INFLUENCE OF FINISHING TECHNIQUES OF TI-BASED ALLOYS USED IN PROSTHETIC RESTORATIONS ON THE FORMATION OF *CANDIDA ALBICANS* BIOFILM

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***Abstract*:** Surface roughness plays an important role in the formation of specific oral cavity biofilm. The paper aims at analyzing the influence of mechanical finishing techniques on the formation of *Candida albicans* biofilms. We studied two different Ti-based alloys used in prosthetic restorations and two finishing techniques: mechanical polishing with rubber polisher and mechanical polishing with cotton polisher and polishing paste. Examinations on roughness have made reference to R_z. The ability to adhere and to form the *Candida albicans* biofilms onto the rough surfaces of the studied samples was highlighted by scanning electron microscopy (SEM).

Microbiological analysis indicates the strong relationship between the surface quality of dental alloys used in prosthetic restorations and the ability of the fungal pathogen of *Candida albicans* type to adhere to these metal surfaces.

Key words: Ti-based alloys, polishing techniques, *Candida albicans* biofilm.

INTRODUCTION

By definition, biofilms are structured microbial communities that get attached to a surface. Biofilms are characterized by structural heterogeneity, genetic diversity, complex community interactions and extracellular matrix with polysaccharide nature [1, 2]. Biofilm formation by the fungal species is a complex and diverse phenomenon. *Candida* species are frequently found in the normal microbiota of the human body [1, 2]. They can easily adhere to the vast majority of biomaterials used in dental prosthetics.

Most research studies on the *Candida* type have considered the formation of *Candida albicans* biofilm.

Candida albicans is common in the oral cavity of elderly patients receiving various forms of removable dentures (removable partial or complete acrylic dentures, removable partial dentures) and it is incriminated as an etiological factor of prosthetic stomatitis [4]. However, there can be stomatitis as a specific entity, because in its etiology several factors interfere, among which we also mention microbial infections, irritations and / or allergies [3].

To colonize any surface, fungal cells must first adhere to the surface of that specific material. Initial attachment of *Candida* cells is a process mediated by nonspecific factors (the hydrophobic character of cell surface and the

electrostatic forces) and specific adhesion. *Candida albicans* has the ability to adhere to human salivary proteins when they cover the biomaterial form which the removable prosthesis is made of [5, 6].

This study aims at identifying the relationship between the characteristics of metal surfaces of the prosthetic restorations, produced by different finishing techniques and the ability of *Candida albicans* to adhere to these surfaces.

MATERIALS AND METHODS

Experimental researches were conducted on two Ti-based alloys generally used in dental prosthetics (Table 1).

Table 1. Non-noble alloys subject to testing

Non-noble alloys	Alloy brand	Chemical composition, [%]						Alloy destination
		Co	Ni	Ti	Cr	Mo	Nb	
Ti – based alloys	Biotan	-	-	99.5	-	-	-	*Bridges/crowns and metallic components of removable partial dentures: major and minor connectors, clasps, attachments.*
	Biotan-Nb	-	-	87.0	-	-	7	

On the cylindrical specimens (Ø7 x 10 mm) made from these alloys, a 3 mm flat facet has been executed upon which roughness measurements were made in three areas located in the same longitudinal direction.

The mechanical polishing applied to the facets was done with a C3 Master Schick micro motor, using a rubber polisher or cotton polisher and a special abrasive paste.

Roughness measurements were performed on special equipment Mitutoyo SJ-301 (Japan), on each probe being conducted in the same direction three determinations.

The strain of Candida albicans isolated from a stomatitis prosthetics was grown on agar Sabouraud for 24 hours at 35ºC; from the developed colonies, we prepared a suspension of levuric cells in a saline peptone buffered solution with pH 7, with a density of 10^6 cells/ml. In this suspension we immersed the sterile specimens from the tested materials for 90 minutes at 35°C – this was the levuric adherence phase. After the time expired, the samples were rinsed in distilled water and then dipped in liquid culture medium - Sabouraud solution with 8% glucose and incubated at 37°C for 24h and 48h respectively. After the two incubation times expired (24 and 48 hours), the samples were extracted from the culture medium, they were rinsed in distilled water to remove the non-adherent cells and they were left for a few minutes at room temperature for drying.

Their surface was then examined by scanning electron microscopy (SEM) on a microscope Quanta 200 3D DUAL BEAM to obtain secondary electron images.

In order not to alter the biological condition of the biofilm obtained, the tests were performed in a LowVac module because this module does not require

metallization or other methods of sample preparation. The working parameters were: working chamber pressure - 60 Pa, working voltage - 10 kV, detector type - LFD (Large Field Detector), working distance ranged from 14 to 25 mm, magnification: 800x, 1200x, 2400x, and platform gradient - 40°.

By using this module, the time elapsed between sample extraction from the culture medium and obtaining the pictures on the microscope did not exceed 15 min.

We took into consideration the following aspects: the surface coverage and biofilm thickness. The surface coverage was examined through image analysis using IQ Materials programme (Media Cybernetics – Canada).

