Temporal dynamics of adhesion of oral bacteria to orthodontic appliances

Radović, Renea; Begić, Gabrijela; Blagojević Lučić, Sanja; Karleuša, Ljerka; Špalj, Stjepan; Gobin, Ivana

Source / Izvornik: **Dental Materials Journal, 2023, 42, 469 - 477**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.4012/dmj.2022-235

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:721801

Dental Materials Journal 2023; 42(4): 469–477

Temporal dynamics of adhesion of oral bacteria to orthodontic appliances

Renea RADOVIC[1]*, Gabrijela BEGIC[2]*, Sanja LUCIC BLAGOJEVIC[3], Ljerka KARLEUSA[4], Stjepan SPALJ[1,5] and Ivana GOBIN[2]

[1] *Department of Orthodontics, Faculty of Dental Medicine, University of Rijeka, Kresimirova 40, 51000 Rijeka, Croatia*
[2] *Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Brace Branchetta 20, 51000 Rijeka, Croatia*
[3] *Department of Surface Engineering of Polymer Materials, Faculty of Chemical Engineering and Technology, University of Zagreb, Savska 16, 10000 Zagreb, Croatia*
[4] *Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Brace Banchetta 20, 51000 Rijeka, Croatia*
[5] *Department of Dental Medicine, Faculty of Dental Medicine and Health, J. J. Strossmayer University of Osijek, Crkvena 21, 31000 Osijek, Croatia*
Corresponding author, Stjepan SPALJ; E-mail: stjepan.spalj@fdmri.uniri.hr

Adhesion of the most common dental biofilm bacteria to alloys used in orthodontics in relation to surface characteristics was analyzed. *Streptococcus mutans* (*S. mutans*), *Streptococcus oralis* (*S. oralis*), *Veillonella parvula* (*V. parvula*), and *Aggregatibacter actinomycetemcomitans* (*A. actynomicetemcomitans*) were incubated for 4 h with nickel-titanium (NiTi) and stainless-steel (SS) wires. The surface roughness and free energy of the alloys, as well as the hydrophobicity of the alloys and bacteria, were assessed. NiTi had higher surface free energy and rougher ($p<0.001$) and more hydrophilic surfaces than SS ($p<0.001$). The hydrophobic properties of the bacteria decreased in the following order: *V. parvula*>*S. oralis*>*S. mutans*>*A. actynomicetemcomitans*. Bacterial adhesion generally increased over time, though this pattern was influenced by the type of alloy and the bacteria present ($p<0.001$). In a multiple linear regression, the principal predictor of adhesion was bacterial hydrophobicity ($p<0.001$), followed by time ($p<0.001$); alloy surface characteristics had a low influence.

Keywords: Oral bacteria, Nickel-titanium, Orthodontics, Stainless steel

INTRODUCTION

Numerous bacterial species in the oral cavity colonize available surfaces, multiply, and form the dental biofilm[1)]. This is how species such as streptococci survive, instead of simply being swallowed[2)]. Orthodontic appliances provide new surfaces suitable for the colonization by oral bacteria and the formation of biofilm, which can cause challenges in oral hygiene and result in enamel demineralization during orthodontic treatment[3-5)]. The most commonly used alloys for orthodontic archwires are nickel-titanium (NiTi) and stainless steel (SS)[6,7)].

Adhesion is the initial step in biofilm formation. It is a complex process that is regulated by bacteria- or substrate-specific factors. Planktonic bacteria approach the surface under the influence of gravitational forces, Brownian motion, and possibly extracellular additives that support bacterial movement[8-10)]. This is followed by an initial reversible attachment and then a transition to irreversible adhesion. The surface of the substrate and the surface of the bacterium are attracted to each other by non-specific forces. As assumed in the extended Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, adhesion is the result of long-range forces (Lifshitz—van der Waals forces), electrostatic interaction forces, and short-range Lewis acid-base interactions, which may be attractive or repulsive[10-12)]. Based on this theory, the characteristics of the material, such as the surface free energy (SFE), hydrophobicity/hydrophilicity, roughness, and topography of the surface, affect bacterial adhesion[1,13-16)].

The total force, that is, the sum of the polar and non-polar components acting on the molecules on the surface, is defined as the SFE. It is calculated from the measured values of the static contact angles of different fluids[10,17,18)]. The contact angle of a water droplet (WCA) on the surface reflects the surface wettability, which correlates with the hydrophobicity of the surface. The smaller the contact angle, the better the wetting of the surface, and vice versa[19)]. Hydrophobic surfaces have an internal WCA greater than 90°, and hydrophilic surfaces have an internal WCA less than 90°. The hydrophobicity of the substrate affects bacterial adhesion. Highly hydrophobic or hydrophilic surfaces are not conducive to bacterial adhesion[10,20)]. However, previous studies have varied in this regard; some concluded that hydrophobicity favours adhesion while others concluded that no correlation was found[21-23)].

In addition, surface roughness and topography at the micrometre and nanometre levels significantly contribute to bacterial adhesion. Roughness can modify the chemistry of a surface by altering its hydrophobicity[10,15,16,20)]. Various studies have reported conflicting results; some suggested that increasing surface roughness increased the adhesion of microorganisms, while others reported that surface roughness has no significant effect[3,4,13,24,25)].

In this study, we analyzed the temporal adhesion of oral bacteria commonly found in the dental biofilm including *Streptococcus mutans* (*S. mutans*), *Streptococcus oralis* (*S. oralis*), *Aggregatibacter actinomycetemcomitans* (*A. actynomicetemcomitans*) and *Veilonella parvula* (*V. parvula*), to orthodontic

*These authors contributed equally to this work.

