Research Article

Fuat Bislimi*, Jagoda Bajevska, Mrinmoy Garai, Arianit A. Reka

A study on the bacterial adhesion of *Streptococcus mutans* in various dental ceramics: *In vitro* study

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Abstract: *Streptococcus mutans* (*S. mutans*) has been identified as a major etiologic agent of human dental caries and forms a significant proportion of oral streptococci in carious lesions. This study investigates the correlation of surface properties (effect of contact angle [CA] and free surface energy) on three restorative materials (zirconia, nickel–chromium–molybdenum alloy and composites) used in dental prosthetics with bacterial adhesion to *S. mutans*. Ten samples of each material (zirconia, nickel–chromium–molybdenum alloy and composites) of 8 mm diameter and 2.5 mm thickness were used. Aqueous CA measurements, free surface energy and bacterial adhesion to the sample surfaces were performed. Bacterial adhesion is determined by planting samples in the blood agar cultures and using an electron microscope (scanning electron microscopy [SEM]). The highest values of bacterial adhesion are found in composites, followed by the metal alloy, while the lowest values are observed in zirconia. Measurements show that zirconia has 17 bacteria; Ni–Cr–Mo alloy has 65, while the composite has 80 bacteria. The composites showed the highest degree of bacterial adhesion, compared to the other investigated materials, which correlates with the free surface energy of the samples (24.31 mJ/m^2 for zirconia, 31.78 mJ/m^2 for Ni–Cr–Mo alloy and 48.82 mJ/m^2 for the composite).

Keywords: *Streptococcus mutans*, bacterial adhesion, hydrophobicity, free surface energy, SEM

1 Introduction

Dental implants and prosthodontic restorations are one of the most routinely used treatment options for the replacement of missing teeth [1–3]. The oral microflora and its interactions with the implant substrata seem to have a crucially impact on the long-term success or failure of dental implants. Once the implant surfaces are exposed to the human oral cavity, they are promptly colonized by microorganisms [4–7].

The biological response to the dental implants or prosthodontic restorations is determined by a number of physical and chemical features. These features include mechanical properties and physiochemical properties such as chemical composition, surface energy, surface wettability and surface topography [8].

Surface topography plays a very important role in bacterial adhesion, such as hydrophobicity, free surface energy, chemical composition and smoothness or roughness of surfaces. Microscopic studies of early dental plaque formations have shown adhesion of the initial colonized bacteria along the cracks and pits in the enamel, indicating the influence of surface structure on bacterial adhesion [9].

The success of esthetic restorations on a long-term basis depends on the quality and quantity of the attached biofilm. Initial attachment and subsequent colonization of bacteria on the surface of restorative materials is key to the pathogenesis of secondary caries promoted in particular by *S. mutans* and *Streptococcus sobrinus* (*S. sobrinus*). *S. mutans* has been identified as the major etiologic agent of human dental caries and forms a significant proportion of oral streptococci in carious lesions [10,11].

* **Corresponding author: Fuat Bislimi,** Department of Prosthodontics, Faculty of Dentistry, s. Cyril and Methodius University, Skopje, Republic of North Macedonia, e-mail: dr.fuati@gmail.com
Jagoda Bajevska: Department of Prosthodontics, Faculty of Dentistry, s. Cyril and Methodius University, Skopje, Republic of North Macedonia
Mrinmoy Garai: Materials Science Centre, Indian Institute of Technology (IIT), Kharagpur 721302, India
Arianit A. Reka: Department of Chemistry, Faculty of Natural Sciences and Mathematics, University of Tetovo, Blvd. Ilinden, 1200, Tetovo, Republic of North Macedonia; NanoAlb, Albanian Unit of Nanoscience and Nanotechnology, Academy of Sciences of Albania, 1000 Tirana, Albania

The various types of materials and their specific textures and physicochemical surface properties influence the quantity and quality of microbial colonization [12–15]. Therefore, knowledge of bacterial adhesion and subsequent biofilm formation (multicellular structures with microbial cells embedded in the extracellular matrix) is important for the study of bacterial pathogenicity. The human mouth provides a unique environment for the formation of complex biofilms; for example, it hosts more than 1,000 species of microorganisms [16]. Among several microorganisms, bacteria are a major group and are capable of forming biofilms. Thus, it is important to know the mechanisms involved in bacterial adhesion in various materials used.