RESULTS AND DISCUSSIONS

The average values of roughness parameter R_z obtained for the mechanically polished metal surfaces are presented in Table 2.

Table 2. The average values of roughness R_z parameter for the metallic surfaces mechanically polished with rubber polisher, cotton polisher and abrasive paste

Non-noble alloys	Alloy brand	R_z values after polishing with rubber polisher, [μm]			Average values after polishing with rubber polisher, [μm]	R_z Values after polishing with cotton polisher and abrasive paste, [μm]			Average values after polishing with cotton polisher and abrasive paste, [μm]
Ti–based alloys	Biotan	4.25	3.59	4.08	3.973	0.90	0.78	0.85	0.843
	Biotan-Nb	3.06	2.47	2.97	2.833	1.05	1.13	0.72	0.967

Comparing the results presented in Table 2, some discussions can be made on the specific values of the R_z roughness parameter:

- after mechanical polishing with rubber polisher, the lowest values were obtained for the Ti-based alloy, Biotan-Nb brand;
- the lowest values (Rz) after mechanical polishing with cotton polisher and abrasive paste were obtained for the Ti-based alloy, Biotan brand;
- the highest values were obtained after mechanical polishing with rubber polisher.

The adhesion capacity and thus the formation of *Candida albicans* biofilms onto the rough surfaces of the studied samples was carried out after 48h from the obtaining of the culture medium within scanning electron microscopy (SEM). The results are presented in Figures 1-8.

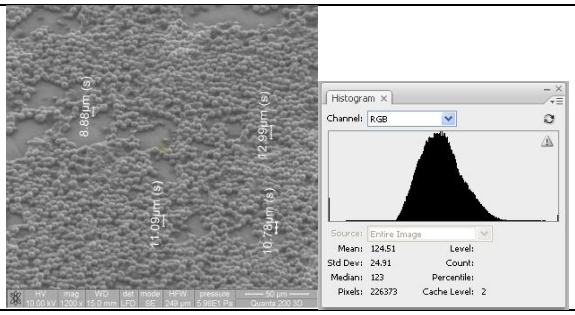

Fig. 1. SEM image of *Candida albicans* biofilm after 48h on the Biotan Nb alloy sample after mechanical polishing with cotton polisher and abrasive paste. The average value of biofilm thickness is *10.93µm.*

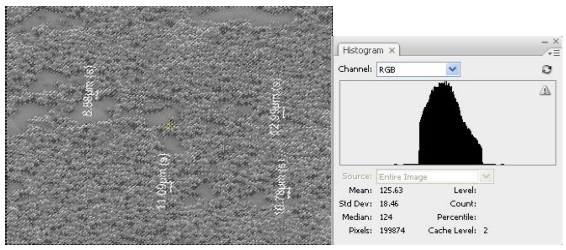

Fig. 2. SEM image of *Candida albicans* biofilm after 48h on the Biotan Nb alloy sample after mechanical polishing with cotton polisher and abrasive paste. The average value of biofilm surface coverage is 84.55%.

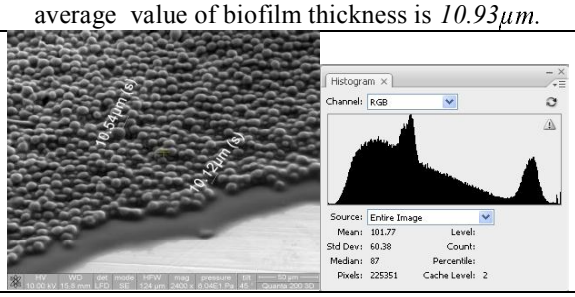

Fig.3. SEM image of *Candida albicans* biofilm after 48h on the Biotan alloy sample after mechanical polishing with cotton polisher and abrasive paste. The average value of biofilm thickness is *10.33µm.*

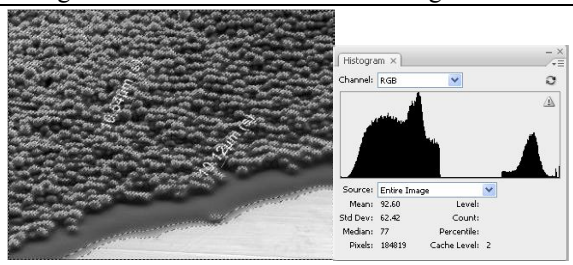

Fig.4. SEM image of *Candida albicans* biofilm after 48h on the Biotan alloy sample after mechanical polishing with cotton polisher and abrasive paste. The average value of biofilm surface coverage is 82.01%.

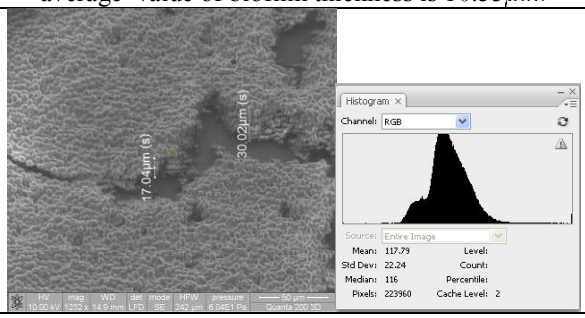

Fig. 5. SEM image of *Candida albicans* biofilm after 48h on the Biotan Nb alloy sample after mechanical polishing with rubber polisher. The average value of biofilm thickness is 23.53µm.

