Multivariate Analysis of Surface Physico-Chemical Properties Controlling Biofilm Formation on Orthodontic Adhesives Prior to and After Fluoride and Chlorhexidine Treatment

Mervyn Y. H. Chin,[1,2] Andrew Sandham,[1] Jonathan Pratten,[3] Joop De Vries,[2] Henny C. van der Mei,[2] Henk J. Busscher[2]

[1] Department of Orthodontics, University Medical Center Groningen, University of Groningen, Hanzeplein 1, Postbox 30 001, 9700 RB Groningen, The Netherlands

[2] Department of Biomedical Engineering, University Medical Center Groningen, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

[3] Division of Microbial Diseases, UCL Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD, UK

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Abstract: Biofilm formation on orthodontic adhesives is a serious clinical problem, as it leads to enamel demineralization around fixed orthodontic appliances, often leaving white spot lesions after their removal. The aim of this work was to determine the influence of surface physico-chemical properties of four commonly used orthodontic adhesives (Concise™, Fuji ORTHO™ LC, Ketac Cem μ, and Transbond™ XT) on early bacterial biofilm formation. In addition, effects of two commercially available mouthrinses (0.05% sodium fluoride and 0.2% chlorhexidine gluconate) on these properties and biofilm formation were determined. Water contact angles on the adhesives decreased after fluoride and chlorhexidine treatment, concurrent with an increase in carbon and a decrease in oxygen surface concentrations, except for Transbond, as determined by X-ray photoelectron spectroscopy. No fluorine was detected on any of the adhesive surfaces after fluoride treatment, while all surfaces showed chlorine after chlorhexidine treatment. Surface roughness of the adhesives measured using three-dimensional optical profilometry was around 4 μm and found not to be a factor governing early biofilm formation. Multiple linear regression analysis indicated that early biofilm formation by *Streptococcus sanguis* could be explained in a model comprising hydrophobicity and the prevalence of oxygen- and nitrogen-rich components on the adhesive surfaces. © 2006 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 78B: 401–408, 2006

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INTRODUCTION

Enamel decalcification and white spot lesion formation are the major clinical problems for patients treated with fixed orthodontic appliances.[1–3] The increased difficulty of biofilm removal around orthodontic brackets and increased biofilm formation on and around resin bonding materials have all been shown to contribute to decalcification adjacent to orthodontic brackets.[4–6]

Extensive research on the bond strength of new orthodontic adhesives is widely available in the literature.[7–9] However, there is a lack of information regarding their plaque-retaining capacities. The development of biofilms is characterized by adsorption of primary colonizers to pellicle-coated surfaces, followed by coadhesion of a community of bacteria with mutual support from inter-dependent species to form a complex three-dimensional structure with physiological and structural heterogeneity. As initial bacterial adhesion and early biofilm formation are important factors governing further colonization,[10] an understanding of the mechanisms involved in the early stages of biofilm formation will be helpful in devising antimicrobial regimes for patients wearing fixed orthodontic appliances.

The benefits of fluoride in the inhibition of enamel decalcification and enhancement of lesion remineralization are well documented.[3,11] The daily use of a fluoride mouthrinse (0.05% sodium fluoride), in addition to the use of a fluoride toothpaste, has been recommended[11] for patients undergoing

Correspondence to: M. Y. H. Chin (e-mail: m.y.h.chin@med.umcg.nl)

TABLE I. Orthodontic Adhesives Used in This Study

Bonding Material	Composition	Manufacturer
Concise™	Two paste chemically-activated composite resin	3M ESPE, St. Paul, MN
Fuji ORTHO™ LC	Light-activated resin-modified glass ionomer cement	GC Corporation, Tokyo, Japan
Ketac Cem μ	Chemically-activated glass ionomer cement	3M ESPE, St. Paul, MN
Transbond™ XT	Light-activated composite resin	3M Unitek, Monrovia, CA

fixed appliances therapy. The use of antibacterial agents like chlorhexidine, triclosan, and zinc has also been reported.[12,13] The bactericidal effect of chlorhexidine was demonstrated in an *in vitro* biofilm model after pulsing chlorhexidine twice daily over a period of 4 days.[14] However, the long-term clinical applicability of chlorhexidine is limited by the discoloration of teeth and tongue and the presence of a metallic taste.[15]

