Influence on Biofilms Formation of Two Types of Orthodontic Brackets with Different Chemical Composition

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Applying of orthodontic brackets will change the environment of the mouth. The purpose of the present study was to compare biofilms formation on stainless steel and sapphire brackets in order to clarify which bracket chemical composition has a higher plaque retaining capacity. For biofilm quantification a strain of Candida spp. and Staphylococcus spp. were selected. Significant differences were observed between the Candida biofilm formed on stainless steel brackets compared to sapphire brackets. Sapphire brackets may be an option in orthodontic treatment not only for aesthetics but also because of aspects related to biofilm formation by Candida spp.

Key words: orthodontic appliance, biofilms, metallic brackets, sapphire brackets

The majority of microorganisms in nature are found attached to different types of surfaces in humid environments, where they grow and form biofilms. The oral cavity is one of the many environments where humidity, nutrients and bacteria are found together, leading to biofilm formation. All surfaces of the mouth are covered by a layer of adsorbed molecules of bacterial and salivary origin, and the initial colonizing bacteria attach to this layer. Initially, these species are held reversibly through weak, long-range physicochemical interactions between charged molecules on the cell and oral surfaces. This interaction can become permanent via strong, short-range stereochemical interactions between adhesins on the bacterium and complementary receptors in the acquired pellicle [1].

Orthodontic appliances change the oral cavity environment by increasing the contact surface which modifies microbial adherence and colonization, acting as foreign reserves and possible sources of infection. The region of the tooth surface around brackets is prone to adhesion of oral bacteria and subsequent biofilm formation. Oral biofilms on dental hard and soft tissues are the main cause of dental diseases, including caries and periodontal disease (fig. 1).

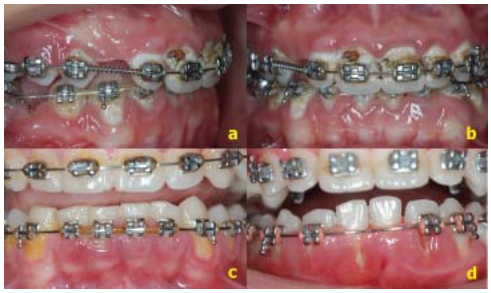

Fig. 1. Lack of proper hygiene during fixed orthodontic treatment leads to the development of oral biofilms, accumulation of dental plaque, caries (a, b) and periodontal disease (c, d)

A single-time, self-performed manual brushing is often insufficient and known to leave biofilm behind in retention sites, such as fissures, interproximal spaces, and gingival margins. Orthodontic appliances make effective biofilm removal even more difficult, and brushing nearly always leaves biofilm behind at the vulnerable bracket-adhesive enamel junction and the sensitive region between brackets and gingival margin, therewith contributing to the occurrence of dental diseases [2].

One of the most frequently found microorganism in infections of the oral cavity is the yeast of *Candida* genus. *Candida* can be found to colonize the cement, enamel and dentine and can survive even on inert surfaces [3].

Staphylococcal biofilms are a major concern in both clinical and food settings because they are an important source of contamination. *Staphylococcus* spp. and especially *aureus* and *epidermidis* are opportunistic pathogens that can adhere to tissues and implant devices and form biofilm leading to infections. Pathogenicity of both species is clearly associated with their ability to form biofilms on biotic and abiotic surfaces, providing high resistance to host defenses and antibiotics, and also to cleaning and disinfection processes [4].

Experimental part

For this study, two different types of orthodontic brackets were chosen in order to test their influence on biofilm formation. We used stainless steel brackets – OmniArch® with Roth prescription (Dentsply GAC International, USA) and a high quality monocrystalline sapphire brackets – PURE® (Ortho Technology, Inc., Florida, USA). For biofilm quantification a strain of *Candida* spp. and *Staphylococcus* spp. were selected.

For isolation and identification of the bacterial strains used in the following experiment, samples were taken from 12 patients, aged 11 to 17 years, six of them wearing metallic brackets and six sapphire brackets using sterile applicators. We collected the samples during the periodic

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Fig. 2. Samples taken from young subjects wearing sapphire brackets (a) and stainless steel brackets (b)

visits for activation of the orthodontic appliance, without prior professional tooth cleaning (fig. 2).

