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Study of the pathogen levels on implant abutments with different coating characteristics

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Abstract

The aim of the present study was to obtain preliminary data on the levels of pathogens according to their DNA concentration in patients with implant abutments of two different surface characteristics.

Material and method: Fifty patients from the city of Sofia treated with dental implants were subjected to a quantitative molecular biological analysis of microorganisms: 25 of the patients received implants with titanium nitride-coated abutments and 25 were treated with implants with titanium nitride-uncoated abutments.

Results: The quantitative molecular biological analysis of microorganisms performed showed no significant association with the presence or absence of coating. Patients in both groups had similar relative proportions of Pg ($p = 0.225$), Td ($p = 0.571$), Tf ($p = 0.333$), Pi ($p = 0.758$), Pm ($p = 0.089$), Fn ($p = 0.087$), En ($p = 0.110$), Cg ($p = 0.774$).

The above results justify the need of further and more detailed quantitative molecular-biological analysis of microorganisms in patients treated with dental implants and abutments of different surface characteristics.

Keywords: oral pathogens, implant abutments, coating characteristics

Introduction

Pure titanium and its alloys demonstrate various advantages - excellent biocompatibility, corrosion resistance, high mechanical strength and low modulus of elasticity. Pure titanium (cpTi) used in dental implantology is available in four different grades, while grade 5 is an aluminum-vanadium-titanium alloy [1]. Titanium surfaces provide epithelial and connective tissue attachment and minimize bacterial colonization. Biofilm formation is influenced by the surface characteristics of the implant abutment, its chemical composition, surface energy and roughness. Increased roughness leads to faster bacterial adhesion, accumulation, formation, and maturation of the bacterial biofilm [2-5].

The mechanisms of biofilm formation around implant abutments and natural teeth are similar. What distinguishes a dental implant from a natural tooth is the presence of a metal, usually titanium abutment, which is in contact with the gingival tissues. Low molecular weight mucins, which are normally isolated from the enamel of natural teeth, are absent around implant abutments. This leads to a qualitative difference in early biofilm formation. This is believed to be one of the reasons for the slower formation of bacterial plaque around implants. However, these differences do not seem to affect the bacterial composition of the early biofilm. Its formation depends on the properties of the surface: chemical composition, roughness and surface energy [3].

Different implant abutments have different chemical and physical properties of the surface on which bacterial biofilm forms. Also, the roughness of the surfaces and the type of material have a significant effect on the volume and composition of the bacterial biofilm [4,6]. Depending on the roughness, a classification of dental implant surfaces has been proposed: smooth (<0.5 μm), minimally rough (0.5–1.0 μm), moderately rough (1.1–2.0 μm), and rough (>2.0 μm) titanium surfaces [7-9]. In a series of studies, increasing surface roughness above the threshold of 0.2 μm and/or increasing surface energy has been found to facilitate biofilm formation [6].

The effect of surface energy on plaque formation and maturation around implants has been studied in high- or low-surface-energy implant abutments [6,11-13]. When comparing surfaces of different roughness, surfaces of higher roughness have higher bacterial adhesion after 2 hours. After 14 hours, the biofilm grows with a similar structure on all investigated surfaces. This indicates that surface condition influences adhesion and biofilm formation [14,15].

Aim

The aim of the present study was to obtain preliminary data on the levels of pathogens according to their DNA concentration in patients with implant abutments of different surface characteristics.

Material And Methods

Fifty patients from the city of Sofia treated with dental implants were subjected to a quantitative molecular biological analysis of microorganisms: 25 of the patients received implants with titanium nitride-coated abutments and 25 were treated with implants with titanium nitride-uncoated abutments.

To achieve the goal, the EURx #E3550 kit was used for the isolation of DNA from body fluids and other tissues. The manufacturer's working protocol was followed. Samples were lysed initially in Lyse T buffer with RNase to degrade RNA. Proteinase K solution was added to the samples to digest the proteins. After 10 minutes of incubation at T = 70 °C, an equivalent amount of 99% ethanol was added to the samples. Purification and extraction of DNA was carried out through minicolumns binding the DNA molecule. The

