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PROSPECTS FOR THE USE OF CHITOSAN BIOACTIVE COATINGS FOR THE PREVENTION OF INFECTIOUS COMPLICATIONS IN DENTAL IMPLANTATION

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Abstract

Background. The study is that for the first time the antibacterial and fungicidal properties of the chitosan-based bioactive coating FAR-5X were evaluated in comparison with traditional titanium materials for dental implantation. It was found that the chitosan coating is able to significantly inhibit the growth of Escherichia coli and Candida albicans due to the mechanism of agglutination of microbial cells, which provides a long-term bacteriostatic and fungicidal effect. The results showed that FAR-5X reduces the level of colonization of titanium surfaces tenfold compared to both pure titanium and control cultures, which confirms its high efficiency as an antimicrobial barrier. For the first time, it has been shown that the use of such a coating allows combining the osseointegration potential of titanium with the pronounced antimicrobial properties of chitosan, opening up new prospects

for the creation of biocompatible implants with an increased level of protection against infectious complications.

Aim. To evaluate the antibacterial and fungicidal properties of the chitosan-based FAR-5X coating in comparison with traditional materials for dental implantation.

Materials and methods. The study was performed on VT-1 titanium disks divided into a control group and a group coated with FAR-5X. The disks were kept in a 50% aqueous solution of chitosan for 24 hours and dried. The antibacterial activity was evaluated against Escherichia coli and the fungicidal activity against Candida albicans according to ISO 27447:2009 by measuring optical density and cell counting. Cultures without contact with materials served as controls. Statistical processing was performed by methods of variation statistics with the assessment of probability by Student's test ($p<0.05$; $p<0.01$).

Results. To reduce the growth of pathogenic microflora and improve osseointegration, VT-1 titanium disks were modified with FAR-5X coating based on a 50% aqueous chitosan solution. The antibacterial activity was evaluated against Escherichia coli and the fungicidal activity against Candida albicans according to ISO 27447:2009 by the method of optical density and cell counting. After 24 hours, the growth of E. coli on FAR-5X was 6 times lower than that of titanium and 12 times lower than that of the control (0.35 ± 0.03; $p<0.01$), and after 96 hours - 28 times lower than that of titanium (0.20 ± 0.02; $p<0.001$). Similarly, the growth of C. albicans on FAR-5X after 14 days was 10 times lower than that on titanium and more than 100 times lower than that on control (0.50 ± 0.04; $p<0.001$). The data obtained confirm the high efficiency of FAR-5X in inhibiting the growth of bacteria and fungi compared to the standard titanium alloy.

Conclusions. The FAR-5X coating significantly inhibits the growth of E. coli and C. albicans compared to the titanium alloy VT-1 and the control ($p<0.001$), demonstrating high antibacterial and fungicidal activity. ANOVA confirmed statistically significant differences, which makes FAR-5X a promising material for improving implant biocompatibility.

Keywords: dental implants; coating; osseointegration; microflora

Introduction. One of the leading causes of dental implant failures is infectious complications associated with the development of pathogenic microflora in the implant site [1]. The proliferation of microorganisms such as Escherichia coli and Candida albicans contributes to inflammatory processes, disrupts the homeostasis of surrounding tissues, and can lead to implant loss [2, 3]. Traditional methods of modifying the titanium surface (machining, phosphate coating, immersion in calcifying solutions) do improve

osseointegration, but they do not always provide sufficient antimicrobial protection and may have side effects, such as the accumulation of zinc cations in the body [4]. In this regard, it is important to search for new biocompatible materials and coatings that can not only stimulate osseointegration but also effectively inhibit the growth of pathogenic microflora without toxic effects on the body [5]. Chitosan, as a natural biopolymer, has pronounced antibacterial and fungicidal properties due to its ability to agglutinate microbial cells, which provides a prolonged bacteriostatic effect [6, 7].

Studies of the chitosan-based FAR-5X coating have shown its effectiveness against E. coli and Candida albicans, as well as the absence of toxic effects on the environment [8]. This makes it promising for use in dental implantology to increase the success rate of implantation, reduce the risk of inflammatory complications and extend the life of implants [9].

Objective of the study. To evaluate the antibacterial and fungicidal properties of the chitosan-based FAR-5X coating in comparison with traditional materials for dental implantation.

Materials and methods. The study was carried out using titanium disks made of VT-1 alloy, which were divided into two groups: control group - VT-1 titanium alloy; experimental group - with FAR-5X coating. Titanium disks were manufactured at the Department of Chemistry and Integrated Technologies of the O.M. Beketov Kharkiv National University of Urban Economy, and kept in a bioactive coating based on a 50 wt% aqueous chitosan solution for 24 hours, after which they were dried under standard conditions. The antibacterial activity of the samples was evaluated against Escherichia coli (a sanitary pathogen capable of forming multidrug resistance) and the fungicidal effect against Candida albicans. Quantitative determination of antibacterial properties was performed in accordance with ISO 27447:2009 [10]. The growth of microorganisms was monitored by measuring optical density and counting cell concentration. Cultures without contact with the test materials served as controls.

