Comparative Study on the Degree of Bacterial Biofilm Formation of Dental Bridges Made from Three Types of Materials

MARIA BOLAT[1], DANA GABRIELA BOSINCEANU[1]*, IOAN GABRIEL SANDU[2,3], DAN NICOLAE BOSINCEANU[1]*, ZINOVIA SURLARI[1], CARINA BALCOS[1], OLEG SOLOMON[4]*, ANCA VITALARIU[1]

[1] Grigore T. Popa University of Medicine and Pharmacy Iasi, 16 Universitatii Str., 700115, Iasi, Romania

[2] Gheorghe Asachi Technical University of Iasi, Faculty of Material Science and Engineering, 61A D. Mangeron Blvd., 700050, Iasi, Romania

[3]Romanian Inventors Forum, 3 Sf. Petru Movila, Bl. L11, III/3, 700089, Iasi, Romania

[4] Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Department Illarion Postolachi, 42 Toma Ciorba St. Post, 2004, Chisinau Moldova

The aim of this study was to compare the biofilm formation on three types of dental crown materials using adenosine triphosphate (ATP) driven bioluminescence as an innovative tool for the rapid chairside enumeration of oral bacteria and assessment of oral hygiene. The study group included 60 patients with fixed prosthodontics, made of three types of dental crown materials (BioHpp - Bredent, Ceramics - VITA VMK Master, and Zirconia - Vita In-Ceram) from which we have collected 60 specimen values using a luciferase-based assay system (system SURE II). The values of ATP were obtained with System SURE II device and statistically analyzed with Anova and Wilcoxon Test. The lowest value was shown for Zirconia, comparing with ceramics and BioHpp, but in time we have seen the increase of ATP for all three dental crown materials.

Keywords: Dental Biofilm, ATP, dental crown, ceramics, zirconia, BioHpp material

Dental plaque, a biofilm present in the oral cavity, causes two of the most common oral infections: caries and gum disease. The mechanism by which plaque adheres to and forms on the surface of teeth and restoration materials has been extensively studied. Saliva-derived pellicles immediately form on the surfaces of thoroughly cleaned teeth and attract bacteria through chemical or electrostatic interactions or by antigens on the surfaces of bacterial cells. The most common organisms present in oral biofilms are the initial colonizer Streptococcus, early colonizer Veillonella, middle colonizer Porphyromonas gingivalis, and Fusobacterium nucleatum [1-4]. The ability of individual bacteria to generate biofilms increases dramatically in the presence of the Veillonella genus**.** It is well known that oral biofilms are complex and dynamic microbial structures. The formation of biofilm in oral cavity has four stages: transport of bacteria, initial bacterial adhesion, attachment, and biofilm maturation. The initial stage of biofilm formation is the adhesion of salivary proteins. The surface of prosthodontic dental materials exposed to the oral environment is covered by salivary proteins [5-7]. Bridges and crowns are fixed prosthetic devices that are cemented onto existing teeth or implants by a dentist or prosthodontist. However, any foreign bodies inserted in oral cavity may provide new niches for the microorganisms, promoting biofilm accumulation. Such biofilm formation on dental materials appears to be like that around natural tooth, which potentially contributes to damage to the mineralized tissues or infections of the soft tissues [8, 9]. Due to the great mechanical properties, biocompatibility, and excellent esthetic properties, zirconia has been widely applied for the fabrication of crowns, bridges, and ceramic posts in dentistry. ZrO_2, a ceramic material used for medical devices, displays good esthetic appearance, high mechanical strength, and high biocompatibility and is used in a wide range of indications, such as frameworks, implants, and abutments. In addition, it's very good long-term stability and reliability was proven in a 10-year clinical study. These excellent material properties and the transformation behavior are explained by the yttrium oxide stabilization of ZrO_2. ZrO_2 has also been demonstrated as a material for primary crowns in the double crown technique and has featured itself as an alternative to a gold alloy [10-15]. Polyetheretherketone (PEEK) is a semi-crystalline linear polycyclic thermoplastic that has been proposed as a substitute for metals in biomaterials. An alternative restoration material (poly ether ether ketone [PEEK]) has been successfully used over the last years in the medical field, and orthopedics, specifically. A modified PEEK material containing 20% ceramic fillers is a high-performance polymer (BioHPP; Bredent GmbH, Senden, Germany), which presents high biocompatibility, good mechanical properties, high temperature resistance, and chemical stability. Due to a 4 GPa modulus of elasticity, it is as elastic as bone and can reduce stresses transferred to the abutment teeth. Furthermore, the white color of BioHPP frameworks provides a different esthetic approach than the conventional metal framework display does. Additional advantages of this polymer material are elimination of allergic reactions and metallic taste, high polishing qualities, low plaque affinity, and good wear resistance [16-19]. Dental ceramics are the restorative material of choice for indirect restorations, mainly due to their biocompatibility, low thermal conductivity, color stability, and aesthetics. Dental ceramics are used in restorative dentistry because of their success rate as well as diverse range of chemical and structural compositions, resulting from recent improvements in biomaterial technology. These materials commonly consist of both glassy and crystalline phases, which are usually heat-treated to provide desirable properties. While the glassy phase contributes to the aesthetics of the ceramics, the crystalline phase is responsible for the mechanical properties of the material [20, 21].