Received Oct 24, 2022: Accepted Jan 6, 2023
doi:10.4012/dmj.2022-235 JOI JST.JSTAGE/dmj/2022-235

archwires made of NiTi and SS. The adhesion of bacteria to solid surfaces is a complex process regulated by the properties of the substrate, environment, and bacterial cells[8]. Therefore, in addition to evaluating the surface characteristics of orthodontic archwires, we assessed the Lewis acid-base properties and hydrophilic/hydrophobic nature of bacterial surfaces by microbial adhesion to solvents (MATS), which was developed by Bellon-Fontaine *et al.*[26]. Furthermore, we examined the SFE of bacterial cells previously discussed[27]. Observing several factors that regulate adhesion can explain the conflicting results in previous studies that focused only on the substrate or a particular bacterial species. We hypothesized that adhesion to the tested materials depended on the interaction of the surface characteristics of the material and the bacterial cell itself. We expected streptococci to have faster adherence than other bacteria and better adherence to NiTi than SS, which is mostly influenced by surface roughness.

MATERIALS AND METHODS

Orthodontic alloys

Sterile orthodontic archwires made of 0.018×0.025″ NiTi (Neo Sentalloy, Dentsply Sirona, Charlotte, USA) and 0.019×0.025″ SS (Stainless Steel, American Orthodontics, Sheboygan, WI, USA) were used.

Atomic force microscopy (AFM)

The morphology of the wire surface was examined quantitatively on five samples by AFM on a NT-MDT Solver Pro AFM device (NT-MDT, Moscow, Russia). The end of each wire was cut to a length of 5 mm and glued to a metal sample holder with quick-setting glue. All samples were purified with 95% ethanol prior to analysis. The recording range was 15×15 μm (3 NSC36 probes without peak, resonant frequency 65 kHz, constant force 0.6 N/m). Each sample was collected from several locations. Subsequently, the average roughness (Ra) was estimated.

Scanning electron microscopy (SEM)

For qualitative analysis of the surface, the JSM-7800F scanning electron microscope (JEOL, Tokyo, Japan) was used by applying a secondary electron detector with an electron beam acceleration voltage of 10 kV and at a working distance of 10 mm. A 4,000× magnification was used.

SFE

The experiment was performed using an OCA 20 goniometer (Data Physics Instruments, Filderstadt, Germany). The contact angle was determined by the sessile drop method. The instrument was equipped with an automatic drop dosing system and software for image analysis and determination of the contact angle between the test liquid droplet and test material. The test liquids were water and diiodomethane (99+%, Acros Organics, Geel, Belgium). Prior to the measurements, the wire surface was cleaned with 95% ethanol (Kemika, Zagreb, Croatia) and allowed to dry. Then, 0.3 μL of the test liquid was applied. Ten measurements per alloy type were performed at a consistent temperature of 25°C. The dispersive and polar components and total SFE of the probe liquids used for the SFE calculation of alloys were as follows: 21.8 (dispersive), 51.0 (polar), and 72.8 (total) for water and 50.8, 0.0 and 50.8 mJm^{-2} for diiodomethane. The free surface energy was determined indirectly by measuring the contact angles of the two liquids and calculated using mathematical models by Owens-Wendt and Wu[28,29].

Bacterial strains and inoculum preparation

Streptococcus oralis ATCC 6249, *Streptococcus mutans* ATCC 25175, *Veillonella parvula* ATCC 10790 and *Aggregatibacter actinomycetemcomitans* ATCC 29522 10790 (Microbiologics, St Cloud, MN, USA) were used to test alloy adhesion. *S. oralis* and *S. mutans* were grown on Mutans-Sanguis (MS) agar, and *V. parvula* and *A. actinomycetemcomitans* were grown on blood agar (Biolife, Milan, Italy) with the addition of 5% sheep blood (Biognost, Zagreb, Croatia) under anaerobic conditions at 37°C for 24–48 h. Bacterial cultures were resuspended in brain heart infusion (BHI) (Becton, Dickinson and Company, Sparks, MD, USA) medium supplemented with 5.0 mg/mL haemin (Sigma-Aldrich, Burlington, MA, USA) and 1.0 mg/mL management (Merck, Darmstadt, Germany). After culturing under anaerobic conditions until the early stationary phase, a bacterial suspension of 10^7 colony-forming units (CFU)/mL was prepared by measuring the optical density at 600 nm (OD600).

Bacterial adhesion to orthodontic wires

Sterile wires were placed in the individual wells of 24 well microtiter plates and incubated in artificial saliva for 4 h at 30°C in 50% artificial saliva, as described in the literature previously[30]. The wires were transferred to new wells, and 200 μL of each individual bacterial suspension was added, followed by incubation for 1, 2, 3 or 4 h at 35°C. After incubation, the wires were rinsed thrice with sterile saline to remove non-adherent bacteria, and the adherent bacteria were peeled off by treatment in a BactoSonic ultrasonic bath (Bandelin, Berlin, Germany) at 40 kHz for 1 min. Ten-fold dilutions were seeded on the appropriate media (MS agar and blood agar) to determine CFU/mL to quantify the adherent bacteria.

Fluorescence microscopy visualization of adherent bacteria on archwires

Adhesion of the tested bacteria to the NiTi and SS archwires for a 4-h period was prepared as previously described. This was followed by staining with the LIVE/DEAD BacLight Bacterial Viability Kit L-7012 (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. SYTO 9 stains all cells a green fluorescent colour, while propidium iodide (PI) stains cells with a damaged membrane red. Adhered bacteria were visualized by epifluorescence microscopy

with filters of GFP/FITC (excitation: 480 nm and emission: 500 nm) and rhodamine (excitation: 490 nm and emission: 635 nm).

