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BACKGROUND

Titanium alloys are widely used for many medical and technical applications due to their exceptional corrosion resistance in various aggressive environments including human body¹⁻⁴. For example, nickel-titanium (NiTi) alloys are applied in the endodontic instruments in form of super-elastic wires for root canal treatment^{4,5}. Among many other, the sulphate-reducing bacteria (SRB) can be present in mouth, mainly in tooth plaque and cavities³⁻⁵. SRB belonging to *Desulfovibrio desulfuricans* species may cause corrosion of many metals, including titanium and its alloys^{1,5-7}. These bacteria can form biofilm on the metal surface and corrosion under their metabolic activity may occur. Up today, there are no satisfactory results of removal already developed mature biofilms. There is a need for research on the possibility to control biofilm formation on various materials including metals. High surface energy connected with the Ti-alloys composition, negative charge and high surface roughness promote the adhesion of bacteria^{1,2,5}. In order to prevent biofilm formation, modifications to the Ti-alloys composition and surface character must be carried out for elimination the effects of these properties.

AIM

The goal of this study was to assess the influence of the titanium alloy type on the biofilm formation by *D. desulfuricans* bacteria.

MATERIALS & METHODS

The biofilms formation by *D. desulfuricans* bacteria on grinded, electropolished and anodized surfaces of three titanium alloys: NiTi, Ti6Al4V and Ti6Al7Nb were investigated during the metal samples immersion in simulated physiological solutions - artificial saliva and artificial saliva under inflammatory conditions).

BACTERIA

- Investigations have been carried out using *Desulfovibrio desulfuricans* bacteria - standard soil strain DSM. Bacteria were cultured in the Postgate's liquid medium.

Ti ALLOY SAMPLES

- NiTi, Ti6Al4V and Ti6Al7Nb alloys were used for this work. The alloy rods of 6-8 mm diameter were cut into samples of 3-4 mm high. Specimens were grinded, electropolished or passivated, then cleaned in ethanol in an ultrasonic bath, air dried, and sterilized at 180°C.

BACTERIA GROWTH

- The sterile samples were placed into tubes containing the artificial or inflammatory saliva and then were inoculated with bacteria *D. desulfuricans* (standard soil strain DSM). As control samples, the sterile systems without bacteria were used. The tubes were incubated at 30°C under anaerobic conditions.

BIOFILM EXAMINATION

- Bacterial biofilms were studied after 48 hours and after 7, 14, 28 days of samples' incubation, using assay of protein concentration in a medium, and biofilm examination in scanning electron microscope (SEM). Biofilms on alloy samples were fixed in 3% water solution of glutaraldehyde, dehydrated in ethanol series, and then examined using SEM.

RESULTS

Table 1. Growth of *D. desulfuricans* strain DSM on grinded, electropolished and anodized surfaces of three titanium alloys: NiTi, Ti6Al4V and Ti6Al7Nb, immersed in artificial or inflammatory saliva (28 days)