In the present study, we analyzed three types of crown materials (zirconia, ceramics and BioHpp), assessing their promotion of biofilm development.

* email: danab1978@yahoo.com; dbossu1@gmail.com, clinicasolodent@gmai.com

Experimental part

Materials and method

The study group included 60 patients (males, females), aged 30-50 years, from urban and rural environment. For all patients, it was explained the materials and method as well as the aim of the study to obtain the informed consent, written accordingly to the regulation. For each patient they have been made dental crowns as follows: BioHpp (Bredent) (n = 20), Ceramics (VITA VMK Master) (n=20), and Zirconia (VITA In-Ceram) (n = 20).

The inclusion criteria were as follows: age 30-50 years; good systemic status; balanced nutrition;

Exclusion criteria: poor systemic status; nutritional imbalance, with excessive consume of sugars; affected salivary function or medication interfering with saliva flow; poor oral hygiene.

The biofilm determination was made one week after definitive cementation and, respectively 6 months.

Working steps: The collecting stick is drawn out from the test tube and the sample is collected by scraping one time the tested surface (crowns occlusal surface and crown-gum interface). The collecting stick is reintroduced in the test tube and is covered, and the environment is released by bending the upper surface of the test tube and by pressing it between fingers. After that the collecting stick is drawn out from the liquid environment of the test tube and is introduced into the reading device. After 15 s the result can be read or can be transferred to the computer. System SURE II will give a score between 0 and 9.999. A score under 1.500 indicates a low activity of the bacterial biofilm. A score over 1500 indicates a high microbial load and accentuated activity of the bacterial biofilm.

Result and discussions

Determinations of ATP values for each material (BioHpp, Ceramics and Zirconia) are shown in table 1. ATP mean values after 7 days for crown-gum interface are as follows: BioHpp - 1173.5, Ceramics - 2265.6, Zirconia - 1951.23. ATP mean values after 6 months for crown-gum interface are as follows: BioHpp - 3965.6, Ceramics - 3694.71, Zirconia - 2573.95.

	Mean
	Statistic
BioHpp 7 days	**1173.5**
BioHpp 6 months	**3965.6**
Ceramics 7 days	**2265.6**
Ceramics 6 months	**3694.71**
Zirconia 7 days	**1951.23**
Zirconia 6 months	**2573.95**
Valid N (listwise)	

Table 1
STATISTICAL ATP VALUES AFTER 7 DAYS AND, RESPECTIVELY 6 MONTHS

To see if there were statistically significant differences between the values obtained from the measurements for the three studied materials, we are using the nonparametric Wilcoxon test, the equivalent of the t test for pair samples. In table 2 -table 6, we can see that the values indicate the significance level of this test. The Z scores are -5.5511 and - 3.817 and have a two-tailed probability of 0.0001. This means that the differences between the two variables - (7 days - 6 months) - are statistically significant, also indicates the Mann-Whitney base statistics, the U value for each pair of materials being 17.00, 379.500, and 495.500, which is statistically significant, $p = 0.0001 < 0.05$.

This study explored the development of dental biofilm around the crown-gum interface, for three types of commercially available ceramic materials. The findings show that in terms of studied materials, low levels of bacterial biofilm determinations after 7 days, have revealed BioHpp and zirconium, as compared with the ceramics. After 6 months, the values obtained for bacterial biofilm were statistically significantly higher compared to those recorded after 7 days.

Comparing the materials between them at the same time intervals, statistically significant differences were found, so at the 7 days determinations, the BioHpp having the lowest values, followed by zirconium and ceramics. After 6 months, the situation of the figures changed significantly, the smallest being for zirconium, followed by BioHpp and ceramics.