MATS

MATS assay is a technique used to measure the surface hydrophobicity and acid-base properties of bacteria. The affinity of the bacterial cells to three solvents was tested: chloroform (an acidic solvent), diethyl ether (a basic solvent), and hexane (a non-polar solvent). An 18–20 h bacterial culture was prepared in a protein-rich BHI medium. The bacterial suspension was centrifuged for 10 min at 7,000× g and 4°C, washed 2×, and resuspended in sterile saline to a concentration of 10^8 CFU/mL. Then 0.2 mL of the test solvent was added to 1.2 mL of the bacterial suspension, which was shaken vigorously for 2 min to form an emulsion. Phase separation was achieved after 15 min of rest, after which the optical density at 600 nm (OD600) was measured in a sample of the separated aqueous phase. The adhesion percentage, that is the affinity of microorganisms to the solvents, was calculated as follows: adherence (%)=$[1-A/A_0]\times100$, where A_0 and A are the optical densities of the bacterial suspension before and after mixing with solvent, respectively[26]. All measurements were performed in triplicates.

Statistical analysis

The surface characteristics were compared using the *t*-test. The degree of adhesion was expressed as the logarithmic value (per base 10) of CFU/mL. A factor analysis of variance (ANOVA) was used to analyze the interaction of time, alloy and bacterial type on adhesion. ANOVA with the Student-Newman-Keuls *post-hoc* test was used to compare adhesion between the four time periods and between the four bacteria. The correlation of adhesion with time, surface characteristics of the alloy, and hydrophobicity of bacteria were analyzed with the Pearson correlation. Predictors of bacterial adhesion were analyzed using multiple regression analysis. The effect size was quantified using the formula $r=\sqrt{(t^2/(t^2+df))}$ for the t-test, and η^2 for ANOVA. The following criteria were used for interpretation: r=0.1–0.3, small effect size; 0.3–0.5, medium; 0.5–0.7, large; and >0.7 very large; squared values were used for the interpretation of η^2.

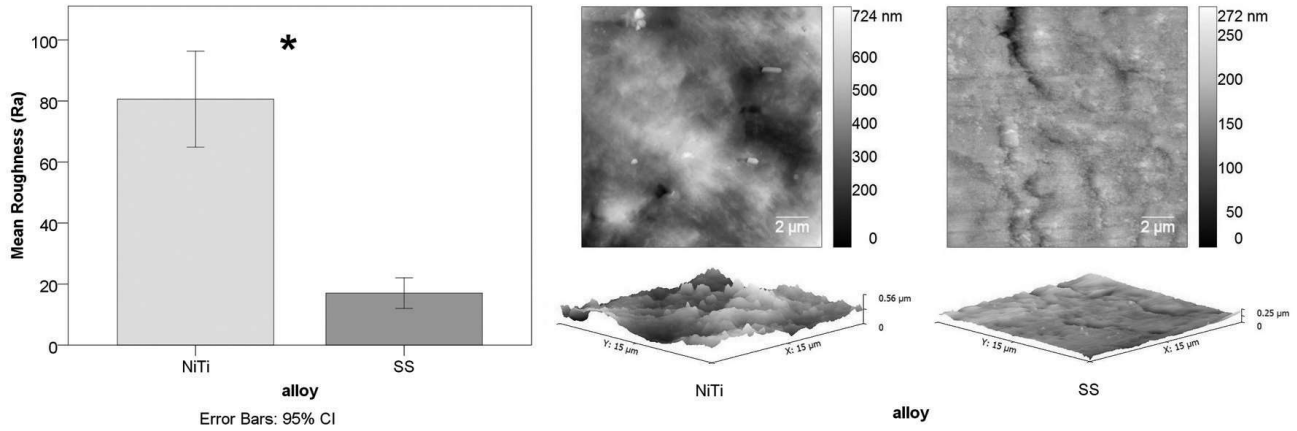

Fig. 1 Comparison of average roughness (Ra) between NiTi and SS alloy with representative AFM topography images (15×15 µm).
*Statistically significant difference

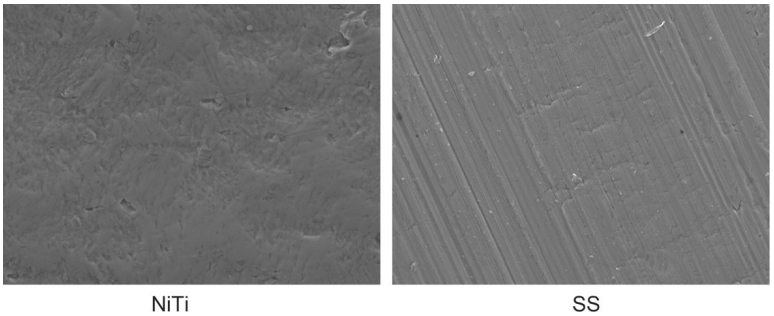

Fig. 2 Representative scanning electron micrographs (×4,000) of the NiTi and SS archwires.
Length of the measuring bar=0.001 mm

All analyses were performed using commercial software SPSS 22 (IBM SPSS, version 22.0, IBM, Armonk, NY, USA).

RESULTS

Surface roughness and topography

1. Microscopic surface roughness by AFM

NiTi archwires had a significantly rougher surface than SS (Ra 80.6±12.7 *vs.* 17.0±4.0; $p<0.001$; r=0.997; Fig. 1).

SEM analysis

Scanning electronic micrographs of the NiTi archwires showed more defects and an increased level of porosity when compared to those of the SS wires (Fig. 2). Various patterns of irregularities were observed, including shallow pores, depressions, and slight elevation. In contrast, the SS wires had clear horizontal scratches parallel to the axis of the wire, likely due to the production process.