DNA was stored in Tris-EDTA solution at T = -20 °C. All the necessary materials, buffers and solutions were available in the EURx #E3550 kit.
The quality of some of the isolated DNA samples was checked by horizontal gel electrophoresis in TAE electrophoresis buffer, 10X Sterile Solution (Tris-Acetate-EDTA, CANVAX). The gel was prepared with 0.8% agarose (SeaKem® LE Agarose, LONZA, Cat. #50004L). When loading the gel, 2 µL of DNA from each sample was mixed with 1 µL of the loading buffer (6X Loading Buffer BLUE, Cat. #EO260-01) and 3 µL of distilled sterile water. SERVA FastLoad 100 bp DNA Ladder (SERVA, Cat. #39316) was used as a marker. PowerPac BASIC (Bio Rad) and an electrophoresis bath (Cleaver Scientific Ltd.) were used to run the gel electrophoresis.
The IBM SPSS Statistics for Windows, Version 27.0 program (Armonk, NY: IBM Corp.) was used for the statistical processing of the data.

Results

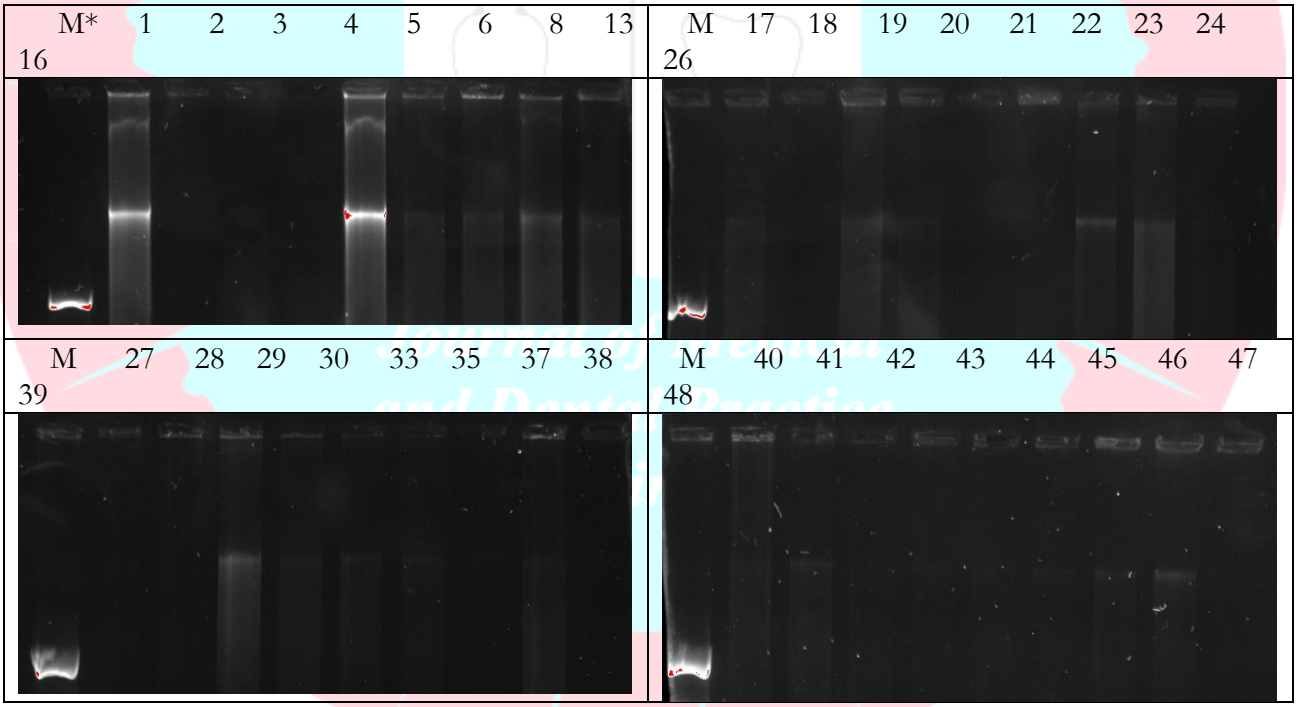
The results obtained by the applied methodology are presented in Tables 1 and 2. Table 1 shows the concentration of DNA isolated from patient samples.

Table 1. Concentration and quality of DNA isolated from patient samples.