The collected samples were spread on Columbia CNA Agar with 5% sheep blood (containing nalitixic acid and colistin), a special media used to isolate gram positive bacterial strains. The CNA Agar was used in order to isolate *Staphylococcus* spp. The bacterial strains were isolated according to typical colonial growth described by the producer. The isolated colonies were spread on BHI agar (Brain Hearth Infusion - Oxoid). For a better isolation of the *Staphylococcus* spp. the Baird Parker agar (BIOKAR Diagnostics - France) with sulfametazin and egg yolk was used.

Isolation of *Candida* strains was done using SDA (Sabouraud Dextrose Agar) culture media. *Candida* colonies were identified based on the colony colour, texture and morphology. The selection of *Candida* spp. and *Staphylococcus* spp. strains was made based on a microtiter plate assay with crystal violet staining [5]. After the microtiter plate assay, one strain of *Candida* spp. and one strain of *Staphylococcus* spp. which produced the highest quantity of biofilm were selected for further experiments.

Biofilm experiments were done on sterilized brackets. First of all, the brackets were sterilized in four different Petri dishes, two for the metal brackets and two for the sapphire brackets, at 120 °C, for 20 min. After sterilization 20 mL of SDB (Sabourand Dextrose Broth) was added in two Petri dishes, one containing stainless steel brackets and one sapphire brackets and then inoculated with 0.5 mL of *Candida* spp. inoculum that matches 0.5 McFarland standards in saline solution (0.9%). In the other two Petri dishes, 20 mL of BHI broth was added and inoculated with 0.5 mL of *Staphylococcus* spp. inoculum that matches 0.5 McFarland standards in saline solution (0.9%). All the Petri dishes were incubated at 37°C for 48h in 100% UR chamber and continuous agitation (20 rpm).

After the incubation period the brackets were rinsed with distillated water, stained with acridine orange (AO) in acetate buffer solution (Sigma) for 2 min at room temperature, rinsed in pure water, air dried at room temperature and examined using the Leica DM 2500 fluorescent microscope's 10x and 20x objectives. Five randomly selected fields (1.77x1.33 mm) on the surface of each bracket were analyzed by microscopy and image processing. The fluorescence images obtained were analyzed using *BioimageL*, special computer software designed for biofilm two dimensional quantification [6]. The coverage percentage and the aria occupied by biofilm was measured and compared. The brackets were also analyzed using a fluorescent stereomicroscope model Leica MZ FLIII, for macroscopic aspects.

Results and discussions

The fluorescent image analysis showed biofilm formation on both types of brackets with *Candida* spp. and *Staphylococcus* spp. respectively. Significant differences were observed between the *Candida* biofilm formed on metal brackets compared to sapphire brackets. Also, macroscopic images showed different aspects of the biofilm on the brackets surface. As can be seen (fig. 3) on the surface of metal bracket the biofilm formed by *Candida* spp. is more abundant and has a pronounced development in comparison to the biofilm formed on sapphire bracket. These differences were also confirmed by the microscopic analysis of the surfaces ($p<0.01$) . Silver nanoparticles bacteriostatic effect over dentures infected with *Candida albicans* was studied in [7].

Table 1
QUANTIFICATION OF *CANDIDA* SPP. BIOFILM

	***Candida* spp. biofilm**			
	Stainless steel brackets		**Sapphire brackets**	
	Coverage %	Biofilm area (μm^2)	Coverage %	Biofilm area (μm^2)
Mean	72.07*	1682538	32.13	750032.7
SD	±6.34	±148439.5	±5.37	±125336.9
Area of an image is 2354100 μm^2; *p=0.0017				

The biofilm formed by *Candida* spp. on metal brackets surface had a mean 72.07 percent coverage capacity (table 1), significantly different when compared to the mean coverage percentage of the same strain on sapphire brackets (32.13%). Similar results were obtained in a study where adhesion of *Candida albicans* was medium on both sapphire brackets – coated wires and sapphire brackets – stainless steel wires and high with both stainless steel brackets-coated wires and stainless steel brackets – stainless steel wire with high significant differences ($p<0.001$) [3].