SFE parameters

The WCA, a measure of surface hydrophobicity, demonstrated that both materials exhibited hydrophilic characteristics. However, NiTi is more hydrophilic, having less WCA than SS (49.7±2.5° *vs.* 71.8±1.2°;

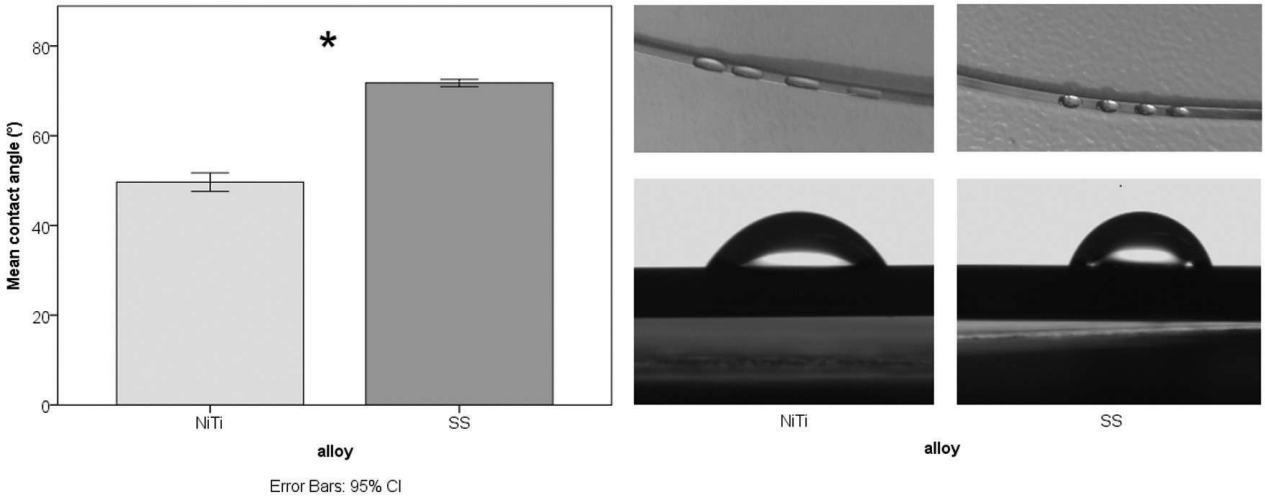

Fig. 3 Comparison of WCA between NiTi and SS archwires.
*Statistically significant difference

Table 1 Total surface free energies (γtot) and surface free energy components (dispersive γd; polar γp) of the wires calculated using the Owens-Wendt and Wu model

Alloy	Surface free energy based on Owens-Wendt method			Surface free energy based on Wu method		
	γd (mJm^{-2})	γp (mJm^{-2})	γtot (mJm^{-2})	γd (mJm^{-2})	γp (mJm^{-2})	γtot (mJm^{-2})
NiTi	30.7	22.8	53.5	32.3	25.4	57.7
SS	34.4	8.2	42.6	35.5	13.1	48.6

Table 2 Microbial adhesion to solvents test to bacterial cells cultured in BHI (bacterial adhesion to hexane, chloroform and diethyl ether)

Bacteria	Solvent		
	Chloroform bacterial adhesion (%)	Hexane bacterial adhesion (%)	Diethyl ether bacterial adhesion (%)
S. mutans	42	48	21
S. oralis	70	58	23.5
A. actynomicetemcomitans	24	0	20
V. parvula	100	100	0

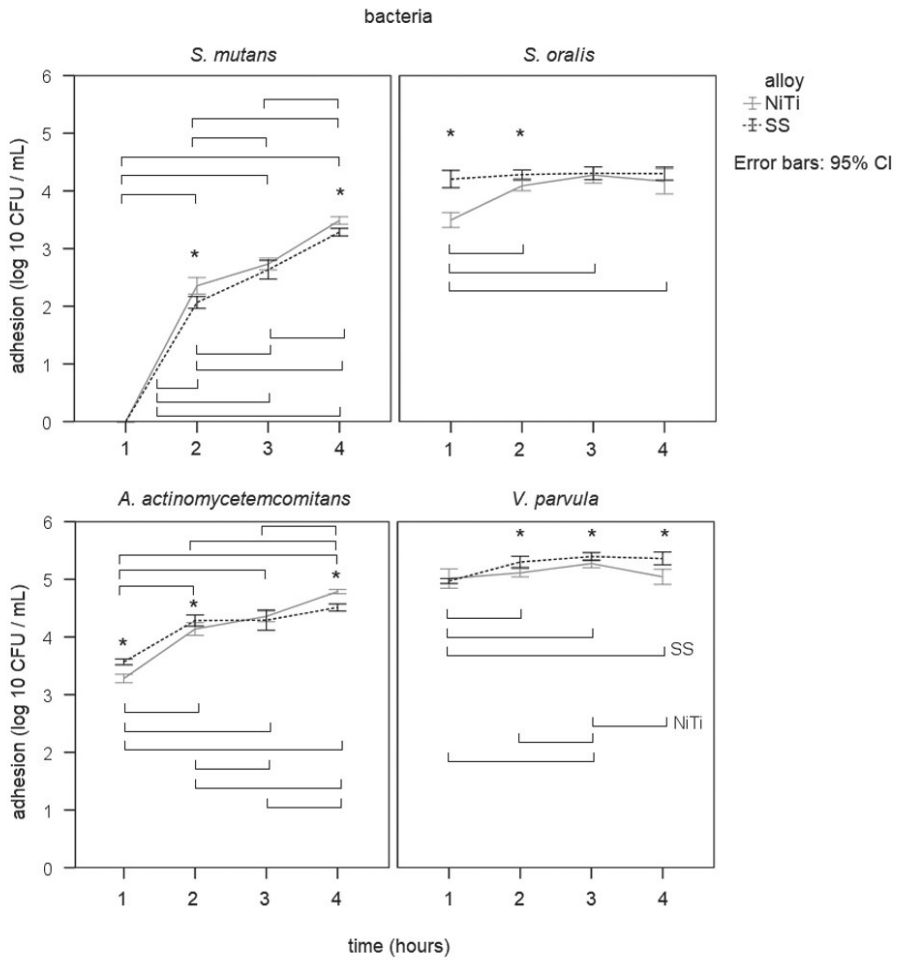

Fig. 4 Comparison of adhesion of bacteria *S. mutans*, *S. oralis*, *V. parvula* and *A. actinomycetemcomitans* to alloys over 4 h.
*Time points where there were significant differences in adhesion between alloys. Results are shown as mean values.

p<0.001; r=0.973; Fig. 3). The contact angle of diiodomethane was lower for SS than for NiTi (49.8±1.4° *vs*. 56.4±2.3°; p<0.001; r=0.869).