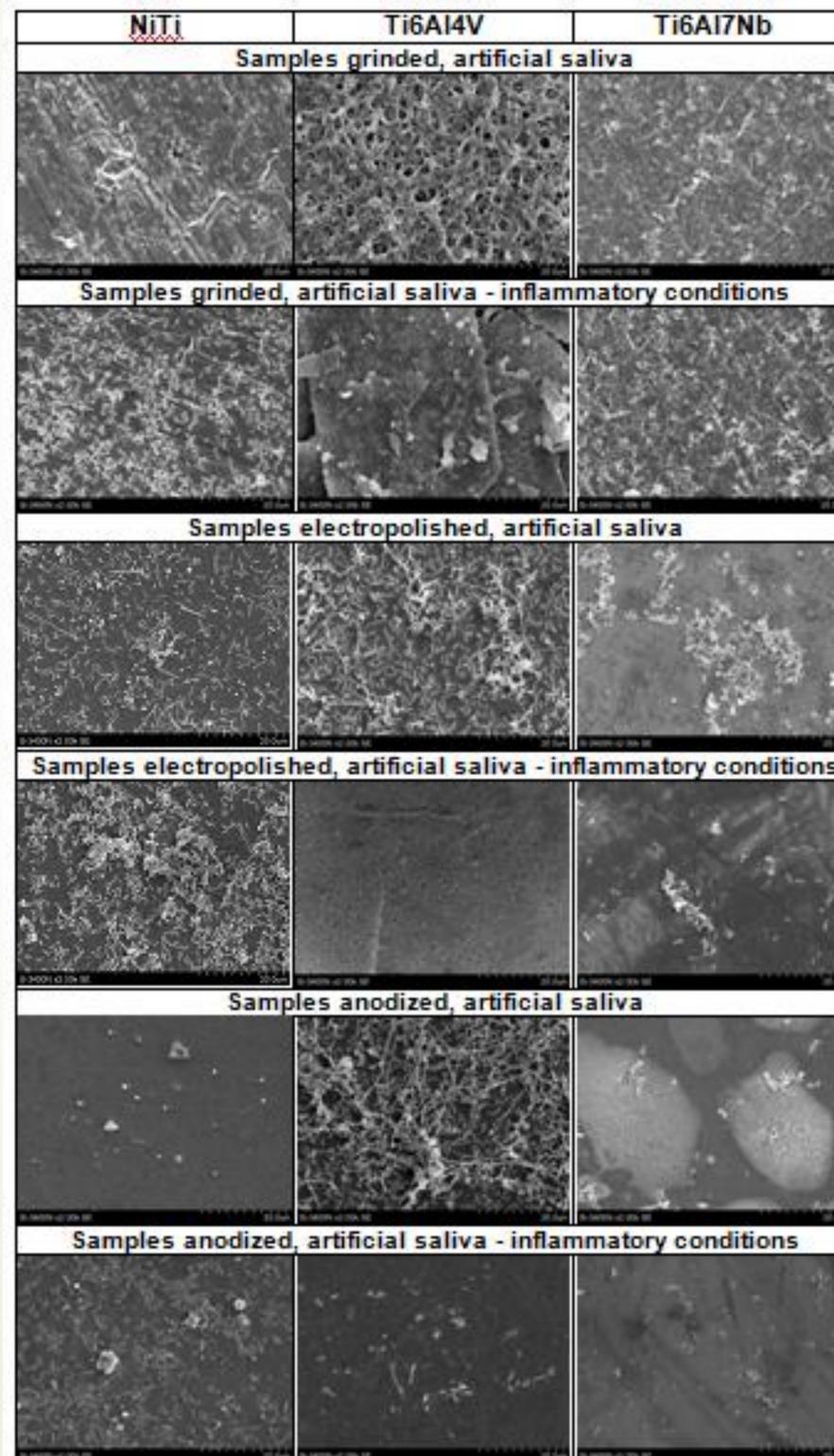


Table 2. Concentration of proteins in liquid media after 28-days growth of *D. desulfuricans* strain DSM on grinded, electropolished and anodized surfaces of three titanium alloys: NiTi, Ti6Al4V and Ti6Al7Nb, immersed in artificial or inflammatory saliva (28 days)

<i>D. desulfuricans</i> strain DSM	Concentration of proteins in medium [µg/ml]	
	Artificial saliva	Inflammatory conditions
Grinded	259.6 ± 18.3	301.6 ± 17.3
Electropolished	277.3 ± 13.3	229.3 ± 43.8
Anodized	296.6 ± 28.6	315.6 ± 43.0

CONCLUSIONS

Based on results of microscopic and biochemical investigations, the influence of the alloy type (NiTi, Ti6Al4V, and Ti6Al7Nb), with different chemical composition, on the biofilm formation by *D. desulfuricans* bacteria has been detected. The bacteria biofilm growth was affected by a state of the alloy surface: grinded, electropolished or anodized.

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