Sample	Conc. [ng/µL]	$A_{260/280}$
1	36.3	1.86
2	10.9	1.73
3	8	1.86
4	4.8	1.77
5	38.6	1.88
6	9.8	1.78
7	18.1	1.88
8	12.7	1.83
9	10.3	1.83
10	19.2	1.83
11	20.5	1.88
12	30.8	1.90
13	12.9	1.81
14	8.2	1.99
15	10.5	1.87
16	17.9	1.95
17	7.8	1.83
18	5.6	1.62
19	10.4	1.87
20	4.6	1.8
21	3.6	1.87
22	6.1	1.7
23	5.0	2.09
24	12.6	1.92
25	13.1	1.83
26	4.9	1.71
27	5.7	1.76
28	12.2	1.8
29	8.4	1.66
30	9.0	1.41
31	13	1.57

32	9.6	1.55
33	4.2	1.82
34	6.6	1.87
35	6.8	2.2
36	6.6	1.9
37	4.35	2.39
38	10.9	1.82
39	4.0	1.63
40	7.3	1.68
41	6.9	1.96
42	8.5	1.89
43	9.7	1.73
44	7.2	1.88
45	10.2	1.88
46	8.3	1.93
47	6.4	1.74
48	9.4	1.81
49	7.0	1.79
50	7.1	1.93

The subsequent horizontal gel electrophoresis in the electrophoresis buffer allowed quality control of some of the isolated DNA samples. The gel electrophoresis results are presented in Figure 1.



Legend: *M – marker; the white bar below M indicates molecular mass of 3000 bp.

Figure 1. Electrophoresis of DNA isolated from patient samples.

The quantitative molecular biological analysis of microorganisms demonstrated no significant association with the presence or absence of coating (Table 2). Patients in both groups had similar relative proportions of Pg ($p = 0.225$), Td ($p = 0.571$), Tf ($p = 0.333$), Pi ($p = 0.758$), Pm ($p = 0.089$), Fn ($p = 0.087$), En ($p = 0.110$), Cg ($p = 0.774$).

Table 2. Presence of microorganism species in patients with coated and uncoated abutments

Microorganisms	Coated n (%)	Uncoated n (%)	p
Porphyromonas gingivalis (Pg)			
○ Yes	19 (76%)	15 (60%)	
○ No	6 (24%)	10 (40%)	0.225
Treponema denticola (Td)			
○ Yes	13 (52%)	11 (44%)	
○ No	12 (48%)	14 (56%)	0.571
Tannerella forsythia (Tf)			
○ Yes	8 (32%)	5 (20%)	
○ No	17 (68%)	20 (80%)	0.333
Prevotella intermedia (Pi)			
○ Yes	8 (32%)	7 (28%)	
○ No	17 (68%)	18 (72%)	0.758
Peptostrep. (Micromonas) micros (Pm)			
○ Yes	16 (64%)	10 (40%)	
○ No	9 (36%)	15 (60%)	0.089
Fusobacterium nucleatum (Fn)			
○ Yes	14 (56%)	8 (32%)	
○ No	11 (44%)	17 (68%)	0.087
Eubacterium nodatum (En)			
○ Yes	4 (16%)	0 (0%)	0.110
○ No	21 (84%)	25 (100%)	
Capnocytophaga gingivalis (Cg)			
○ Yes	15 (60%)	14 (56%)	
○ No	10 (40%)	11 (44%)	0.774

The presence of different types of microorganisms demonstrated no significant association with the presence or absence of coverage.

Discussion

The results are in compliance with the data of a number of studies on the accumulation of bacteria on metal restorations and in the mouth, according to their surface structure (16-21). The total number of microorganisms in the studied samples did not show significant association between the factors “presence” and “absence” of coating. Porphyromonas gingivalis (Pg) showed the highest frequency, followed by Treponema denticola (Td).

Today, various coatings are known to reduce bacterial adhesion, regardless of whether they are exposed to saliva or not. Their use in the transmucosal components of implant systems may affect the amount and composition of the bacterial biofilm. The results of a number of authors have shown that the number of bacterial cells is higher on pure titanium surfaces than on TiN or ZrN coated ones. The lowest number of bacterial cells was present on the ZrN coating. It was also found that the metabolic activity of bacteria on such coatings was lower than that on pure titanium surfaces. Components of implant systems with a TiN layer have shown a significant reduction in the number of bacteria and this fact may be relevant for the condition of the peri-implant gingival tissues [16].

Conclusion

The above results justify the need for a wider quantitative molecular-biological analysis of microorganisms in patients treated with dental implants and abutments of different surface characteristics. Obtaining a larger volume of scientific data may allow the construction of a map of infectious risks and create prerequisites for a better prognosis of treatment outcomes.

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