These differences may be due to the chemical composition, surface free energy and surface roughness of the tested materials. It is well known that stainless steel brackets undergo major chemical alterations due to variation in temperature and *p*H, presence of saliva, organic acids produced by microorganisms and enzymes. The combined action of these biological factors can significantly alter the integrity of orthodontic materials surface by increasing roughness, formation of craters and pits, corrosion, metal ions release (nickel and chromium) and formation of an organic layer containing Na, P, S, Cl, K, and Ca [8, 9]. All these alterations combined can lead to bioaccumulation of metal ions in tissues, increase the contact surface due to corrosion processes, provide the right conditions for biofilm formation and can lead to biodegradation of materials, changing some of their properties, which can compromise their clinical performances [8, 9]. Other studies found that sapphire brackets have a smoother surface compared to metal brackets, therefore more bacterial adhesion was found on metal brackets surface [10]. An *in vivo* study concluded that ceramic brackets exhibit less long-term biofilm accumulation than metal brackets [11]. Another studies showed that the adherence of *Candida albicans* was increased by the composite bracket, whereas the use of metallic brackets decreased the number of colony-forming units [12].

Image analysis of the biofilm formed by *Staphylococcus* spp. (fig. 4) on both types of brackets showed no significant difference in regards to coverage percentage and area occupied by biofilm ($p>0.01$). Also, the amount of biofilm formed on both type of brackets was very low in comparison to the amount of biofilm formed by *Candida* spp (table 2).

These differences may be due to the chemical composition of the culture medium or the physical and chemical proprieties of the brackets that can influence bacterial adhesion, growth and biofilm formation. It is well

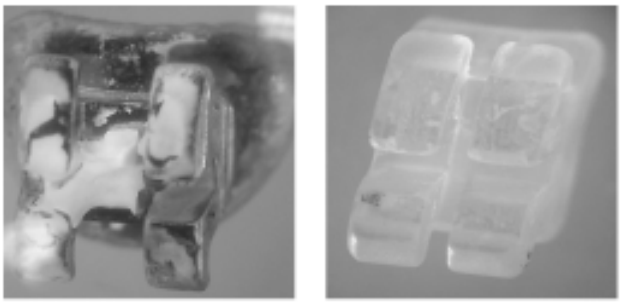

Fig. 3. Macroscopic aspects of *Candida* spp. (A) stainless steel bracket, (B) sapphire bracket

Table 2

QUANTIFICATION OF STAPHYLOCOCCUS SPP. BIOFILM

	Staphylococcus spp. biofilm			
	Stainless steel brackets		Sapphire brackets	
	Coverage %	Biofilm area (μm²)	Coverage %	Biofilm area (μm²)
Mean	15.26*	356068.3	36.7	857025.8
SD	±11.93	±278542.2	±14.47	±338160.9
Area of an image is 2354100 μm²; *p=0.085				

known that culture media or the surrounding environment is influencing bacterial growth and biofilm formation. For example, metallic orthodontic brackets have been found to induce specific changes in the oral environment such as reduced levels of *p*H and affinity of bacteria to a metallic surface because of electrostatic reactions, also it increased plaque accumulation [3]. Also, when examining the adhesion of lipopolysaccharides of *Escherichia coli* and *Porphyromonas gingivalis*, stainless steel brackets exhibited significantly stronger bonds [13]. Factors that influence the adhesion and formation of biofilm are the presence of other bacterial strains and the conditioning of the surfaces with saliva [14]. Because no differences of *Staphylococcus* spp. biofilm were observed between brackets, suggests that attachment of some micro-organisms are not influenced by brackets chemical composition and that conditioning of the surfaces is favoring it.

The bacteria responsible of primary colonisation and the secondary microorganisms generate an extracellular matrix of polymers related to biofilm growing.The biofilm bacteria have an active metabolism causing *p*H variations. These fluctuations can cause mineral loss when *p*H decreases and mineral gain when *p*H increases [15].

The critical surface tension of the substrate is considered a key factor in modulating the attraction of species on the surface. In an previous study the authors investigated the wettability and microbial attachment on different bracket materials and concluded that: (a) stainless steel brackets presented the highest critical surface tension, indicating an increased potential for microorganism attachment on metallic brackets, (b) the lowest surface tension values obtained from the fiber-reinforced polycarbonate and ceramic alumina brackets indicated reduced plaque retaining capacity compared to stainless steel brackets [16].