Today, in dental prosthetics, different materials are used, which have different physicochemical properties and act differently on the bacterial adhesion of *S. mutans*. In nickel–chromium alloys, despite the good physical chemical properties of the material, biofilm formation on its surface is crucial. The same problem is present in composite resins used in dentistry; as in some complex resins, bacterial adhesion is stimulated by the released residual monomer [17].

This study investigates the correlation of surface characteristics (effect of contact angle [CA] and free surface energy) on three restorative materials (zirconia, nickel–chromium–molybdenum alloy and composites) used in dental prosthetics with bacterial adhesion (by means of the test specie *S. mutans*).

2 Materials and methods

For the purpose of studying the bacterial adhesion of *S. mutans* in various dental ceramics, three various starting materials were used: zirconium (Ceramill, Zolid FX White, Germany), metal alloy (Kera N, Eisenbacher Dentalwaren ED-GMBH, Germany) and composite (Sr ADORO – Ivoclar Vivadent, Liechtenstein). Ten specimens in cylindrical shape (dimensions: 8 mm in diameter and 2.5 mm in thickness) from all three types of starting are used during this research. The surfaces of the specimens were treated with standard polishing methods as specified by the material manufacturer.

To form biofilm formations for further analysis, we used *S. mutans* bacteria, reference strain ATCC 35668, which according to the manufacturer's instructions was developed in laboratory conditions in Petri dishes with 5% blood agar incubation of the plates at 37°C in an incubator with microaerophilic conditions at 10% CO_2 for 24 h. Bacteria obtained in culture are used to make a bacterial suspension at a concentration of 0.5 Mc Farland. The concentration of bacteria in the suspension is measured with the DensiCHEK Plus-bio Merieux apparatus. The samples will then be cleaned and sterilized in an autoclave for 30 min at 121°C. Each set of samples (3 sets of 10 samples) will be covered with the bacterial suspension for 15 min at 37°C; the samples were removed from the suspension and washed with physiological solution, then incubated in Petri dishes containing the blood agar culture at 37°C for 48 h under microaerophilic conditions with 10% CO_2. Then, after 48 h, specimens are prepared for scanning electron microscopy (SEM).

The CA measurements were performed on the basis of an average of three consecutive measurements of each sample according to a standard procedure with distilled water droplets, ethylene glycol and glycerol. CA measurements were made using the See System E instrument with Software 7.0, Advex Instrument. Measurements were performed on three types of samples, and the results were obtained by averaging three consecutive measurements of each sample according to the standard distilled water drop procedure.

Surface-free energy (SFE) calculations are performed according to the Owens–Wendt Regression model. For this purpose, CA measurements were performed on the basis of an average of three consecutive measurements of each sample according to a standard procedure with distilled water droplets, ethylene glycol and glycerol.

To observe the morphology of bacterial adhesion, specimens are prepared for SEM. The samples were fixed in 2% glutaraldehyde for 24 h at room temperature, washed three times with buffer phosphate solution (pH 7.4) and dehydrated through a series of sorted ethanol solutions (20%, 40%, 60%, 80% and 100%). The samples are then dried, coated with gold spray and scanned using an electron microscope VEGA3 LMU coupled with energy-dispersive X-ray spectroscopy (INCA Energy 250 Microanalysis System). The accelerating voltage of the SE detector was set to 20 kV.

XRPD analysis was performed on Rigaku Ultima IV X-ray diffractometer equipped with D/teX high-speed 1-dimensional detector using CuKα radiation (λ = 1.54178 Å) in 2θ range from 5° to 60°. The accelerating voltage and the current power were set to 40 kV and 40 mA, respectively.

The chemical compositions of the starting materials as provided by the producer for all three starting materials are shown below (Table 1).

Ethical approval: The conducted research is not related to either human or animal use.