Initial adhesion of bacteria to surfaces and early biofilm formation, including adhesion to orthodontic adhesives, is influenced by the surface physico-chemical properties of the bacterial cell and substratum surfaces, such as surface roughness, hydrophobicity, and elemental surface composition.[16,17] Cell surface hydrophobicity of bacteria is an important physical factor for their adhesion to surfaces. According to surface thermodynamics, hydrophilic strains would preferentially adhere to hydrophilic surfaces, while hydrophobic strains would have a preference for hydrophobic surfaces.[17,18] Generally, however, hydrophobic strains adhere to a greater extent than hydrophilic bacteria.[19] Not all orthodontic composites possess identical properties in relation to hydrophobicity and surface roughness.[20] A strong linear correlation between bacterial colonization and hydrophobicity and a moderate correlation between bacterial adhesion and surface roughness of various orthodontic bonding materials have been reported.[21] In addition, biofilm formation by oral bacteria is far less on hydrophobic surfaces than on hydrophilic surfaces both in the human oral cavity[22] as well as in the oropharynx,[23] possibly due to fluctuating shear forces. Yamauchi et al.[24] studied the influence of surface roughness of denture resin on bacterial adhesion and found that *Streptococcus mutans* adhered in higher amounts on smooth surfaces, whereas *Streptococcus oralis* were found in higher proportions on rough sites.

The great majority of studies attempt to relate bacterial adhesion and biofilm formation on surfaces with a single property of the surface, such as hydrophobicity, roughness, or chemical composition. In reality, however, these properties work in concert and a multiple regression analysis would be more appropriate to perform. Multiple linear regression analysis using the elemental surface composition, presence of proteins, surface hydrophobicity, and surface roughness as inputs demonstrated that adhesion of *Pseudomonas aeruginosa* to contact lenses could be described by the hydrophobicity, surface roughness, percentage oxygen and nitrogen, O═C/O—C ratio, and the presence of proteins on the surface of contact lenses.[25] The complexity of the interaction between biofilm formation and surface properties of orthodontic adhesives and the changes brought about by the introduction of antimicrobial agents is yet to be unveiled.

This *in vitro* study aimed to investigate the surface physico-chemical properties (hydrophobicity, roughness, and composition) factors involved in early *S. sanguis* biofilm formation on four commonly used orthodontic adhesives, and to determine the effects of two commercially available fluoride- and chlorhexidine-containing mouthrinses. Biofilm formation was studied in a parallel plate flow chamber, while the influence of the surface physico-chemical properties was analyzed using multiple linear regression analysis.

MATERIALS AND METHODS

Specimen Preparation

Orthodontic adhesives outlined in Table I were selected for this study. Six discs, 5 mm in diameter and 1 mm in thickness, were fabricated from each material using a polytetrafluoroethylene mould. The adhesives were packed into the mould using the individual mixing spatula provided by the manufacturers, after which the mould was compressed between two glass plates, covered with copier overhead film (MC 110, Océ, The Netherlands) to ensure maximal flatness and ease of removal after setting. All experimental discs were made following the manufacturers' instructions by one operator (M.Y.H.C.) during one session to maximize consistency, although it should be noted that an alternative choice, that is, preparing multiple samples out of different packages in multiple sessions, could be defended as well. No additional surface finishing procedure was performed to mimic the clinical situation. Fuji ORTHO LC and Transbond were light activated with 3M Unitek Curing Light XL3000 (400–500 nm, irradiance 580 mW cm^{-2}; Monrovia, CA) for 20 s while Concise and Ketac Cem were allowed to bench cure for 5–7 min. Discs were disinfected before each experiment by immersing into 1 mL 70% ethanol for 5 min, after which they were sterilely handled using latex gloves and forceps. Discs of each material were randomly assigned to three groups.

To determine the effect of fluoride and chlorhexidine mouthrinse on early biofilm formation, two groups of discs were treated with 2 mL Ortho Swirl (0.05% sodium fluoride; Ortho-Care, Bradford, UK) and Corsodyl (0.2% chlorhexidine gluconate; GlaxoSmithKline Consumer Healthcare, Brentford, UK) for 1 min in a plastic vial, respectively. After removal of the discs from the rinses, they were thoroughly washed in deionized water and blotted dry.

Surface Physico-Chemical Properties of the Adhesives

Hydrophobicities. Water contact angles were measured to characterize hydrophobicities of the adhesives prior to and after fluoride and chlorhexidine treatment. Contact angles were measured at 25°C from sessile drops (3 μL), employing a homemade contour monitor. This monitor registers the contour of a liquid droplet based on grey-value thresholding after which contact angles are calculated from the height and base width of a droplet.