The values of the polar component for the SS wires were lower than those for NiTi, resulting in lower values of the total SFE of the SS wires (Table 1). Higher polarity and SFE values suggest stronger bacterial adhesion; however, it should be noted that the difference between the SFE of the bacteria and the surface is inversely proportional to bacterial adhesion[28,31].

Microbial adhesion

1. MATS

V. parvula demonstrated a high affinity for a non-polar solvent (hexane), indicating extremely hydrophobic properties. *S. oralis* and *S. mutans* were found to be moderately hydrophobic and *A. actynomicetemcomitans* demonstrated hydrophilic properties (Table 2).

Microbial adhesion to orthodontic archwires

The results are shown in Fig. 4. A three-factor ANOVA indicated a significant interaction of bacteria, time and alloy on adhesion, with a moderate effect size (p<0.001; η2=0.158). A two-factor ANOVA showed that the interaction between time and alloy was present in *A. actinomycetemcomitans* (p<0.001; η2=0.369), *S. oralis* (p<0.001; η2=0.273), and *V. parvula* (p=0.005; η2=0.068), but not in *S. mutans*. In addition, interactions between bacteria and time were found in NiTi (p<0.001; η2=0.754) and SS (p=0.005; η2=0.708), with a smaller effect size in SS. Further analysis of the results demonstrated, the dependence of adherence on time, type of alloy, and type of bacteria.

A two-factor ANOVA showed that the alloy-bacterial interaction was present after the first (p<0.001; η2=0.342), second (p<0.001; η2=0.210), and fourth hours (p=0.005; η2=0.286), but not after the third hour. Adherence depended on the alloy, but not equally in all bacteria and in all time periods. The asterisks in Fig. 4 indicate the

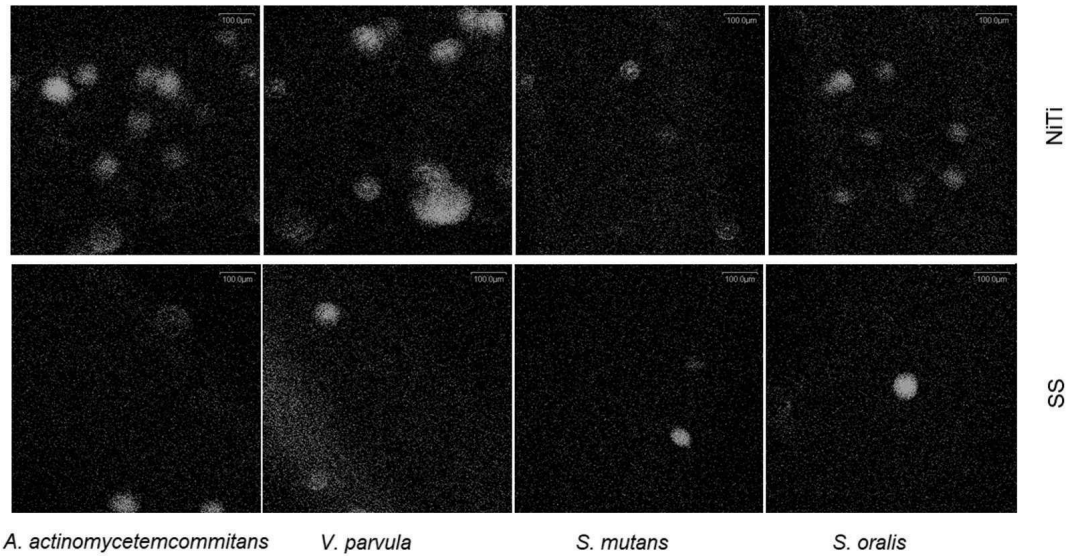

Fig. 5 Representative images of bacteria adhering within 4 h to NiTi and SS wires.
Biofilms were stained with the LIVE/DEAD Sustainability Kit (FilmTracer™ LIVE/DEAD® Biofilm Viability Kit, Invitrogen). Green cells represent viable, live bacteria, while red cells represent dead bacteria.

time points at which there were significant differences in the adhesion between the alloys. *S. oralis* and *V. parvula* adhered more strongly to SS, whereas *S. mutans* and *A. actinomycetemcomitans* adhered more strongly to the NiTi alloy. *S. oralis* demonstrated significantly higher adhesion to SS in the first 2 h ($p \leq 0.002$; r=0.534 and 0.796), while *V. parvula* demonstrated higher adhesion to SS after 2 h ($p \leq 0.029$; r=0.256–0.519). *A. actinomycetemcomitans* demonstrated higher adhesion to SS in the first 2 h; however, after 3 h, it demonstrated higher adhesion to NiTi ($p \leq 0.037$; r=0.428–0.838). *S. mutans* demonstrated significantly higher adhesion to NiTi after 2 and 4 h ($p \leq 0.002$; r=0.680 and 0.686, respectively).

Adhesion depended on the bacterial species, with equal effect sizes for each period and on both alloys ($p<0.001$; $\eta 2$=0.893–0.974). *V. parvula* had the highest adhesion to both NiTi and steel, and *S. mutans* had the lowest adhesion at all four time intervals. *S. oralis* and *A. actinomycetemcomitans* exhibited similar adhesion dynamics. Significant differences were found in the initial adhesion, after the first hour, when *S. oralis* adhered more strongly to SS and the fourth hour, when *A. actinomycetemcomitans* adhered more strongly to NiTi. Adhesion of *S. mutans* took more than an hour, after which adhesion increased while *S. oralis* and *V. parvula* adhered significantly in the first hour. *A. actinomycetemcomitans* showed intense adhesion during the first 2 h and between the third and fourth hours.