Table 1: Chemical composition of starting materials

Materials	Manufacturer	Composition
Zirconium	Ceramill* Zolid fx white, Germany	Y_2O_3 (9.15–9.55%), HfO_2 (<5%), Al_2O_3 (<0.5), others (0.1%)
Metal alloy	Kera N, Eisenbacher-Dentalwaren ED-GMBH, Germany	Ni (61.4%), Cr (25.9%), Mo (11%), Si (1.5%), others (<0.1%)
Composites	Sr ADORO, Ivoclare Vivadent, Liechtenstein	Uretan dimetakrilat (UDMA), (SiO_2, 70%)

3 Results and discussion

3.1 XRPD analysis of the starting materials

The XRPD analysis (Figure 1a) shows the presence of amorphous phase present in the sample, manifested with a wide bump in the region 15–30° 2θ. This bump is due to the amorphous silica [18,19]. Evident is the presence of crystalline phases: quartz in the following 2θ 26.58° (d 3.35 Å), 50.10° (d 1.81 Å) and 59.70° (d 1.54 Å) [20,21]; peaks at 30.10° (d 2.96 Å) and 34.82° (d 2.57 Å) due to the presence of cubic zirconia. Figure 1b shows the presence of two peaks at 43.86° (d 2.06 Å) and 50.86° (d 1.79 Å) as a result of the Ni–Cr–Mo alloy present in the sample. Figure 1c depicts crystalline behavior of the sample manifested by the appearance of peaks characteristic for cubic zirconia as well as quartz peaks. The peaks at 2θ 20.92° (d 4.24 Å) and 26.70° (d 3.34 Å) are due to the presence of quartz, while 30.20° (d 2.95 Å), 35.06° (d 2.55 Å), 50.26° (d 1.81 Å), 59.82° (d 1.54 Å) and 62.72° (d 1.47 Å) are as a result of cubic zirconia [20–22].

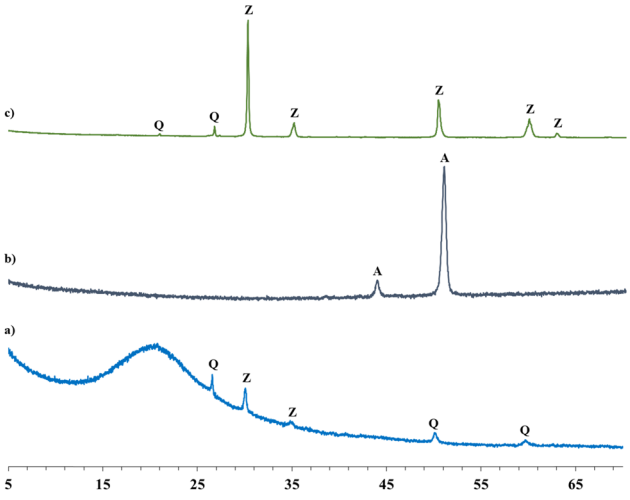

Figure 1: XRPD of a – composite (Q – quartz, Z – zirconia), b – alloy (A – Ni–Cr–Mo alloy) and c – zirconium (Q – quartz, Z – zirconia).

Table 2: CA of zirconium, metal alloy and composite

Specimen	CA (°)
Zirconium	90.34
Metal alloy (Ni–Cr–Mo alloy)	86.41
Composite	87.59

3.2 CAs and free surface energy

Table 2 shows the results of the CA for the three tested samples (zirconium, alloy and composite). Based on the CA measurements, the following is observed: the highest CA is observed with zirconium sample. The CA of the metal alloy is characterized by a CA lower by 4.35% than the zirconium sample, while the CA of the composite material is characterized by an even lower angle by 3.04%. This observed difference is not statistically significant. Therefore, based on the measurements made, the following can be concluded: based on the CA measurements, it can be concluded that the most hydrophobic characteristics are the samples with ordinal numbers: 1, 3 and 2.

Table 3 shows the results of the SFE of three tested samples subject of this *in vitro* study. The data obtained from the SFE according to the OWR model show highly significant differences among the different materials. From the measurements of the SFE, it can be concluded that the highest values show the samples in ordinal numbers: 3, 2 and 1. These results, especially the CA for the composite, are most likely due to the amorphous matter present in the sample (Figure 1a).