Surface Roughness. A Proscan 2000 optical profilometer (Scantron Industrial Products, Monarch Centre, Taunton, UK) was used to determine the average surface roughness (R_a) prior to and after fluoride and chlorhexidine treatment. Proscan 2000 is an optical profilometer capable of non-contact three-dimensional surface profiling down to 10 nm depth of field, and a submicron resolution at a rate of 2000 points per second. The use of the (S-Type) chromatic sensor or the (L-Type) scattering laser triangulation allows examination of dark and rough surfaces with the object viewed in any orientation, zoomed in upon or leveled. A chromatic sensor (S5/03) with a white light source was used in this study. R_a indicates the average absolute distance of the roughness profile to the center plane of the profile.

X-ray Photoelectron Spectroscopy. The chemical compositions of the adhesive surfaces pre- and post-treatment with chlorhexidine and fluoride mouthrinses were determined by X-ray photoelectron spectroscopy (XPS) using an S-Probe spectrometer (Surface Science Instruments, Mountain View, CA) equipped with an aluminium anode (10 kV, 22 mA) and a quartz monochromator. The direction of the photoelectron collection angle (θ) was at an angle of 35° to the surface of the sample, and the electron flood gun was set at 10 eV. A survey scan was made with a 1000 $\times$ 250 μm^2 spot and a pass energy of 150 eV. Detailed scans of the C_{1s}, O_{1s}, and N_{1s} electron-binding energy peaks were obtained using a pass energy of 50 eV. Binding energies were determined by setting the binding energy of the C_{1s} component due to the carbon–carbon bond at 284.8 eV. The experimental peaks were integrated after nonlinear background subtraction and the peaks were decomposed assuming a Gaussian/Lorentzian ratio of 85/15 by using the SSI PC software package. The elemental surface compositions were expressed in atomic %, setting %C + %O + %N + %Si to 100%.

Early Biofilm Formation in the Parallel Plate Flow Chamber

Bacterial Strain, Culture Conditions, Harvesting, and Media. *S. sanguis* SK36, one of the primary colonizers in oral biofilm architecture, was used in this study. For each adhesion experiment, the organism was precultured from Columbia blood agar (Oxoid, Basingstoke, UK). Cultures were prepared by inoculating a fresh colony into 20 mL tryptone soy broth (TSB, Oxoid) containing 5% yeast extract (Oxoid) and incubating in 5% carbon dioxide atmosphere for 24 h at 30°C. Bacterial cells were harvested by centrifugation (10 min, 3000 rpm, 21°C) and washed twice with ultrapure water (Milli-Q Water Purification System, Millipore Corporation, Bedford, MA). Subsequently, optical density was measured (560 nm) with a Spectronic genesys (Spectronic instruments, USA) and the bacteria suspended in 0.85% saline (NaCl) to a final density of 1×10^8 cells mL^{-1}, as determined using a calibration curve. One milliliter of standardized inoculum was added to 500 mL of artificial saliva for each flow chamber experiment. A mucin-containing artificial saliva with a pH of 6.9 was used as the growth medium for the biofilm.[26] The composition per 500 mL of distilled water was 0.5 g of "lab-lemco" beef extract (Oxoid), 1 g of yeast extract (Oxoid), 2.5 g of proteose peptone (Oxoid), 1.25 g of mucin (Hog gastric) (Sigma, Poole, UK), 0.175 g of sodium chloride (Sigma), 0.1 g of calcium chloride (Sigma), 0.1 g potassium chloride (Sigma), and 0.313 mL of 40% urea (Sigma).

Parallel Plate Flow Chamber and Biofilm Formation. A parallel plate flow chamber (FC 71; BioSurface Technologies Corporation, Bozeman, MT) was employed, as described before in detail.[27] Adhesion studies were performed on substrata prior to and after treatment with a fluoride or chlorhexidine mouthrinse. All experiments were carried out at 37°C in duplicate, with separately cultured strains. Four experimental discs were placed in the flow chamber simultaneously. A peristaltic pump (Watson-Marlow, Falmouth, UK) was used to draw media through the chamber at a rate of 0.05 mL min^{-1}, corresponding to a shear rate of 11 s^{-1}, which is comparable to physiologically occurring shear rates in the human oral cavity.[28] A pulse damper was installed between the peristaltic pump and the flow chamber to compensate pulsing of the pump. Artificial saliva was pumped through the flow chamber for 5 min allowing conditioning film formation on the adhesive surfaces. Broth containing *S. sanguis* was then added into the artificial saliva and the experiment was allowed to proceed for 6 h. The culture was maintained at 37°C and stirred for the duration of each experiment.