Increased bacterial adhesion to the alloys was noted with prolonged exposure, but not equally in all bacteria or to both alloys. *V. parvula* and *S. oralis* demonstrated higher adhesion at the start, followed by a slow rate of increase. *S. mutans* and *A. actinomycetemcomitans* demonstrated lower adhesion at the start, but a higher rate of increase with time. In *S. oralis*, there was a significant increase in adhesion in the NiTi wires, primarily 2 h after exposure ($p<0.001$; $\eta 2$=0.674), whereas in the SS wires, there was no significant increase between the first and fourth hours. In *S. mutans*, the increase was significant at each time point, and the increase was the most significant after 2 h on both alloys ($p<0.001$; $\eta 2$=0.981 for SS and $\eta 2$=0.974 for NiTi). In *V. parvula*, a significant increase was recorded after 2 h in SS, whereas in NiTi, a significant increase was only recorded after 3 h, and then actually decreased ($p \leq 0.007$; $\eta 2$=0.376 for NiTi and $\eta 2$=0.142 for SS). In *A. actinomycetemcomitans*, adhesion increased significantly on NiTi for 4 h, while on SS there was no significant increase between 2 and 3 h ($p<0.001$; $\eta 2$=0.954 for NiTi and $\eta 2$=0.828 for SS).

Initial adhesion (at 1 h) was related to the hydrophobicity of the bacteria (adhesion to hexane and chloroform, r=0.850 each; diethyl ether, r=–0.629; all $p<0.001$). Overall adhesion was also related to bacterial hydrophobicity (adhesion to hexane, r=0.496; chloroform, r=0.623; diethyl ether, r=–0.682; all $p<0.001$). The multiple regression predictors of bacterial adhesion were primarily bacterial hydrophobicity, followed by time; the least predictive factor was the surface characteristics of the alloy. The model with bacterial adhesion to hexane, time, and alloy characteristics explained 29% of the variance (R=0.542; R^2=0.294; adjusted R^2=0.289; $p<0.001$). An increase of bacterial adhesion to hexane (with a higher hydrophobicity) by one percentage point increased bacterial adhesion to the alloy by 0.01 CFU/mL ($p<0.001$) and an increase of exposure by 1 h increased adhesion by 0.19 CFU/mL ($p<0.001$). The surface

characteristics of the alloy did not have a significant effect on bacterial adhesion. The unique contribution of bacterial hydrophobicity adhesion variability was 25%; the contribution of time to adhesion variability was 4%. Similar results were obtained when bacterial adhesion to chloroform and diethyl ether were used in the model.

Fluorescence microscopy visualization of adherent bacteria on archwires

Bacteria that adhered to NiTi and SS within 4 h were visualized using fluorescent LIVE/DEAD staining (Fig. 5). The bacterial cells were stained with SYTO 9 green fluorescent dye, indicating their viability. Red-stained cells were not observed, indicating that the bacterial membrane was preserved, which prevented PI dye penetration of the cell.

DISCUSSION

This study analyzed several factors influencing the adhesion of oral bacteria to alloys used in orthodontics and revealed that adhesion is mostly influenced by bacterial characteristics, not the surface of the alloy.

We found that of the four bacterial species examined, *S. mutans* and *A. actynomicetemcomitans* adhered better to the NiTI wires. *A. actynomicetemcomitans* did not adhere in first 2 h, while *S. mutans* was detected from the second hour onwards. *V. parvula* and *S. oralis* adhered to SS wires in large numbers, though *S. oralis* adhered from start and *V. parvula* only from the second hour. NiTi wires were found to have a rougher surface, higher SFE, lower contact angle with water, and are increased hydrophilicity when compared to SS wires. According to previous studies, these characteristics would suggest stronger adherence of the bacteria to NiTi wires[4,15,21,32]. The adhesion of *S. mutans* is in line with these expectations and results to date. However, it is important to note that *S. mutans* is a cariogenic bacterium and a major oral pathogen; therefore, so it is understandable that it is frequently used in research on dental materials. The results of such studies may suggest that SS orthodontic wires are less susceptible to bacteria adhesion and thus, from a microbiological point of view, more desirable than NiTi wires[33-35]. However, adhesion is defined by various parameters, including surface-specific characteristics and the biophysical and biochemical properties of the bacteria themselves. Therefore, different results are often obtained by changing some of the research conditions[36]. In our study, *V. parvula* showed significantly stronger adhesion to SS wires despite their antiadhesive properties. Although not statistically significant, the same trend was observed with *S. oralis*. It is obvious that some of their characteristics contributed to this, and we clarify the differences below. The results of bacterial adhesion to hexanes showed that these species were strongly hydrophobic, whereas *S. mutans* was moderately hydrophobic, and *A. actinomycetemcomitans* was hydrophilic. The hydrophobicity of bacterial cells is known to favour their adhesion to surfaces[37]. This would explain why *V. parvula* and *S. oralis,* with the most hydrophilic properties, demonstrated adhesion primarily in the first hour.

Although hydrophobic cells adhere more strongly to hydrophobic surfaces, the heterogeneity of the bacterial surface under various environmental factors and adhesion forces should be considered. Bacterial wall deformation stimulates bacterial phenotypic responses that may also manifest as changes in hydrophobicity[38,39]. In our study, the hydrophobic species adhered more strongly to the less hydrophilic SS wires. In addition, with the exception of *A. actinomycetemcomitans*, bacterial hydrophobicity correlated with adhesion strength in general, with *V. parvula* demonstrating the strongest adherence to both materials and *S. mutans* the weakest. In addition, it is often stated that solid surfaces with high SFE are conducive to adhesion and biofilm formation[1,40]. However, when comparing the free energies of the interacting surfaces, we found that lower free adhesion energy, which is the difference between the SFE of the bacterial cells and solid substrate, indicated a higher degree of adhesion. Because the same medium was used for all species, SFE was constant and thus does not need to be considered[11,31]. In a previous study, we reported the SFE values of various bacteria[27]. When we compared these values with the SFE values of the NiTi and SS wires used in this study, we observed certain correlations. The largest difference was observed between the SFE wires and *S. mutans*, which is consistent with its low adhesion to both test materials. *S. oralis* and *V. parvula* demonstrated equal differences, but the difference was smaller compared to SS wires, to which these species adhered in higher amounts. The smallest difference was noted for *A. actinomycetemcomitans,* which demonstrated strong adhesion to both the materials. A slightly lower degree of adhesion compared to *V. parvula* can be explained by the fact that a laboratory strain with smooth colonial morphology was used in this study. It has been previously shown that in such strains, there is a lack of expression of the outer membrane proteins, which are related to adhesion[41]. We believe that this is why *V. parvula* and *S. mutans* were more sensitive to the surface characteristics of the material, which results in stronger adhesion to the NiTi wires.