The results were statistically analyzed using methods of variation statistics. The significance of differences between the groups was assessed by Student's t-test at significance levels of $p<0.05$ and $p<0.01$.

Results and discussion. In order to reduce the development of pathogenic microflora and avoid intense inflammation of the surrounding soft tissues to ensure improved osseointegration of dental implants, there are various methods of surface modification, such as mechanical and/or chemical treatment and calcium phosphate coatings. The success rate of implantation and enhancement of osseointegration can be addressed by immersing titanium

disks in various calcifying solutions: CaP (positive control), F-CaP, Zn-CaP, and FZn-CaP. However, the use of this method can contribute to the accumulation of zinc cations in the body as an additional factor in reducing immunity, increasing allergies and the development of various diseases.

To ensure safe antibacterial protection, the FAR-5X test material was treated with 50 wt. % aqueous chitosan solution for 1 day. Chitosan is known to be active against pathogenic staphylococci, streptococci, enterobacteria, Escherichia coli, corynebacteria, micrococci, and fungi of the genus Candida. The growth of pathogenic flora is stopped due to agglutination (gluing) of microbial bodies with chitosan. The agglutination mechanism is identical to the gluing of red blood cells by polycations. The binding of chitosan to saccharide receptors on the cell membrane provides a bacteriostatic effect.

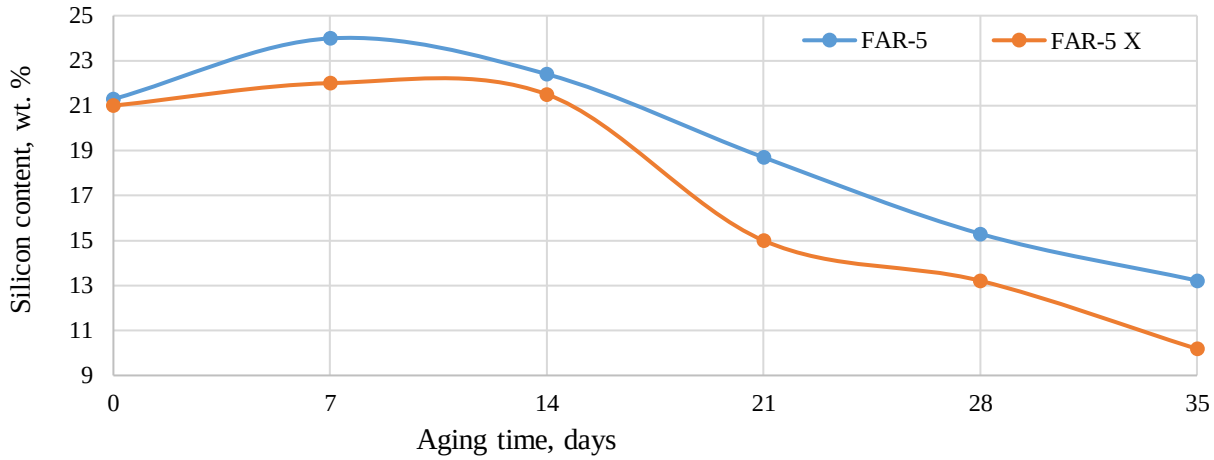
Escherichia Coli was chosen for the study of antibacterial properties as a sanitary-indicative pathogenic microorganism that causes a significant number of diseases and can contribute to a decrease in immunity and the development of bone tissue infections. It should be noted that Escherichia Coli develops multidrug resistance to antibiotics and prevents the use of antiseptic procedures in endoprosthetics. The bactericidal properties of the developed FAR-5X coating were determined according to ISO 27447:2009 by the quantitative method with the initial concentration of Escherichia Coli cells C= 1.0·104 kl/ml (Table 1) with an exposure time of 24 and 96 days.

After 24 hours of observation in the control culture, a sharp increase in the number of bacterial cells was noted (2.1±0.11, which corresponded to $4.2 \cdot 10^5$ cl/ml). In the group of titanium VT-1, this figure was lower (0.71±0.05; $1.4 \cdot 10^5$ cl/ml), but still remained within the range of active microbial growth. Instead, the FAR-5X coating provided a pronounced inhibition of bacterial proliferation (0.35±0.03; $6.0 \cdot 10^4$ kl/ml), which was 6 times lower than titanium and more than 12 times lower than the control ($p<0.01$). Analysis of variance confirmed statistically significant differences between the groups ($F=15.42$; $p<0.01$) (Table 1 and Fig. 1).