Image Analysis. The flow chamber was positioned on the stage of a phase contrast microscope (Olympus BH-2) equipped with an ultra-long working distance objective (40×; Olympus ULWD-CD Plan 40 PL, Japan; producing a size of field 140 $\times$ 100 μm^2). The discs were removed at the end of each experiment from the flow chamber and stained with SYTO® 13 live-cell nucleic acid stain (Molecular probes Europe BV, Leiden, The Netherlands). A CCD camera (JVC TK-C1360B) was attached to the microscope and images were collected onto a computer using FlashBus MV-lite frame grabber. Five images per disc were captured at random. Image analysis was performed using ImageJ (National Institute of Health, Bethesda, MD). Deposited bacteria were first discriminated from the background by converting the images to 256 shades of gray (8-bit grayscale). The resulting grayscale images were then threshold into binary black and white images, which were subsequently stored on disk for later analysis. The grayscale cut-off point for each image was defined by the intensity levels of the original image, which was

TABLE II. Water Contact Angles (θ_w, Degrees), Percentage Elemental Surface Compositions, and Mean Surface Roughnesses (R_a, μm) of the Four Orthodontic Adhesives Used in This Study Prior to and After Fluoride (F^-) and Chlorhexidine (CHX) Treatment

Substratum	Water Contact Angle[a] (θ_w, degrees)	Elemental Surface Composition[b] %C	%O	%N	%Si	%F	%Cl	R_a[c] (μm)
Concise	97 ± 3.6	64.6	30.1	0.0	5.4	0.0	0.0	1.9 ± 1.0
Concise + F^-	64 ± 4.2	83.0	14.6	0.0	1.9	0.0	0.0	1.8 ± 0.8
Concise + CHX	88 ± 1.0	79.6	19.3	0.8	1.1	0.0	0.2	2.1 ± 0.9
Fuji ORTHO LC	121 ± 3.5	60.2	25.8	3.1	4.5	1.5	0.0	3.6 ± 1.6
Fuji ORTHO LC + F^-	59 ± 6.0	75.1	17.3	4.4	2.6	0.0	0.0	3.0 ± 1.9
Fuji ORTHO LC + CHX	52 ± 5.8	75.9	18.2	2.9	2.5	0.0	0.6	3.6 ± 1.2
Ketac Cem	83 ± 3.5	64.1	26.3	3.0	1.6	1.5	0.0	4.1 ± 1.6
Ketac Cem + F^-	46 ± 16.5	74.2	22.4	2.7	0.0	0.0	0.0	4.2 ± 1.5
Ketac Cem + CHX	68 ± 2.5	80.5	14.7	2.3	2.5	0.0	0.4	3.5 ± 2.1
Transbond	127 ± 1.6	80.7	15.4	2.0	2.8	1.1	0.0	6.4 ± 2.0
Transbond + F^-	50 ± 4.7	73.0	25.1	0.9	2.3	0.0	0.0	5.5 ± 1.9
Transbond + CHX	68 ± 8.5	74.1	20.6	1.0	4.0	0.0	0.4	5.9 ± 2.7

[a] ± denotes standard deviations over three contact angles measured on one adhesive surface.
[b] Because of heavy use of the extremely expensive XPS, only done in single-fold.
[c] ± denotes standard deviations over five surface roughness measurements done on one adhesive surface.

in turn influenced by the presence of cells in the captured image. The percentage of the surface in the field of view covered by biofilm was calculated by counting the pixels on threshold images to determine the proportion of pixels representing *S. sanguis* cells.

Statistical Analysis

To analyze differences in the contact angle measurements, adhesion numbers, and mean surface roughnesses of the adhesive surfaces, independent sample *t*-tests were performed using a significance level of 0.01. Backward multiple linear regression analysis was performed with SPSS for Windows (Version 12.0, SPSS, Chicago, IL) to identify the surface properties most predictive for biofilm formation prior to and after mouthrinse treatment. The percentage surface coverage (PSC) was used as a dependent variable, while the water contact angle, mean surface roughness, the percentage surface composition %C, %O, %N, %Si, %F, and %Cl were entered as explanatory variables. The absence or presence of surface fluorine and chlorine were entered as explanatory variables after binary-coding as 0 or 1. Variables were excluded when equal in both situations or if they correlated significantly with other variables, as determined with Pearson's correlation test.

