Study Regarding the Modulation Capacity of Oral Bacterial Biofilms Community Climax of Different Restorative Materials

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The aim of this study was to demonstrate the use of adenosine triphosphate– (ATP–) driven bioluminescence as an innovative tool for the rapid chairside enumeration of oral bacteria (including plaque streptococci) and assessment of oral hygiene and caries risk. The study group included 60 patients with 120 direct coronal restorations with three tipes of restorative materials (resin-modified glass ionomer cement GC Fuji VIII, giomer Beautifill Flow Plus Shofu and composite resin (GC Gradia) from witch we have collected 120 specimens using a luciferase-based assay system(system SURE II). The values of ATP were obtained with System SURE II device and statisticaly analised with Wilcoxon Test. The lowest value was shown for glass ionomer cement GC Fuji VIII, comparing with composite resins, but in time we have seen the increase of ATP for all three restorative materials.

Keywords: ATP, restorative materials, oral biofilm, caries risk

The dental caries was considered a nutritional-bacterial disease, infectious and transmissible [1]. The carious lesions are considered the signs of an imbalance between calcium and phosphate ions in the dental tissues and saliva, mediated by the microorganisms of the bacterial plaque and influenced by ecological factors [2].

In the last years, the modern dentistry shifted from the predominant surgical approach to a preventive-therapeutical approach focused on prevention, early diagnosis of the incipient carious lesions and customized medical treatment of the carious disease.

The demineralisation processes are associated to proper conditions for the bacterial metabolic processes [3]. The dental caries start when these conditions select a cariogenic bacterial plaque, uncontrolled by specific means. The demineralised organic matrix can be ireversible degraded by the proteolitic bacteries, process followed by the loss of hard dental tissue and cavity formation [2].

All the implied factors interact to maintain the dynamic balance of the oral ecosystem. Each alteration of one factor determines new changes in the ecological niches, inducing reactions from the bacterial communities to reestablish a new balance. This new state of balance will be defined by new parameters of the bacterial population (composition, density, metabolic activity, interactions with oral ecosystem). Non-pathogenic bacterial plaque can change, in certain conditions, in a cariogenic or periodontopathic bacterial plaque [2].

The dental medicine requests both early, accurate, complete diagnostic and the development of the methods used in the determination of the cariogenic risk. The orientation to the medical concept of the carious disease with the precise detection of the risk factors could identify the cariogenic risk patients, allowing the orientation of the therapy to less invasive and more effective preventive treatments. The carious process is initiated by bacterial biofilm produced on any hard surface exposed to adequate quantities of water and nutrients. 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 [4-7].

The instruments for the assessment of the cariogenic risk are required for the diagnostic of the carious disease and the choice of the preventive-therapeutical options. Most previous researches were focused on the social factors, behavioral factors, diet and clinical parameters. Most of these variables, like frequency of dental check-ups, sugars consume, fluoride exposure, brushing habits, clinical assessment of dental plaque, are subjective observations [8]. To improve the assessment instruments related to the cariogenic risk, American Academy of Pediatric Dentistry (AAPD) promoted the use of the microbiological tests as an additional instrument for the assessment of the cariogenic risk [9-12].

The assessment of ATP by bioluminescence is one of these microbiological tests. This quantitative method is fast, accurate and noninvasive and is recommended for the to measurement of the bacterial load in various research fields. In the last years, it was also used in dentistry to assess the microorganisms in saliva and dental biofilm [13-17].

ATP (adenosine triphosphate) is a chemical substance acting as an energy source for all living organisms. The presence of ATP is considered the proof for the presence of a living organism or a substance produced by this organism.

ATP (Adenosine Tri-Phosphate) measurement was developed to estimate bacterial cell numbers in biological samples to ensure microbiological quality. The bioluminescent luciferin-luciferase method has largely been used in order to evaluate the ATP content in living cells from numerous biological media including dental plaque [18]. As it turns out, when looking to identify the presence of the bacteria at play in the caries infection, ATP is an extremely predictive biomarker for acidogenic and aciduric organisms. These bacteria survive and thrive in acidic pH environments because they have the ability to pump the hydrogen ions (protons) out of their cell. This requires a tremendous expenditure of ATP [19]. Therefore

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ATP bioluminescence provides one clear look into the acidic nature of the biofilm and its potential to cause the net mineral loss associated with cavity formation.

The system SURE II uses the bioluminescence measurement technology of ATP to detect the levels of the acid-producers bacteria and the bacteria implied in the demineralization of the hard dental tissues.