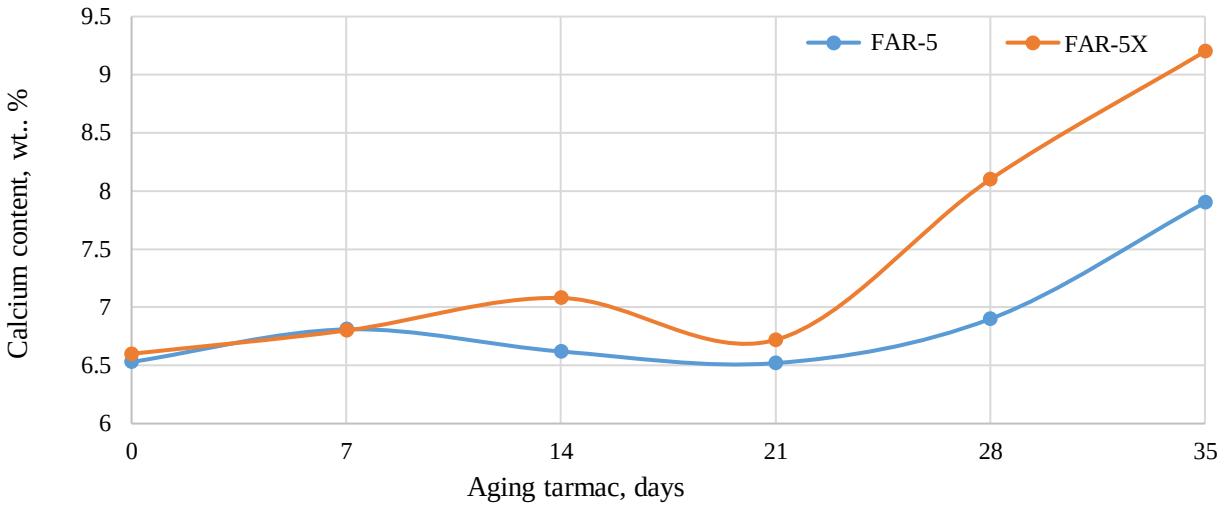
At the 96th hour of the experiment, the difference between the groups became even more pronounced. In the control culture, the maximum growth of the bacterial population was observed (14.0±1.07; $2.75 \cdot 10^6$ cfu/ml). On the surface of titanium VT-1, a significant increase in cell mass was also observed (5.73±0.04; $1.5 \cdot 10^6$ cells/ml), although it was twice lower than in the control ($p<0.01$). Instead, in the group coated with FAR-5X, bacterial growth practically did not develop (0.20±0.02; $0.1 \cdot 10^5$ cfu/mL), which was 70 times less than in the control and 28 times less than in titanium ($p<0.001$). The analysis confirmed the reliability of the differences ($F=48.63$; $p<0.001$).

Table 1 - Control of Escherichia Coli cell growth in contact with test samples

Research materials	The beginning of the exposition (0 hours)		Exposure time, hours			
			24		96	
	Optical density	C, cl/ml	Optical density	C, cl/ml	Optical density	C, cl/ml
VT-1 titanium alloy	0,05±0,01	$1,0 \cdot 10^4$	0,71±0,05	$1,4 \cdot 10^5$	5,73±0,04	$1,5 \cdot 10^6$
FAR-5X	0,05±0,03	$1,0 \cdot 10^4$	0,35±0,03	$6,0 \cdot 10^4$	0,20±0,02	$0,1 \cdot 10^5$
Cultures	0,05±0,04	$1,0 \cdot 10^4$	2,1±0,11	$4,2 \cdot 10^5$	14,0±1,07	$2,75 \cdot 10^6$