RESULTS

Surface Physico-Chemical Properties of Adhesive Surfaces

Surface properties of the adhesives prior to and after fluoride and chlorhexidine treatment are presented in Table II. Water contact angles revealed the hydrophobic nature of the orthodontic adhesives used in this study (Transbond ≥ Fuji ORTHO LC > Concise ≥ Ketac Cem) and decreased for all adhesives after mouthrinse treatment. Transbond, Fuji ORTHO LC and Concise showed significant reductions ($p < 0.01$) in water contact angles after immersion in fluoride mouthrinse, whereas a 45% reduction in water contact angle of fluoride-treated Ketac Cem disc was found not statistically significant ($p > 0.01$). Significant reductions in hydrophobicities of the adhesive surfaces were also evident after chlorhexidine treatment ($p < 0.01$), except for Concise.

The changes in hydrophobicity observed were accompanied by changes in surface chemistry of the adhesive surfaces. From the single-fold XPS analysis, all four orthodontic adhesives contained carbon as a major constituent, and an increase in the amount of carbon was observed after exposure to mouthrinses (on average 14.5% after fluoride and 15.7% after chlorhexidine treatment) with the exception of Transbond. Transbond presented a higher surface oxygen content after exposure to both mouthrinses, whereas the other adhesives showed a lower amount of oxygen. The detection of silicon on all adhesive surfaces both before and after mouthrinse treatment (except for fluoride-treated Ketac Cem) confirmed the presence of silica-containing filler particles incorporated within the materials. No fluorine was detected by XPS in any adhesive surface after fluoride treatment. All adhesives had surface chlorine after chlorhexidine treatment.

The mean surface roughnesses (R_a) of the adhesive surfaces were in descending order: Transbond (6.4 ± 2.0 μm), Ketac Cem (4.1 ± 1.6 μm), Fuji ORTHO LC (3.6 ± 1.6 μm), and Concise (1.9 ± 1.0 μm). The use of fluoride and chlorhexidine mouthrinses did not significantly alter the surface roughness of the adhesives ($p > 0.01$).

Early Biofilm Formation

An image of *S. sanguis* SK36 biofilm captured by the CCD camera is presented in Figure 1(a), and the PSC by biofilm

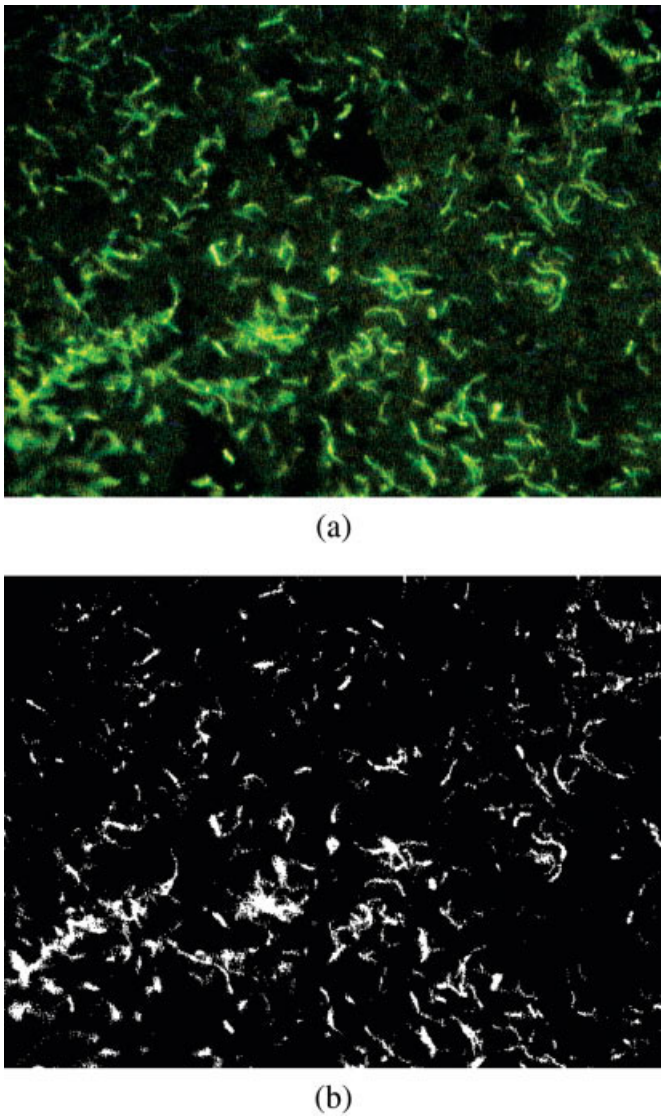

Figure 1. A representative image of *S. sanguis* biofilm formed on Transbond after (a) fluorescence staining with SYTO® 13 live-cell nucleic acid and (b) binary thresholding into black and white image ready for PSC calculation (×1000 magnification). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