The acidogenic bacteria contain up to 100 times more ATP than non-producers acid bacteria. The light produced when this ATP is in contact to the reference liquid will be emitted in direct relation to the ATP quantity [20]. System SURE II measures the quantity of the light emitted in this reaction and offers information about the potential levels of cariogenic bacteria presented in the bacterial biofilm. The reading appears on System SURE as a number of relative light units (RLU), giving information about the presence of a noncariogenic biofilm or a biofilm with the cariogenic bacterial high load.

The aim of this study is to assess, using ATP bioluminescence method, the carioactivity of the biofilm adjacent to a resin-modified glass ionomer cement, a giomer and a composite resin (Gradia, GC Company).

Experimental part

Materials and Method

The study group included 60 patients (males, females), aged 20-45 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.

120 direct coronal restorations were performed as follows: resin-modified glass ionomer (Fuji VIII, GC Company) (n = 40), giomer (Beautifill Flow Plus, Shofu) (n = 40), and composite resin (Gradia, GC Company) (n = 40), accordingly to the producer protocol.

The inclusion criteria were as follows: age 20-45 years; good systemic status; balanced nutrition; medium cariogenic risk; active dental caries localized on occlusal and buccal surfaces, without pulp complications.

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

Following the clinical examen, the next parameters were assessed:

a.DMFT, DMFS indices.

b.Carioactivity of the biofilm adjacent to the direct restorations, using ATP bioluminescence method after 24 h and after 6 months after filling placement.

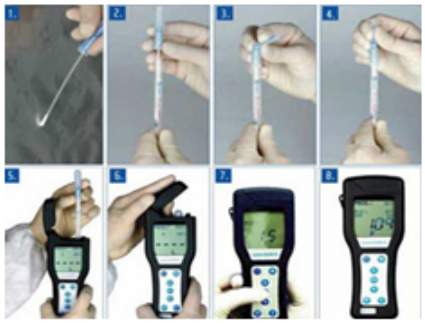

Fig. 1. Working steps using SURE II for ATP assessment

Working steps:

The collecting stick is drawn out from the test tube and the sample is collected by scraping one time the tested surface (to the interface enamel-restoration). 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 (fig. 1). 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 seconds 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 cariogenic activity of the bacterial biofilm. A score over 1500 indicates a strong cariogenic community climax of the bacterial biofilm.

Result and discussions

The mean ATP values for each restorative material (glass ionomer, giomer, composite resin) were determined and presented in table 1. ATP mean values for interface enamel-restoration, after 24 h, were as follows: 864.73 for glass ionomer, 2999 for giomer, 4237.2 for composite. ATP mean values for interface enamel-restoration, after 6 months, were as follows: 1080.8 for glass ionomer, 3702.53 for giomer, 4654 for composite. The comparative analysis,

	Mean
	Statistic
G24hours	864.73
G6months	1080.80
CF24hours	2999.00
CF6months	3702.53
C24hours	4237.20
C6months	4654.00
Valid N (listwise)	

Table 1
STATISTICAL ATP VALUES AFTER 24 HOURS AND 6 MONTHS

Ranks

		N	Mean Rank	Sum of Ranks	
G6luni - G24ore	Negative Ranks	0[a]	.00	.00	a. G6luni < G24ore
	Positive Ranks	40[b]	20.50	820.00	b. G6luni > G24ore
	Ties	0[c]			c. G6luni = G24ore
	Total	40			
CF6luni - CF24ore	Negative Ranks	0[d]	.00	.00	d. CF6luni < CF24ore
	Positive Ranks	40[e]	20.50	820.00	e. CF6luni > CF24ore
	Ties	0[f]			f. CF6luni = CF24ore
	Total	40			
C6luni - C24ore	Negative Ranks	5[g]	25.20	126.00	g. C6luni < C24ore
	Positive Ranks	35[h]	19.83	694.00	h. C6luni > C24ore
	Ties	0[i]			i. C6luni = C24ore
	Total	40			

Table 2
RESULTS OF WILCOXON TEST. INDICATION OF THE NUMBER OF NEGATIVE, POSITIVE AND ABSENT DIFFERENCES RELATED TO THE DATES IN RELATION TO THE TYPE OF RESTORATIVE MATERIAL

Test Statistics[b]

	G6months-G24hours	CF6months-CF24hours	C6months-C24hours
Z	-5.511[a]	-5.511[a]	-3.817[a]
Asymp. Sig. (2-tailed)	.000	.000	.000

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

Table 3
RESULTS OF WILCOXON TEST. STATISTICAL VALUES OF EACH RESTORATIVE MATERIAL, RECORDED AFTER 24 h AND 6 MONTHS

after 24 h and 6 months, shows a significant increase of ATP values to the interface enamel-restoration.

