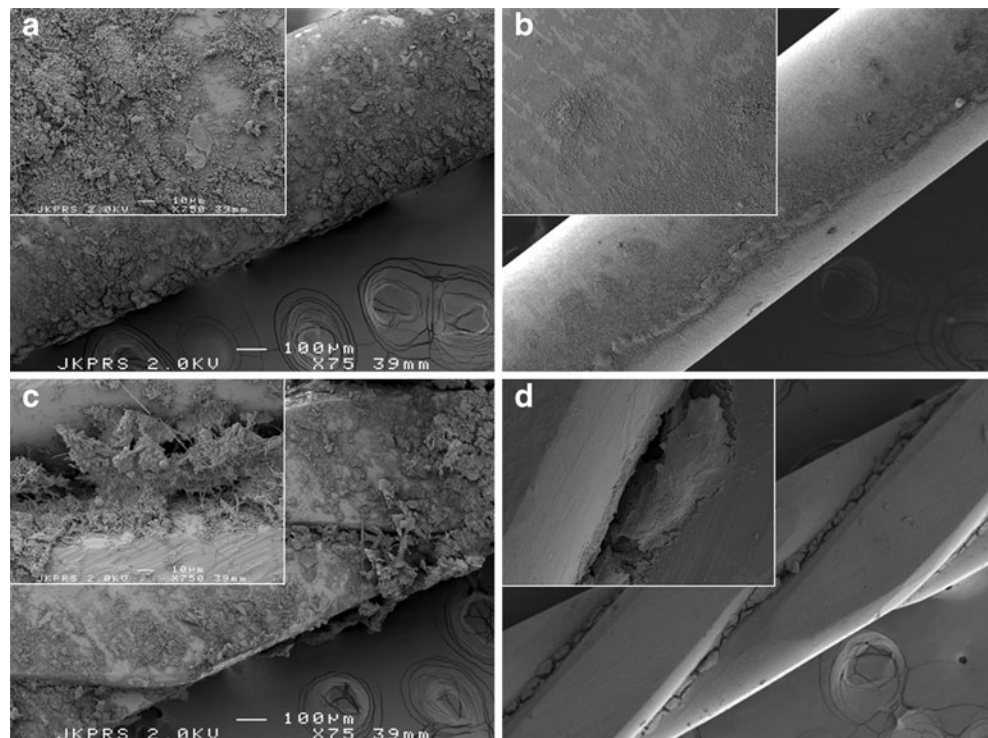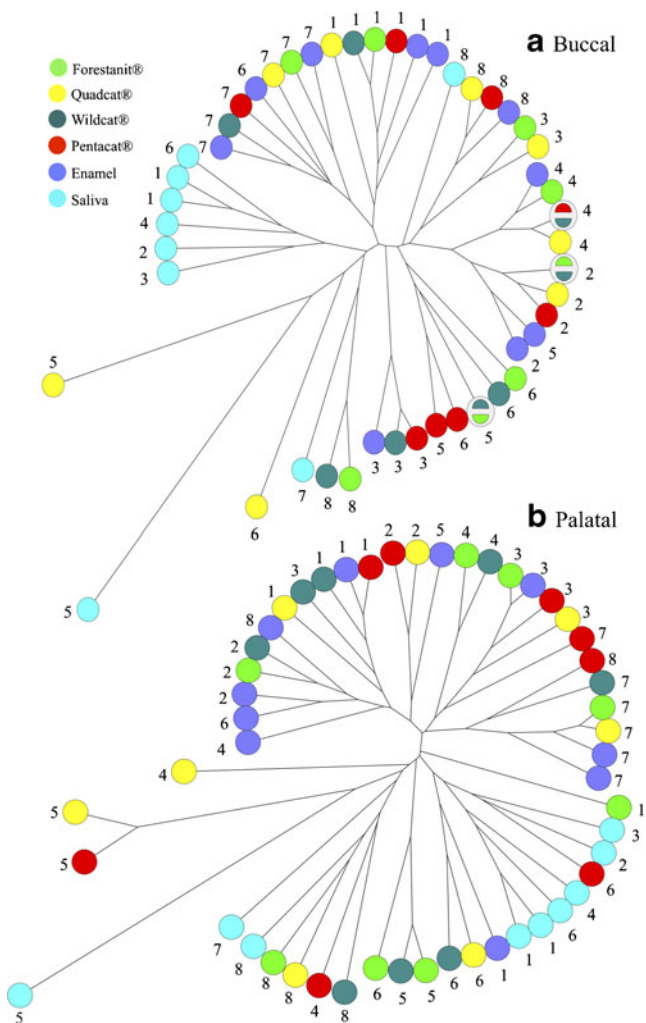
Fig. 5 Dendograms indicating the similarities in the microbial composition of biofilms formed on the different wire types (Forestanit®, Quadcat®, Wildcat® and Pentacat®), enamel and the microbial composition of saliva. Clustering of biofilms with a similar microbial composition was shown per volunteer, including the composition of saliva of each volunteer. No clustering was observed for any special wire type or enamel. *Numbers* denote different volunteers. **a** Buccal samples. **b** Palatal samples