was calculated by counting the pixels on the thresholded image in Figure 1(b). Table III summarizes the PSC by *S. sanguis* biofilms for the four adhesives prior to and after mouthrinse treatment. Concise had significantly lower PSC than Transbond ($p < 0.01$) prior to mouthrinse exposure, while biofilm formation was most extensive on the two most hydrophobic surfaces prior to mouthrinse treatment, that is, Transbond and Fuji ORTHO LC.

The use of the fluoride mouthrinse generally discouraged early biofilm formation on all adhesive surfaces, with Ketac Cem and Transbond reaching a level of statistical significance ($p < 0.01$). All adhesives treated with chlorhexidine mouthrinse revealed reduced biofilm formation, except Ketac Cem, but without reaching statistical significance ($p > 0.01$).

Multiple Linear Regression Analysis

Pearson's correlation test demonstrated a high correlation between %C and %O, which led to the removal of %C from backward multiple regression analysis. Furthermore, since %F was below the detection limit of XPS after fluoride and chlorhexidine treatment, it was also excluded from the analysis.

Table IV shows the standardized regression coefficients (β) and their significance values (p) after backward multiple linear regression analyses. Hydrophobicity together with the amount of oxygen and nitrogen present on the adhesive surfaces result as dominant factors that jointly accounted for 41% of the total variance (R^2) in PSC prior to and after exposure to fluoride and chlorhexidine mouthrinses. The set of β-coefficients suggests that after adjusting for the effects of other explanatory variables, hydrophobicity has the strongest effect on PSC by *S. sanguis* biofilms. Consequently, early *S. sanguis* biofilm formation on orthodontic adhesives can be described by

$$\mathrm{PSC} = -0.04 + 0.01 \times \theta_w(^\circ) - 0.02 \times \%\mathrm{oxygen} + 0.13 \times \%\mathrm{nitrogen}$$

Figure 2(a–c) provides a graphical display of the single regression relationships between the PSC and the three variables determined in multiple linear regression analysis to be influential on early biofilm formation. However, only hydrophobicity and %nitrogen correlated significantly ($p < 0.01$) to early biofilm formation, with a lack of correlation ($p > 0.01$) between biofilm formation and the level of oxygen on the adhesive surfaces.

TABLE III. Percentage Surface Coverage (PSC) of Early *S. sanguis* SK36 Biofilms on Orthodontic Adhesives Prior to and After Fluoride and Chlorhexidine Treatment

	Treatment[a]		
Substrata	Untreated	0.05% Sodium Fluoride	0.2% Chlorhexidine Gluconate
Concise	0.25 ± 0.16	0.12 ± 0.05	0.17 ± 0.10
Fuji ORTHO LC	1.17 ± 0.85	0.63 ± 0.16	0.42 ± 0.15
Ketac Cem	0.47 ± 0.25	0.11 ± 0.07	0.76 ± 0.14
Transbond	1.20 ± 0.82	0.15 ± 0.05	0.50 ± 0.14

[a] ± denotes standard deviations over duplicate runs, with separately cultured bacterial strains and different discs.

TABLE IV. Standardized Regression Coefficients (β) and *p*-Values After Backward Multiple Linear Regression Analyses

Variable	β	*p*
θ_w	0.489	0.000
R_a	–	–
%O	−0.193	0.036
%N	0.306	0.001
%Si	–	–
%Cl	–	–
R^2	41%	

The percentage surface coverage (PSC) of the adhesives prior to and after mouthrinse treatment by early *S. sanguis* biofilms was taken as the dependent variable and the water contact angle (θ_w), mean surface roughnesses (R_a), percentage surface composition %C, %O, %N, %F, and %Cl were taken as explanatory variables