a



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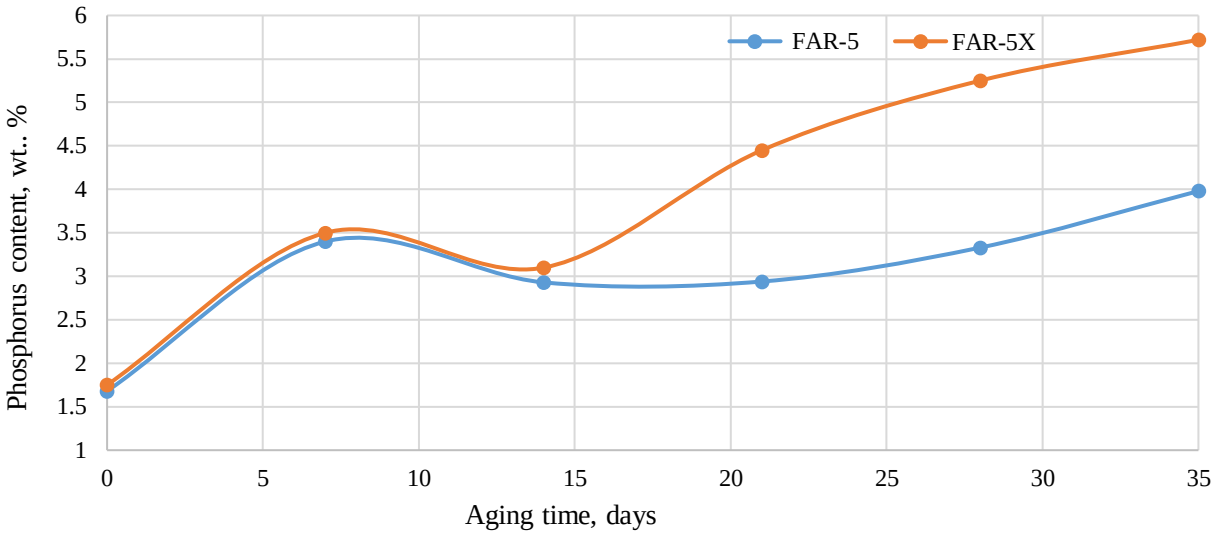

c

Figure 1. The content of silicon (a), calcium (b) and phosphorus (c) elements in the surface layers of the experimental coatings after exposure to the MPO

The study of fungicidal activity was carried out using the fungi Sandida albican with an initial concentration of C= 2.4·106 kl/ml during 7 and 14 days of exposure. The results of the study of the effect of the experimental materials on the growth of Sandida albican cells allowed us to establish their fungicidal effect on the pathogenic microorganism, which is similar in its tendency to the interaction of materials with Escherichia Coli.

After 7 days in the control culture, an intensive increase in the number of Candida albicans cells was noted, which was reflected in an increase in optical density to 0.65±0.05 ($3.9 \cdot 10^8$ cfu/mL). A similar trend was observed on the VT-1 titanium alloy, where the index was 1.10±0.08 ($1.94 \cdot 10^8$ cfu/ml), which, although lower than the control culture, still indicated active growth of microorganisms. In the FAR-5X-coated group, the results were fundamentally different: the optical density increased to only 0.30±0.02 ($1.65 \cdot 10^7$ cfu/mL), which was 12 times lower than titanium and more than 20 times lower than the control ($p<0.01$). The analysis confirmed the presence of statistically significant differences between all groups ($F=22.14$; $p<0.001$) (Table 2).

After 14 days, the difference between the samples became even more pronounced. In the control culture, the level of optical density reached 1.20±0.09 ($6.7 \cdot 10^8$ cfu/ml), which confirms the massive development of fungal colonies. For the titanium alloy VT-1, this indicator was 1.70±0.11 ($3.5 \cdot 10^8$ cfu/ml), which is two times less than the control ($p<0.05$), but still reflected a high level of colonization. In contrast, only a minimal increase in optical density to 0.50±0.04 ($3.1 \cdot 10^7$ cfu/ml)

was recorded for the FAR-5X coating, which was more than 100 times lower than the control values and 10 times lower than titanium ($p<0.001$). The analysis of variance confirmed the high reliability of the differences between the groups ($F=57.82$; $p<0.001$).

Table 2: Control of the growth of Sandida albican cells in contact with the experimental samples

Research materials	Start of exposure (0 days)		Exposure time, day			
			7		14	
	Optical density	C, cl/ml	Optical density	C, cl/ml	Optical density	C, cl/ml
VT-1 titanium alloy	0,40±0,02	$2,4 \cdot 10^6$	1,10±0,08 ($\times 10^2$)	$1,94 \cdot 10^8$	1,70±0,11 ($\times 10^2$)	$3,5 \cdot 10^8$
FAR-5X	0,40±0,03	$2,4 \cdot 10^6$	0,31±0,02 ($\times 10$)	$1,65 \cdot 10^7$	0,50 ±0,04 ($\times 10$)	$3,1 \cdot 10^7$
Cultures	0,40±0,02	$2,4 \cdot 10^6$	0,65±0,05 ($\times 10^2$)	$3,9 \cdot 10^8$	1,2±0,09 ($\times 10^2$)	$6,7 \cdot 10^8$

Conclusions. The FAR-5X coating exhibits pronounced antibacterial and fungicidal activity against E. coli and C. albicans compared to the titanium alloy VT-1 and the control. After 24-96 hours, bacterial growth on FAR-5X was 6-70 times lower than on titanium and 12-28 times lower than on the control ($p<0.001$). After 7-14 days, the growth of C. albicans decreased by 12-100 times compared to other groups ($p<0.001$). Therefore, FAR-5X can be considered as a promising antimicrobial coating to improve the biocompatibility of implants.

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