and fully covering the substratum surfaces though with a viability of less than 2 % [13]. Possibly, full coverage by a relatively thick biofilm hampers the supply of nutrients to the biofilm, leading to a low viability extending to the deeper layers of the biofilm [31] while allowing antimicrobials to remain active on the outer layer [32, 33]. The present in vitro study, though confined to 48 h, shows a larger clustering of bacteria on gold than on stainless steel, which may be considered as the on-set of a thick and fully covering biofilm. The difference can probably be attributed to the higher surface roughness of gold wires compared with stainless steel single-strand wires (compare Fig. 3a, b) since a surface roughness above a threshold of 2 μm is already known to facilitate biofilm formation on restorative

materials [34]. The larger clustering of bacteria on gold probably offers protection against oral antimicrobials. Positive effects of antimicrobials, such as NaF, sodium lauryl sulphate in toothpastes and essential oil in mouthrinses on biofilm inhibition have been extensively described for oral biofilms on smooth surfaces [9–12]. In the present study, however, exposure to toothpaste supernatant reduced biofilm formation only on both single-strand wires. Additional effects of the antimicrobial mouthrinse compared with the toothpaste supernatant were observed for the stainless steel single-strand wires. This supports the above suggestion that the increased roughness of gold wires compared with stainless steel ones as well as the crevices and niches in multi-strand wires protect oral biofilm organisms against chemical challenges. Unfortunately, gold wires became unavailable during the course of this study, impeding inclusion of gold wires in our in vivo analysis. The current in vitro study has been carried out using biofilms grown from human whole saliva. Therewith, a larger number of strains can be grown from more controlled experiments using clinical isolates and the chances of bacteria to adhere are therefore increased [25]. Moreover, biofilms grown from saliva are more representative of in vivo biofilms, whereas at the same time it may be considered a disadvantage that we had a lower control of the biofilm composition than when using single strains of bacteria [35].

Despite differences between salivary protein adsorption in vitro and in vivo [36] and possible differences in the selective growth of bacteria from saliva in vitro and in vivo, our in vivo comparison of biofilm formation on different retention wires confirms that on the palatal side, less biofilm is formed on single-strand than on multi-strand retention wires, likely due to the protection offered by growth in crevices and niches of multi-strand wires against mechanical removal (brushing) and oral antimicrobials. Although volunteers did not brush the wire itself with toothpaste and used a toothpaste without antibacterial claims, it cannot be avoided that antimicrobials are involved in in vivo biofilm formation on the wires. The toothpaste used contains fluoride and sodium lauryl sulphate, both known to be antimicrobial that spread through the oral cavity during brushing. Moreover, saliva contains several antimicrobial peptides and proteins that affect biofilm formation [37]. In vivo protection against mechanical removal is furthermore implicated by the fact that no significant differences were observed between buccally placed wire types, but only for palatally placed ones, within reach of frictional removal forces exerted by the tongue [30]. These results imply that for this type of research, buccal placement, though preferred by volunteers, is to be avoided in comparative studies on oral biofilm formation on retention wires since differences only become evident under clinical conditions when wires are placed palatally.

Microbial compositions of saliva from different volunteers obtained using DGGE cluster more strongly than the compositions of the adhering biofilms in different volunteers. Moreover, no clustering is observed for the composition of biofilms formed on different retention wires, including enamel in different volunteers. This demonstrates that inter-individual differences control the composition of the oral microflora [38], for instance through dietary influences, which is difficult to standardise in any clinical study [24, 39].

Conclusions

Recent studies showed an increased incidence of lingual gingival recession, biofilm retention and bleeding on probing of teeth with bonded retainers [7, 8]. Based on the current results, it is concluded that single-strand wires attract only slightly less biofilm in vitro than multi-strand wires, with no significant difference between single-strand stainless steel and gold wires. In vivo however, single-strand, palatally placed stainless steel wires attracted significantly less biofilm compared with the other wires, indicating that with respect to biofilm formation and its prevention, single-strand stainless steel wires should be the first choice. Single-strand stainless steel wires attract less biofilm in vivo, not because they are less adhesive to oral biofilm, but because biofilms on single-strand retention wires is less protected by growth in crevices and niches against oral antimicrobials than when formed on multi-strand wires.

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