DISCUSSION

Adsorbed components from the fluoride and chlorhexidine mouthrinses on surfaces of orthodontic adhesives evaluated in this study changed the elemental surface composition and hydrophobicity of the adhesive surfaces. The water contact angles of the mouthrinse-treated surfaces exhibited convergence to an intermediate hydrophobicity (average water contact angle = 62°± 14°) as compared with their untreated counterparts (average water contact angle = 107°± 19°). Heterogeneous dissolved organic carbon compounds in the mouthrinses, for example, ethanol, sorbitol, hydrogenated castor oil, and peppermint oil, can generate organic films of different properties, which individually influences biofilm formation in a different way. Also, the surface properties of the underlying adhesive dictate selective adsorption[29] and conformational changes of adsorbed macromolecules.[30]

Considering the multitude of physico-chemical changes occurring at the adhesives surfaces associated with the application of mouthrinses, it becomes unlikely that a single surface characteristic could, on its own, account for biofilm formation. As a virtue of this study, employing a number of different experimental techniques, the multiple regression analyses indicate that biofilm formation on orthodontic adhesives is a multifactorial process and analysis in terms of single factors (Figure 2) merely yields scatter plots. Therefore, the use of a multiple linear regression analysis is a powerful and suitable statistical tool to unravel the complex interplay among many explanatory variables acting on a dependent variable, as elaborated in this study.

Multiple linear regression analysis revealed that hydrophobicity became the main determinant for *S. sanguis* biofilm formation prior to and after mouthrinse treatment, as evidenced by the high standardized coefficient. The use of fluoride and chlorhexidine mouthrinses made the hydrophobicity of orthodontic adhesives less hydrophobic and accordingly the adhesive surfaces attracted less *S. sanguis* biofilm. Topical application of fluoride solutions has been proven to reduce the surface energy of enamel, making bacterial colonization unfavorable. This effect, along with the well-substantiated biological action of fluorides, may further support the role of surface physico-chemical properties on oral microbial attachment.[31]

S. sanguis is one of the primary colonizers of human tooth surfaces, abundantly present in dental plaque and possessing a hydrophobic cell surface (water contact angle 108°, unpublished). The result from the present study is in line with the general notion that bacteria with hydrophobic surface properties prefer hydrophobic material surfaces and those with hydrophilic surface properties prefer hydrophilic surfaces.[18,20,32] It also agrees with the thermodynamic effects described in an *in vivo* study on the influence of surface free energy of substrata with respect to supragingival plaque formation.[22]

Several *in vivo* studies[25,33] indicated that increasing surface roughness could lead to more extensive bacterial adhesion and retention, possibly due to the protective and sheltering role of rough surfaces against shear forces and even against oral hygiene measures.[18] The role of surface roughness in biofilm formation was insignificant in our study as determined by the multiple linear regression analyses, which is in agreement with observations that surface roughness played a less important role in bacterial colonization on various orthodontic bonding agents.[21] Although the disc surfaces were left unpolished in the present study to mimic the clinical situation, they were formed against glass surfaces, which were inevitably smoother than the orthodontic adhesives used *in vivo*.

The exposure of the adhesive surfaces to sodium fluoride and chlorhexidine mouthrinses resulted in the loss of detection of fluorine and other minor elements from the adhesive surfaces. Fluoride release from resin-modified glass ionomer cement is substantially lower than from other types of glass ionomer cements. This may be associated with the higher resistance to dissolution at the initial setting stage demonstrated by the resin-modified glass ionomer cements.[34]

In addition, the possibility that these elements including fluoride were eluted from the surfaces cannot be ruled out, although the deposition of a film blocking their detection is another likely explanation.[16] It can easily be envisaged and it is in line with the XPS observation that such a film develops as a result of adsorption of carbonaceous components like ethanol, sorbitol, hydrogenated castor oil, peppermint oil, and methyl salicylate from the solutions. Because the photoelectrons in XPS are strongly attenuated by passage through the sample material itself, the information obtained comes from a sampling depth reaching up to 15 nm or less under the conditions applied in this study.