The table 2 indicates the number of negative, positive and absent differences related to the dates in relation to the type of restorative material. The analysis of G6months - G24 hshows, for all 40 tests, values G6months > G24 h. The same result was obtained for the analysis CF6months - CF24 h. A different result was found for the analysis C6months-C24 h, with 5 tests showing C6months < C24 h, and 35 tests showing C6months > C24 h.

To determine if ATP values are significant, we used non-parametric test Wilcoxon, analyzing the values for each restorative material (glass ionomer, giomer, composite resin), after 24 h, and 6 months. The values for table 3 show the statistical values of each restorative material, recorded after 24 h and 6 months: G24 h is 0.8 from the value G6months, CF24 h is 0.8099 from the value CF6months, and C24 h is 0.910 from the value C6months.

The result of table 3 indicates the significance level of this test. The scores Z are -5,5511 and -3,817, and have 0,0001 two-tailed probability. This result mean that the difference between the two variables (24 h, 6 months) is statistically significant.

This in vivo study was designed to quantify the retention of dental plaque to the interface tooth-restoration using the direct measurement technique of ATP by bio-luminescence. Comparing with other techniques, with measurement periods up to 5 days, this method is a fast and appropriate screening alternative.

The study performed by H.J. Busscher et.al. demonstrat the association between biofilms and the surface deterioration of composites and glass ionomer cements, which enhances biofilm formation again [21]. The residual monomer released from composites influences biofilm growth *in vitro*, but effects *in vivo* are less pronounced, probably due to the large volume of saliva into which compounds are released and its continuous refreshment [22]. Nurit Beyth analyses the development of bacterial biofilm on the surfaces of the composite direct restorations in comparison with amalgam and demonstrates the antibacterial properties of amalgam, and the absence of antibacterial properties for composite resins. This may explain the clinical observation of biofilm accumulated more on composites compared to amalgams [23]. Masaomi IKEDA studies the bacterial biofilm formed on the direct composite resins restorations [17, 24, 26]. Muktar A. Elalem performed a study that quantifies, by ATP bioluminescence, the bacterial biofilm around the orthodontic brackets performed by different materials [27]. Numerous studies assessed the bacterial biofilm localized on the surface of the composite resins restorations, but just a few studies were focused on the carioactivity of the biofilm adjacent to the coronal direct restorations.

The results of this study showed significant statistical differences related to ATP quantity measured on different types of material restorations after 24 hours and 6 months. This can be explained by the wear phenomenon of restorative materials which may suffer, in time, multiple degradation, such us corrosive, abrasive, adhesive and fatigue [26].

This result highlights the recommendation to avoid composite resins as restorative materials for the patients with poor oral hygiene.

The high sensitivity of the bioluminescence method facilitates the analysis of small samples from dental plaque collected from the teeth surfaces.

The use of the bioluminescence method offers the possibility to perform a more comprehensive analysis of the bacterial content than it was possible using the conventional bacteriological methods. The data presented both in this study and recent researches show that we can analyze the carioactivity from small samples collected from individual teeth surfaces.

The sensitivity and the speed of bioluminescence method combined with the ability to process the data, extend further the analysis possibilities both of the viable cells and total cell mass for a high number of dental bacterial plaque samples [25].

The ATP assessment using bioluminescence method was used as a bacterial quantitative method in various research fields, but only recently it was introduced for saliva and dental bacterial plaque determinations (Surre II, Cariscreen ATP Meter, luminometrul Veritas) [22].

Our clinical data agree with the hypothesis that ATP measurements present a statistically significant association with the number of bacteria in dental plaque and saliva (including oral streptococci), and can be used as a potential assessment instrument for the oral hygiene and the cariogenic risk. The clinical use of this technology highlights other features of the dental biofilm by the quantitative measurement of the cariogenic risk, allowing the dentist to diagnose dental caries in relation to the cariogenic risk and to implement an individualized treatment against carious disease [27]. Early caries detection and quantification of lesions to establish their progression or arrest is crucial if dental approach is going to be changed from mainly operative to preventive. Early caries diagnosis is also important for clinical dental researches - the ability of accurate detection and determination of the size of early lesions may permit the use of shorter intervals and lesser number of patients to obtain the effectiveness of caries preventive measures [5].

Conclusions

The bacterial load of the biofilm adjacent to the composite resins restorations is related to the type of restorative materials used. The lowest ATP values are recorded for glass ionomer cement. Regarding the changes in oral biofilm after 24 h and 6 months, an increasing of the mean ATP values was observed for all restorative materials, demonstrating the importance of the time factor in the apparition and the evolution of the caries lesions. The assessment and early diagnostic of the caries risk will allow the application of individualized treatment against caries disease.