The effect of surface roughness on biofilm growth has been assessed by both in vitro and in vivo assays. The current study is in line with previous research in the field that shows that, the bacterial biofilm development in different ways depending on the material of manufacture of dental crowns. Also, as expected, the number of bacteria adhered on the dental crowns surface increased in time, which literature studies conclude that the correlation between surface properties, which degrades as time passes, and bacteria adhesion are in close contact [4].

Bollen et al. [17] found that the range in surface roughness of different intraoral hard surfaces was found to be wide, and the impact of dental treatments on the surface roughness is material-dependent, some clinical techniques result in a very smooth surface (compressing of composites against matrices), whereas others made the surface rather rough. Hahnel et al. [5] conducted research on the surface characterization of dental ceramic and the correlation of initial adherence of three oral streptococcal strains, but no correlation has been observed. Discordance may be derived from several different factors relating to both bacteria and substratum. Pita et al. [6] studied the behavior to form biofilm of five oral streptococci species on various dental implant surface topographies. Their data showed that S. cricetus, S. mutans, and S. sobrinus exhibited higher biofilm formation compared to S. salivarius and S. sanguinis, suggesting that biofilm

	Days7-6M	N	Mean Rank	Sum of Ranks
ATP-7D	BioHpp	20	20.50	820.00
	Zirconia	20	60.50	2420.00
	Total	40		

Mann-Whitney U	17.000
Wilcoxon W	820.000
Z	-7.698
Asymp. Sig. (2-tailed)	**.000[a]**

a. Grouping Variable: days7

Table 2
BIOHPP'S COMPARATIVE VALUES AND ZIRCONIA AFTER 7 DAYS

	Days7	N	Mean Rank	Sum of Ranks
ATP-7D	Ceramics	20	29.99	1199.50
	Zirconia	20	51.01	2040.50
	Total	40		
Mann-Whitney U		379.500		
Wilcoxon W		1199.500		
Z		-4.046		
Asymp. Sig. (2-tailed)		.000[a]		

a. Grouping Variable: days7

Table 3
CERAMICS'S COMPARATIVE VALUES AND ZIRCONIA AFTER 7 DAYS

	Months 6	N	Mean Rank	Sum of Ranks
ATP6	BioHpp	20	**20.60**	824.00
	Zirconia	20	**60.40**	2416.00
	Total	40		
	Mann-Whitney U	456.000		
	Wilcoxon W	1263.000		
	Z	-3.115		
	Asymp. Sig. (2-tailed)	.001[a]		

a. Grouping Variable: 6 months

Table 4
BIOHPP'S COMPARATIVE VALUES AND ZIRCONIA AFTER 6 MONTHS

Table 5
BIOHPP'S COMPARATIVE VALUES AND CERAMICS AFTER 6 MONTHS

	Days7	N	Mean Rank	Sum of Ranks
ATP6	BioHpp	20	**36.01**	737.00
	Ceramics	20	**54.83**	2503.00
	Total	40		
Mann-Whitney U		17.000		
Wilcoxon W		737.000		
Z		-7.535		
Asymp. Sig. (2-tailed)		.000[a]		

a. Grouping Variable: 6 months

	Months 6	N	Mean Rank	Sum of Ranks
ATP6	Ceramics	20	**32.89**	1315.50
	Zirconia	20	**48.11**	1924.50
	Total	40		
	Mann-Whitney U	495.500		
	Wilcoxon W	1315.500		
	Z	-2.930		
	Asymp. Sig. (2-tailed)	.003[a]		

a. Grouping Variable: 6 months

Table 6
CERAMIC'S COMPARATIVE VALUES AND ZIRCONIA AFTER 6 MONTHS

formation depends on not only the surface topography but also the bacteria species involved.

The findings of this study are consistent with those of Elter et al. [20], who argues that in all surface properties plaque adhesion in supragingival areas was significantly higher than in subgingival areas. Biofilm accumulation in supragingival areas was significantly increasing by higher surface roughness, whereas this influence was not detected in subgingival areas.

Conclusions

Within the limitations of this research, we concluded that the materials used in dental bridges may have the ability to store bacterial biofilm from the first days of in vivo use. The current study deepened our understanding on the mechanism of bacterial adhesion and biofilm formation on the indirect dental materials. More researches *in vitro* and *in vivo* are needed with respect to other oral bacteria and factors that may affect the results. Biofilm formation on various types of dental ceramics differed significantly, zirconia exhibited low plaque accumulation.