The nature of the conditioning film on substratum surfaces is an important factor affecting early biofilm formation. Along with changes in chemical composition of a substratum surface after conditioning film formation, physico-chemical properties of the surface such as its hydrophobicity, roughness, charge, and elasticity may alter.[35] The surface irregularities present on the adhesive surfaces may be masked and leveled by the conditioning film and hence the protective role of the rough surface against shear forces present in the flow chamber could be eliminated. In contrast, the conditioning

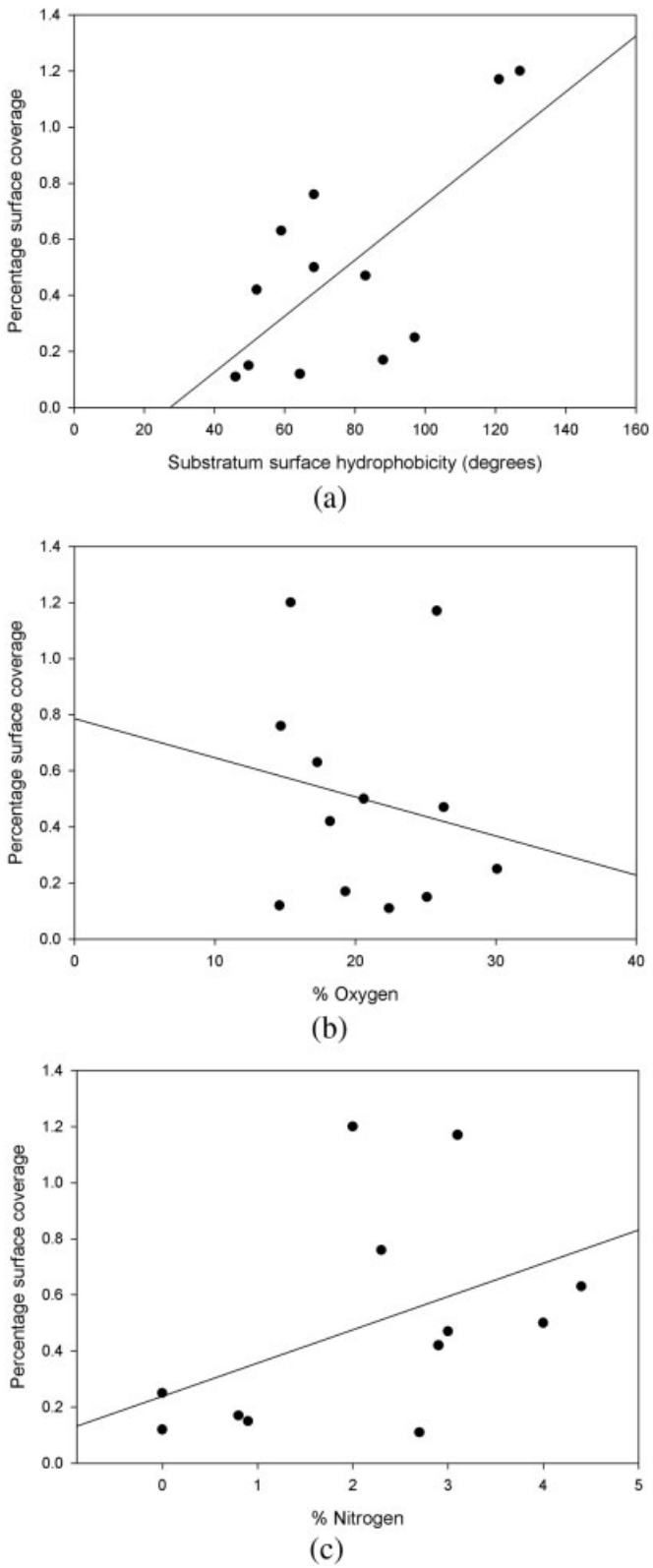

Figure 2. Single linear regression analyses demonstrating the PSC of *S. sanguis* SK36 as a function of (a) surface hydrophobicity, measured by water contact angle (θ_w), (b) percentage oxygen (%O), and (c) percentage nitrogen (%N) on the surface of orthodontic adhesives prior to and after exposure to a fluoride and chlorhexidine containing mouthrinse for 1 min. The 12 data points are deduced from data on the four different adhesive surfaces prior to and after treatment with fluoride and chlorhexidine mouthrinse. Lines indicate the best fit in a single-parameter linear regression model.

film has been shown to reduce the fluoride transport out of glass ionomer cements,[36] as a possible reason for high bacterial adherence on Fuji Ortho. Future research should be carried out to understand how the multiple changes brought about by the adsorption of a conditioning film impact bacterial adhesion and subsequent biofilm formation on orthodontic adhesives, with particular focus on interaction of conditioning film and antibacterial therapy.

In summary, this study demonstrated that early biofilm formation on orthodontic adhesives is a multi-factorial phenomenon. The use of a multivariate analysis revealed that antibacterial mouthrinse treatment had an impact on early biofilm formation on these adhesive surfaces, which were due to changes in hydrophobicity and the amount of oxygen and nitrogen present on these surfaces.

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