Table 3: SFE of zirconium, metal alloy and composite

Specimen	SFE according to OWR model [mJ/m^2]
Zirconium	24.31
Metal alloy (Ni–Cr–Mo alloy)	31.78
Composite	48.82

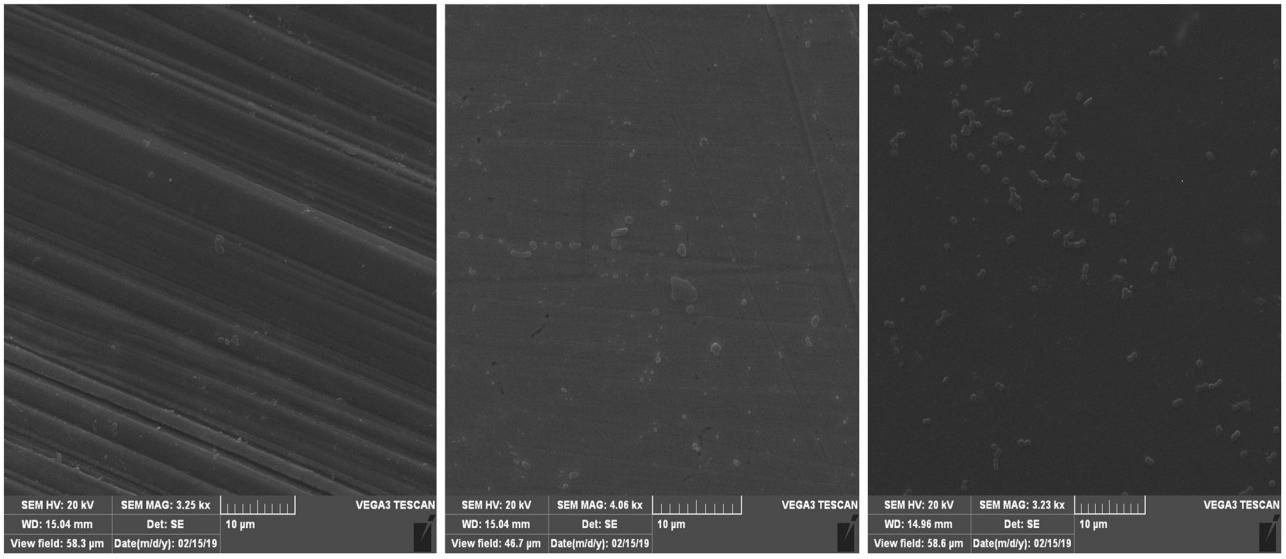

Figure 2: SEM micrographs of a – zirconia composite, b – metal alloy, c – composite.

3.3 SEM analysis

The SEM micrographs of the surfaces of zirconium, metal alloy and the composite are shown in Figure 2. Based on the provided results, it is observed that the surfaces of all three materials are remarkably different. The composite seems to be with the smoothest surface, while zirconia has a rigid surface topography.

While surface morphology plays a crucial role in the process of bacterial adhesions; in this study, the opposite is observed. Although the rigidness of the surface of zirconia is clearly observable compared to the other two, yet the average number of bacteria is observed to be significantly higher in the composite material. Table 4 shows the results obtained based on the number of bacteria developed in all three specimens. It is evident that the number of bacteria in metal alloys is around 3.8 times higher than zirconia, while this number is around 4.5 times higher in the composite material.

Table 4: Number of bacteria in different specimens (zirconium, metal alloy and composite)

Specimen	Number of bacteria
Zirconium	17
Metal alloy (Ni–Cr–Mo alloy)	65
Composite	80

4 Conclusions

This *in vitro* study evaluated the bacterial adhesion of *S. mutans* to zirconia, nickel–chromium–molybdenum alloys and composite materials. The obtained results show different values of CA, free surface energy and bacterial adhesion for all three materials. No correlation was found between CA and bacterial adhesion, whereas SFE correlated with bacterial adhesion, so that with increasing free surface energy, the bacterial adhesion of *S. mutans* increased. The analysis of the susceptibility of the selected dental materials to the adhesion of microorganisms as part of this study showed that the composite material is more susceptible to the adhesion of microorganisms. The hydrophobicity of the surfaces shows no association with bacterial adhesion in the test materials, whereas the free surface energy is correlated with the bacterial adhesion of *S. mutans*. Differences in the adhesion of microorganisms to the surface zirconia vs composite were statistically significant. Based on the above-mentioned results, it can be concluded that zirconia as dental ceramics has the lowest values for the presence of bacteria, followed by nickel–chromium–molybdenum alloy, while the highest values are found in the composites.

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References

[1] Wassmann T, Kreis S, Behr M, Buergers R. The influence of surface texture and wettability on initial bacterial adhesion on titanium and zirconium oxide dental implants. Int J Implant Dent. 2017;3:32.