Reference

1. PAES LEME, A.F., KOO, H., BELLATO, C.M., BEDI, G., CURY, J.A., J. Dent. Res., **85,** 2006, p. 878.

2. IOVAN, G., Caria dentarã repere etiologice si patogenice, Ed. Gr.T.Popa, Iasi, 2011, p. 6, 39.
3. ZERO, D., FONTANA, M., LENNON, A.M., J. Dent. Educ., **65,** 2010, p. 32.
4. STOLERIU, S., IOVAN, G., PANCU, G., GEORGESCU, A., SANDU, A.V., ANDRIAN, S., Rev. Chim (Bucharest), **66,** no. 9, 2015, p. 1120.
5. CIRLIGERIU, L., SINESCU, C., BOARIU, M., NEGRUTIU, M.D., NICA, L., Rev. Chim (Bucharest), **66**, no. 9, 2015, p. 1479.
6. BOSINCEANU, D.N., SANDU, I.G., BOSINCEANU, D.G., FORNA, N.C., Rev. Chim (Bucharest), **65**, No. 4, 2014, p. 466.
7. BALAN, A., STOLERIU, S., ANDRIAN, S., SANDU, A.V., IOVAN, G., SERBAN-PINTILICIUC, V., Rev. Chim (Bucharest), **66**, no. 2, 2015, p. 182.
8. CAROUNANIDY, U., SATHYANARAYANAN, R., J. Conserv. Dent., **12**, no. 3, 2009.
9. REICH, E., LUSSI, A., NEWBRUN, E., Int. Dent. J., **49,** no. 1, 1999, p. 15.
10. ISMAIL, A.I., NAINAR, S.M., SOHN, W. Pediatr. Dent., **25,** no. 5, 2003, p. 425.
11. TSANG, P., QI, F., SHI, W., Pediatr. Dent., **28,** no. 2, 2006 p.188.
12. CRALL, J., J. Pediatr. Dent., **27,** no. 4, 2005, p. 323.
13. LI, Y., GE, D. SAXENA, Y., CAUFIELD, P.W., J. Clin. Micro., **45**, no. 1, 2007, p. 81.
14. PELLEGRINI, P., SAUERWEIN, R., FINLAYSON, T., MCLEOD, J., COVELL, D.A., MAIER, T., Am. J. Orthod. Dentofacial Orthop., **135,** 2009, p. 29.
15. ALICE, C.L. HARTY, D.W.S., JAQUES, A.J., Microbiol, **150**, 2004, p. 159.
16. 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.
17. MURARIU, A., SAVIN, C., FEIER, R., BALAN, A., Rev. Chim (Bucharest), **67**, No. 9, 2015, p. 1876.
18. GALLEZ, F., FADEL, M., SCRUEL, O., CANTRAINE, F., COURTOIS, P., Cell. Biochem. Funct., **18,** no. 2, 2000, p. 103.
19. ALICE, C.L. HARTY, D.W.S., JAQUES, A.J., Microbiol, **150**, 2004, p. 1339.
20. ARAKAWA, H., KARASAWA, K., IGARASHI, T., SUZUKI, S., GOTO, N., MAEDA, M., Anal. Biochem., **333,** 2004, p. 302.
21. GHARECHAHI, M., MOOSAVI, H., FORGHANI, M., Journal of Biomaterials and Nanobiotechnology, **3,** 2012, p. 541.
22. BUSSCHER, H.J., RINASTITI, M., SISWOMIHARDJO, W., VAN DER MEI, H.C., Journal of Dental Research., **89**, No. 7, 2010, p. 657.
23. NURIT, B., J. DOMB, A., WEISS, E.I., Journal of Dentistry, **35,** 2007, p. 201.
24. IKEDA, M., MATIN, K., NIKAIDO, T., TAGAMI, J., Dental Materials Journal **26,** no 6, p. 915.
25. SANCHEZ, M.C., LLAMA-PALACIOS, A., BLANC, V., LEON, R., HERRERA, D., SANZ, M., J. Periodontal Res., **46**, 2011, p. 60.
26. NICA, I., IOVAN, G., GHIORGHE, A., STOLERIU, S., PANCU, G., ANDRIAN, S., Mat. Plast., **51,** no. 3, 2014, p. 284.
27. FAZILAT, S., SAUERWEIN, R., MCLEOD, J., FINLAYSON, T., ADAM, E., ENGLE, J., Pediatr. Dent., **32,** 2010, p. 19

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