Thus, the present study demonstrated that the interaction between bacterial surface and dental materials must be understood when modifying materials to decrease bacterial adhesion. However, the results of this study cannot be applied to all bacterial strains in dental biofilm because of their biophysical and biochemical diversity as well as their interaction, resulting in different adhesion mechanisms. In addition, present study demonstrated that the initial colonizers are the essential link in biofilm formation, and they are important in research of application of new materials.

This is in line with previous studies that suggest that initial colonizers, which are in direct contact with the surface, stimulate phenotypic responses that are transmitted to other inhabitants of the biofilm[9,42]. So, the influence of the surface characteristics of the material on

bacterial adhesion will depend on the bacterial species, that is, on the hydrophobicity and SFE of the bacterial cells. Bacteria used in this study were chosen because they are the first colonizers and we expected their fast adhesion to the examined wires. What is known from the literature is the fact that the later colonizers of the oral biofilm, without these first colonizers and their receptors, as well as metabolites, hardly adhere to artificial surfaces. Therefore, only after a detailed study of the dynamics of adhesion of oral biofilm pioneers individually, we can expand the research to the dynamics of adhesion in a mixed population of bacteria and investigate the dynamics of adhesion in the oral cavity.

So, in the future, it would be interesting to investigate the adhesion of a mixed suspension of oral bacteria to different materials.

CONCLUSION

The NiTi and SS wires differed in terms of their surface roughness, hydrophobicity, and SFE and the NiTi wires should have been more receptive for bacterial adhesion, but the adhesion depended on the bacterial species tested. *S. mutans* and *A. actynomicetemcomitans* adhered better to NiTi wires, while *V. parvula* and *S. oralis* adhered rather to SS wires. So, the hydrophobicity and SFE of the bacterial cells in conjunction with the surface characteristics had a significant effect on the strength of bacterial adhesion. Our results contribute to the understanding of the early bacterial colonization of dental materials. Furthermore, the present study can contribute to the optimization of the antiadherent properties materials, specifically with respect to early oral colonizers.

AKNOWLEDGMENTS

Funded by Croatian Science Foundation (IP-2020-02-4027) “Environmental factors and microbiological interaction in structure of dental biofilm”, PI Stjepan Spalj.

REFERENCES

1) Liber-Kneć A, Łagan S. Surface testing of dental biomaterials —Determination of contact angle and surface free energy. Materials (Basel) 2021; 14: 2716.
2) Saini R, Saini S, Sharma S. Biofilm: A dental microbial infection. J Nat Sci Biol Med 2011; 2: 71-75.
3) Abraham KS, Jagdish N, Kailasam V, Padmanabhan S. Streptococcus mutans adhesion on nickel titanium (NiTi) and copper-NiTi archwires: A comparative prospective clinical study. Angle Orthod 2017; 87: 448-454.
4) Lee S-P, Lee S-J, Lim B-S, Ahn S-J. Surface characteristics of orthodontic materials and their effects on adhesion of mutans streptococci. Angle Orthod 2009; 79: 353-360.
5) Oliveira DC, Thomson JJ, Alhabeil JA, Toma JM, Plecha SC, Pacheco RR, *et al*. In vitro Streptococcus mutans adhesion and biofilm formation on different esthetic orthodontic archwires. Angle Orthod 2021; 91: 786-793.
6) Huang H-H. Variation in corrosion resistance of nickel-titanium wires from different manufacturers. Angle Orthod 2005; 75: 661-665.
7) Daems J, Celis J-P, Willems G. Morphological characterization of as-received and in vivo orthodontic stainless steel archwires. Eur J Orthod 2009; 31: 260-265.
8) Berne C, Ellison CK, Ducret A, Brun YV. Bacterial adhesion at the single-cell level. Nat Rev Microbiol 2018; 16: 616-627.
9) Chawla R, Gupta R, Lele TP, Lele PP. A skeptic’s guide to bacterial mechanosensing. J Mol Biol 2020; 432: 523-533.
10) Sterzenbach T, Helbig R, Hannig C, Hannig M. Bioadhesion in the oral cavity and approaches for biofilm management by surface modifications. Clin Oral Investig 2020; 24: 4237-4260.
11) Bos R. Physico-chemistry of initial microbial adhesive interactions —Its mechanisms and methods for study. FEMS Microbiol Rev 1999; 23: 179-229.
12) Hermansson M. The DLVO theory in microbial adhesion. Colloids Surf B Biointerfaces 1999; 14: 105-119.
13) Musa Trolic I, Todoric Z, Pop Acev D, Makreski P, Pejova B, Spalj S. Effects of the presence of probiotic bacteria in the aging medium on the surface roughness and chemical composition of two dental alloys. Microsc Res Tech 2019; 82: 1384-1391.
14) Kreve S, Dos Reis AC. Bacterial adhesion to biomaterials: What regulates this attachment? A review. Jpn Dent Sci Rev 2021; 57: 85-96.
15) Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res 2006; 17 Suppl 2: 68-81.
16) Yoda I, Koseki H, Tomita M, Shida T, Horiuchi H, Sakoda H, *et al*. Effect of surface roughness of biomaterials on Staphylococcus epidermidis adhesion. BMC Microbiol 2014; 14: 234.
17) Gräfe W. A quantum mechanical model for the calculation of the surface free energy and surface stress of nanoparticles. Prot Met Phys Chem Surf 2019; 55: 872-879.
18) Starostina IA, Stoyanov OV, Makhrova NV, Deberdeev RYa. Use of probe polymer surfaces for determining the surface free energy parameters. Dokl Phys Chem 2011; 440: 162-164.
19) Fang Q, Cui H-W, Du G-B. Surface wettability, surface free energy, and surface adhesion of microwave plasma-treated Pinus yunnanensis wood. Wood Sci Technol 2016; 50: 285-296.
20) Simpson JT, Hunter SR, Aytug T. Superhydrophobic materials and coatings: a review. Rep Prog Phys 2015; 78: 086501.
21) Li J, McLandsborough LA. The effects of the surface charge and hydrophobicity of Escherichia coli on its adhesion to beef muscle. Int J Food Microbiol 1999; 53: 185-193.
22) Rincic Mlinaric M, Karlovic S, Ciganj Z, Acev DP, Pavlic A, Spalj S. Oral antiseptics and nickel–titanium alloys: Mechanical and chemical effects of interaction. Odontology 2019; 107: 150-157.
23) Zita A, Hermansson M. Effects of bacterial cell surface structures and hydrophobicity on attachment to activated sludge flocs. Appl Environ Microbiol 1997; 63: 1168-1170.
24) Bevilacqua L, Milan A, Del Lupo V, Maglione M, Dolzani L. Biofilms developed on dental implant titanium surfaces with different roughness: Comparison between in vitro and in vivo studies. Curr Microbiol 2018; 75: 766-772.
25) de Melo F, do Nascimento C, Souza DO, de Albuquerque RF. Identification of oral bacteria on titanium implant surfaces by 16S rDNA sequencing. Clin Oral Implants Res 2017; 28: 697-703.
26) Bellon-Fontaine M-N, Rault J, van Oss CJ. Microbial adhesion to solvents: A novel method to determine the electron-donor/electron-acceptor or Lewis acid-base properties of microbial