Aknowledgement: This work was supported by a grant of the Romanian Ministery of Research and Innovation, CCCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0239/ 60PCCDI 2018, within PNCDI III

References

1.TERANAKA, A., TOMIYAMA, K., OHASHI, K., MIYAKE, K., SHIMIZU, T., HAMADA, N., MUKAI, Y., HIRAYAMA, S., NIHEI, T., Journal of Oral Science., **60,** no. 1, 2018, p. 129.

2.MERK, S., WAGNER, C., STOCK, V., EICHBERGER, M., SCHMIDLIN, P.R., ROOS, M., STAWARCZYK, B., Materials, **9**, no. 11, 2016, p. 908.

3.KYOUNG, H.K., LOCH, C., WADDELL, J.N., TOMPKINS, G., SCHWASS, D., Int. J. Dent., **2017**, 2017, ID Article: 7627945. doi: 10.1155/2017/7627945.

4.ZHAO, B., VAN DER MEI, H. C., SUBBIAHDOSS, G., Dental Materials, **30**, no. 7, 2014, p. 716.

5.HAHNEL, S., ROSENTRITT, M., HANDEL, G., BURGERS, R., *Dental Materials*, **29**, no. 8, 2009, p. 969.

6.PITA, P.P.C., RODRIGUES, J.A., OTA-TSUZUKI C., BioMed Research International, **2015**, 2015, ID Article 159625.

7.TSVETESLAVA, I.I., MARGARITA, B., IBRJM, S., DAMIAN, E., RADOSLAV, I., Research J. of Pharmaceutical Biological & Chemical Sciences, **7,** no**.** 6, 2016, p. 736.

8.SANCHEZ, M.C., TOLEDANO-OSORIO, M., BUENO, J., et al., Dental Materials, **35**, no. 1, 2019, p.156.

9.MARTU, I., BOSINCEANU, D.G., FORNA, N.C., VITALARIU, M.A., LUCHIAN, I., BOSINCEANU, D.N., Rom. J. Oral Rehab., **9**, no. 2, 2017, p. 60.

10.BOSINCEANU, D.N., BOSINCEANU, D.G., BOLAT, M., BACIU, R., FORNA, N.C., Rom. J. Oral Rehab., **8**, no. 1, 2016, p. 7.

11.BOSINCEANU, D.G., SANDU, I.G., BOSINCEANU, D.N., FORNA, D.A., BOLAT, M., FORNA, N.C., Mat. Plast., **54**, no. 4, 2015, p. 733.

12.STAWARCZYK, B., BEUER, F., WIMMER, T., JAHN, D., SENER, B., ROOS, M., Applied Biomaterials., **7**, 2013, p. 1209.

13.VITALARIU, A.M., DIACONU-POPA, D., TATARCIUC, M., BARLEAN, L., Rev. Chim. (Bucharest), **66**, no. 10, 2015, p. 1720

14.FORNA, N.C., TATARCIUC, M., VITALARIU, A.M., BACIU, R.E., DIACONU-POPA, D., Rom. J. Oral Rehab., **10**, no. 4, 2018, p. 13.

15.DIACONU-POPA, D., VIALARIU, A.M., TATARCIUC, M., Rom. J. Oral Rehab., **9**, No. 2, 2017, p. 54.

16.BOLLEN, C.M.L., LAMBRECHTS, P., QUIRYNEN, M., Dental Materials, **13**, no. 4, 1997, p. 367.

17.MARTU, I., GORIUC, A., MARTU, M.A., VATA, I., BACIU, E.R., MOCANU, R., SURDU, A.E., POPA, C., LUCHIAN, I., Rev. Chim.(Bucharest), **68**, no.10, 2017, p. 2407.

18.OGODESCU, A.S., MORVAY, A.A., OGODESCU, A.E., LUCA, M.M., DINU, S., ZETU, I., Rev. Chim. (Bucharest), **66**, no.1, 2015, p. 101.

19.EARAR, K., GRIGOROIU, R., SCUTARIU, M.M., VASILE, E., ANTONIAC, A., DRAGOMIR, L., GRADINARU, S., Rev. Chim. (Bucharest) **68**, no.7, 2017, p. 1560.

20.ELTER, C., HEUER, W., DEMLING, A., HANNIG, M., HEIDENBLUT, T., BACH, F.W., STIESCH-SCHOLZ, M., Int. J. Oral & Maxillofacial Implants, **23**, no. 2, 2008, p. 327.

Manuscript received: 7.12.2018