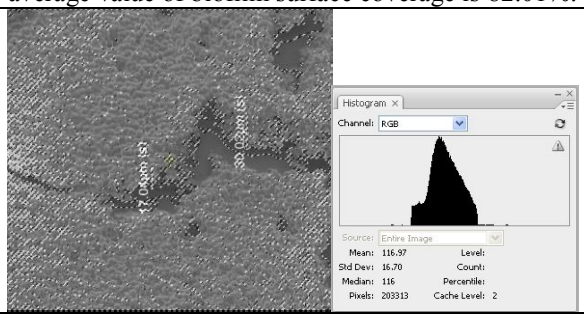

Fig. 6. SEM image of *Candida albicans* biofilm after 48h on the Biotan Nb alloy sample after mechanical polishing with rubber polisher. The average value of biofilm surface coverage is 90.78%.

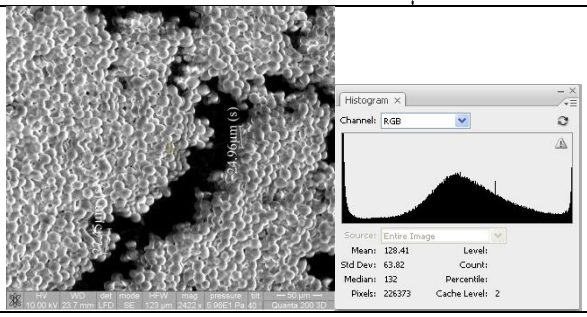

Fig.7. SEM image of *Candida albicans* biofilm after 48h on the Biotan alloy sample after mechanical polishing with rubber polisher. The average value of biofilm thickness is 26.02µm.

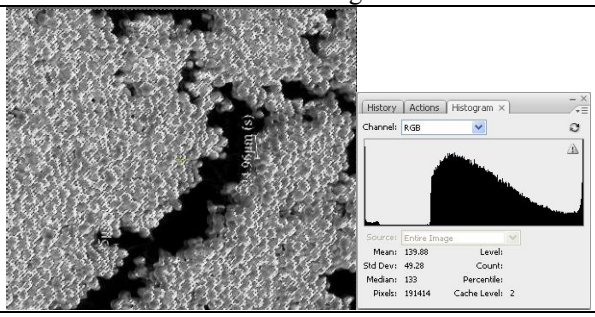

Fig. 8. SEM image of *Candida albicans* biofilm after 48h on the Biotan alloy sample after mechanical polishing with rubber polisher. The average value of biofilm surface coverage is 84.55%.

The surface coverage and biofilm thickness formed on the surfaces of samples under analysis varied depending on the finishing method (Table 3).

Table 3. Biofilm thickness formed on the tested sample surfaces

Non-noble alloys	*Alloy brand*	*Finishing techniques*	*Mean deviation (µm)*	*Candida albicans biofilm thickness after 48h (µm)*	*Candida albicans biofilm surface coverage (%)*
Ti-based alloys	Biotan	mechanical polishing with rubber polisher	3.973	26.02	84.55
		mechanical polishing with cotton polisher and abrasive paste	0.843	10.33	82.01
	Biotan-Nb	mechanical polishing with rubber polisher	2.833	23.53	90.78
		mechanical polishing with cotton polisher and abrasive paste	0.967	10.93	88.29

We may notice significant differences between the biofilm thickness obtained on the mechanically polished samples with cotton polisher and abrasive paste, as compared to the mechanically polished with rubber polisher. As for the biofilm surface coverage, there was no difference between the samples polished by the two techniques.

CONCLUSIONS

Based on the results obtained both by measuring roughness and by microbiological evaluation, some general conclusions may be drawn:

- we studied two brands of Ti- based alloys frequently used in dental prosthetics;
- the finishing by polishing the surfaces of the analyzed samples was performed mechanically;
- polished surface roughness was evaluated by specific roughness parameter R_z;
- the adherence capacity of *Candida albicans* onto the studied samples was shown using SEM analysis;
- the analysis of the average values highlights one important technical aspect:
- for all alloys, the lowest roughness is obtained after mechanical polishing with cotton polisher, and the highest roughness appears after mechanical polishing with rubber polisher
- the microbiological analysis revealed the following aspects:
- on the surface samples of Ti-based alloys mechanically polished with cotton polisher and abrasive paste, the biofilm thickness was much smaller than for the mechanical polishing with rubber polisher;
- maximum thickness of biofilms formed by *Candida albicans* has been found on the surface of Biotan alloy sample mechanically polished with rubber polisher;
- polished surface roughness is one of the factors responsible for *Candida albicans* biofilm formation on the surfaces of fixed or removable prosthetic restorations.

In conclusion, in vitro results of this research emphasize the strong relationship between the surface quality of dental alloys used in prosthetic restorations and the ability of *Candida albicans* to adhere onto those metal surfaces.

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