cells. Colloids Surf B Biointerfaces 1996; 7: 47-53.
27) Begić G, Petković Didović M, Lučić Blagojević S, Jelovica Badovinac I, Žigon J, Perčić M, *et al*. Adhesion of oral bacteria to commercial d-PTFE membranes: Polymer microstructure makes a difference. Int J Mol Sci 2022; 23: 2983.
28) Wu S. Calculation of interfacial tension in polymer systems. J Polym Sci Part C Polym Symp 2007; 34: 19-30.
29) Owens DK, Wendt RC. Estimation of the surface free energy of polymers. J Appl Polym Sci 1969; 13: 1741-1747.
30) Pratten J, Smith AW, Wilson M. Response of single species biofilms and microcosm dental plaques to pulsing with chlorhexidine. J Antimicrob Chemother 1998; 42: 453-459.
31) Zhang X, Zhang Q, Yan T, Jiang Z, Zhang X, Zuo YY. Quantitatively predicting bacterial adhesion using surface free energy determined with a spectrophotometric method. Environ Sci Technol 2015; 49: 6164-6171.
32) Krishnan M, Seema S, Tiwari B, Sharma HS, Londhe S, Arora V. Surface characterization of nickel titanium orthodontic arch wires. Med J Armed Forces India 2015; 71 (Suppl 2): S340-S345.e5.
33) Kim I-H, Park H-S, Kim YK, Kim K-H, Kwon T-Y. Comparative short-term in vitro analysis of mutans streptococci adhesion on esthetic, nickel-titanium, and stainless-steel arch wires. Angle Orthod 2014; 84: 680-688.
34) Espinosa-Cristóbal LF, López-Ruiz N, Cabada-Tarín D, Reyes-López SY, Zaragoza-Contreras A, Constandse-Cortéz D, *et al*. Antiadherence and antimicrobial properties of silver nanoparticles against streptococcus mutans on brackets and wires used for orthodontic treatments. J Nanomater 2018; 2018: 9248527.
35) Arango-Santander S, Gonzalez C, Aguilar A, Cano A, Castro S, Sanchez-Garzon J, *et al*. Assessment of streptococcus mutans adhesion to the surface of biomimetically-modified orthodontic archwires. Coatings 2020; 10: 201.
36) Rzhepishevska O, Hakobyan S, Ruhal R, Gautrot J, Barbero D, Ramstedt M. The surface charge of anti-bacterial coatings alters motility and biofilm architecture. Biomater Sci 2013; 1: 589-602.
37) Heilmann C. Adhesion mechanisms of staphylococci. Adv Exp Med Biol 2011; 715: 105-123.
38) Heipieper HJ, Cornelissen S, Pepi M. Surface Properties and Cellular Energetics of Bacteria in Response to the Presence of Hydrocarbons. In: Timmis, K.N. (eds) Handbook of Hydrocarbon and Lipid Microbiology. Berlin, Heidelberg: Springer; 2010. p. 1615-1624.
39) Bujdáková H, Didiášová M, Drahovská H, Černáková L. Role of cell surface hydrophobicity in Candida albicans biofilm. Open Life Sci 2013; 8: 259-262.
40) Quirynen M, Bollen CML. The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. J Clin Periodontol 1995; 22: 1-14.
41) Inouye T, Ohta H, Kokeguchi S, Fukui K, Kato K. Colonial variation and fimbriation of *Actinobacillus actinomycetemcomitans*. FEMS Microbiol Lett 1990; 69: 13-17.
42) Wang C, Hou J, Mei HC van der, Busscher HJ, Ren Y. Emergent properties in streptococcus mutans biofilms are controlled through adhesion force sensing by initial colonizers. mBio 2019; 10: e01908-e01919.