[2] Poon CY, Bhushan B. Comparison of surface roughness measurements by stylus profiler, AFM and non-contact profiler. Wear. 1995;190:76–88.

[3] Hahnel S, Rosentritt M, Handel G, Bürgers R. Surface characterization of dental ceramics and initial streptococcal adhesion *in vitro*. Dent Mater. 2009;25:969–75.

[4] Abrahamsson I, Berglundh T, Lindhe J. Soft tissue response to plaque formation at different implant systems. A comparative study in the dog. Clin Oral Implants Res. 1998;9:73–9.

[5] Scarano A, Piattelli M, Caputi S, Favero GA, Piattelli A. Bacterial adhesion on commercially pure titanium and zirconium oxide disks: an *in vivo* human study. J Periodontol. 2004;75:292–6.

[6] Elter C, Heuer W, Demling A, Hannig M, Heidenblut T, Bach FW. Supra and subgingival biofilm formation on implant abutments with different surface characteristics. Int J Oral Maxillofac Implants. 2008;23:327–34.

[7] Oh TJ, Yoon J, Misch CE, Wang HL. The causes of early implant bone loss: myth or science? J Periodontol. 2002;73:322–33.

[8] Mabbouxa F, Ponsonnetb L, Morriera J, Jaffrezicb N, Barsottia O. Surface free energy and bacterial retention to salivacoated dental implant materials – an *in vitro* study. Colloids Surf B. 2004;39:199–205.

[9] Lee BC, Jung GY, Kim DJ, Han JS. Initial bacterial adhesion on resin, titanium and zirconia *in vitro*. J Adv Prosthodont. 2011;3:81–4.

[10] Soliman WE, Ali AI. Adhesion of *Streptococcus mutans* biofilm on the surface of indirect resin composites restoration. Int J Adv Res. 2017;5:1217–23.

[11] Fontana M, Gffel LE, Gregory RL. Characterization of preparations enriched for *Streptococcus mutans* fimbriae: salivary immunoglobulin A antibodies in caries-free and caries-active subjects. Clin Diagnolab Immunol. 1995;2:719–25.

[12] Van Brakel R, Cune MS, Van Winkelhoff AJ, De Putter C, Verhoeven JW, Van Der Reijden W. Early bacterial colonization and soft tissue health around zirconia and titanium abutments: an *in vivo* study in man. Clin Oral Implants Res. 2011;22:571–7.

[13] Al-Ahmad A, Wiedmann-Al-Ahmad M, Faust J, Bachle M, Follo M, Wolkewitz M. Biofilm formation and composition on different implant materials *in vivo*. J Biomed Mater Res B Appl Biomater. 2010;95:101–9.

[14] 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;17:68–81.

[15] Quirynen M, Bollen CM. The influence of surface roughness and surface-free energy on supra and subgingival plaque formation in man, a review of the literature. J Clin Periodontol. 1995;22:1–14.

[16] Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8:623–33.

[17] Aykent F, Gunal S, Avunduk M, Ozkan S. Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion. J Prosthet Dent. 2010;103:221–7.

[18] Reka AA, Anovski T, Bogoevski S, Pavlovski B, Boškovski B. Physical-chemical and mineralogical-petrographic examinations of diatomite from deposit near village of Rožden, Republic of Macedonia. Geologica Macedonica. 2014;28:121–6.

[19] Reka AA, Pavlovski B, Makreski P. New optimized method for low-temperature hydrothermal production of porous ceramics using diatomaceous earth. Ceram Int. 2017;43:12572–78.

[20] Reka AA, Pavlovski B, Ademi E, Jashari A, Boev B, Boev I, et al. Effect of thermal treatment of trepel at temperature range 800–1200°C. Open Chem. 2019;17:1235–43.

[21] Cekova B, Pavlovski B, Spasev D, Reka A. Structural examinations of natural raw materials pumice and trepel from Republic of Macedonia. Proceedings of the XV Balkan Mineral Processing Congress. Sozopol, Bulgaria; 2013. p. 73–75.

[22] Reka AA, Pavlovski B, Cekova B. Special ceramics based on partially stabilized ZrO2. Proceedings Symposium of novel technologies and economic development; 2013. p. 73–81.