Conclusions

Biofilm formed by *Staphylococcus* spp. on both types of brackets showed no significant difference in regards to coverage percentage and area occupied by biofilm which suggests that attachment of some microorganisms,

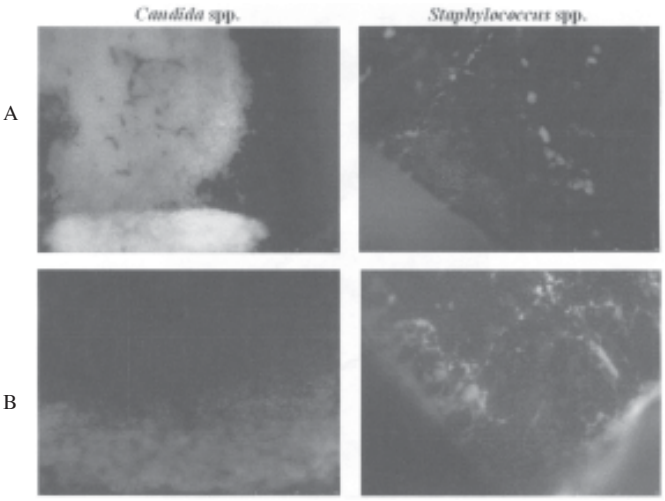

Fig. 4. Aspects of the *Candida* spp.and *Staphylococcus* spp. biofilm on stainless steel bracket (A) and safire bracket (B)

including *Staphylococcus* spp. are not influenced by brackets chemical composition but conditioning of the surfaces is favoring it. Sapphire brackets may be an option in orthodontic treatment not only for aesthetics but also because of aspects related to biofilm formation by *Candida* spp. Orthodontic brackets change the oral cavity environment both chemically and physically, which can lead to biofilm formation, increase risk of caries and periodontal disease, thus oral hygiene is very important.

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References

1.DO, T., DEVINE, D., MARSH, P.D., Clin. Cosmet. Investig. Dent., **28**, no. 5, 2013, p. 11.
2.REN, Y., JONGSMA, M.A., MEI, L., VAN DER MEI, H.C., BUSSCHER, H.J., Clin. Oral. Invest., **18**, 2014, p. 1711.
3.SALOOM, H.F., MOHAMMED-SALIH, H.S., RASHEED, S.F., J. Clin. Exp. Dent., **5**, no. 1, 2013, p. 36.
4.GUTIERREZ, D., RUAS-MADIEDO, P., MARTINEZ, B., RODRIGUEZ, A., GARCIA, P., Plos One, **9**, no. 9, 2014, p. 107307.
5.DJORDJEVIC, D., WIEDMANN, M., MCLANDSBOROUGH, L.A., Appl. Environ. Microbiol., **68**, no. 6, 2002, p. 2950.
6.CHAVEZ DE PAZ, L.E., Appl. Environ. Microbiol., **75**, no. 6, 2009, p. 1734.
7.MENDES, B.A.B., FERREIRA, R.A.N., PITHON, M.M., HORTA, M.C.R., OLIVIERA, D.D., J. Appl. Oral Sci. **22**, no. 3, 2014, p. 194.
8.MIKULEWICZ, M., WOLOWIEC, P., MICHALAK, I., CHOJNACKA, K.,CZOPOR, W., BERNICZEI-ROYKO, A., VEGH, A., GEDRANGE, T., Med. Sci. Monit., **20**, 2014, p. 860.
9.LEE, S.P., LEE, S.J., LIM, B.S., AHN, S.J., Angle Orthodontist, **79**, no. 2, 2009, p. 353.
10.LINDEL, I.D., ELTER, C., HEUER, W., HEIDENBLUT, T., STIESCH, M., SCHWESTKA-POLLY, R., DEMLING, A.P., Angle Orthodontist, **81**, no. 5, 2011, p. 907.
11.BRUSCA, M.I., CHARA, O., STERIN-BORDA, L., ROSA, A.C., Angle Orthodontist, **77**, no. 2, 2007, p. 331.
12.KNOERNSCHILD, K.L., ROGERS, H.M., LEFEBVRE, C.A., FORTSON, W.M., SCHUSTER, G.S., Am. J. Orthod. Dentofacial Orthop., **115**, no. 6, 1999, p. 634.
13.PAPAIOANNOU, W., GIZANI, S., NASSIKA, M., KONTOU, E., NAKOU, M., Angle Orthodontist, **77**, no. 6, 2007, p. 1090.
14.STOLERIU S, IOVAN G., PANCU G., GEORGESCU A., SANDU A.V., ANDRIAN S., Rev. Chim.(Bucharest), **63**, no. 11, 2012, p.1120.
15.ELIADES T., ELIADES G., BRANTLEY W.A., Am. J. Orthod. Dentofacial. Orthop., **108**, 1995, p. 351

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