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Research Article

Study of Interaction of *Staphylococcus aureus* with Nanostructured Barrier Layers based on Fluorocarbon Films

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Abstract

Introduction: The problem of polymeric materials and products surface modification for various purposes in order to provide resistance to microbial colonization is currently one of the most important problems. Adhesion of microorganisms is a key factor which determines the prospects of subsequent processes of biodegradation or biocorrosion of polymer materials and products. From this point of view fluorocarbon materials which are now widely used in various fields of science and technology, as well as fluorocarbon films formed by ion-plasma technology, which can be the basis of barrier layers, attract a lot of attention. The aim of this work is to study the interaction between *Staphylococcus aureus* and nanostructured barrier layers based on fluorocarbon films formed by ion-plasma technology under different conditions.

Materials and Methods: The work includes the study of the adhesion of *Staphylococcus aureus* on the surface of the barrier layers formed on polytetrafluoroethylene by ion-plasma technology under different conditions, using a two-beam ion scanning electron microscope Quanta 200 3D (FEI Company, USA) at an accelerating voltage of 5 kV and 10 kV after deposition a thin gold film (999) on their surface in the SPI-Module Sputter / Carbon Coater System (PI Inc., USA).

Results: This study revealed that the adhesion ability of *Staphylococcus aureus* to the nanostructured surface of the barrier layer is largely dependent on the pretreatment conditions of the polymer surface that affect the surface topography. Wherein, structures identical to biofilms have not been obtained on investigated fluorocarbon materials.

Conclusion: This study clearly showed the presence of the transient area (from films deposition to etching) in the deposition of fluorocarbon films using plasma-forming mixture of CF_4 + C_6H_{12} and demonstrated the possibility of creating a relief on the surface of fluorocarbon materials, ensuring the absence of adhesion of gram-positive microflora on the surface on the nanostructured barrier layers. It was also shown that the adhesion of *Staphylococcus aureus* is determined by the conditions of formation of the fluorocarbon film surface.

Keywords: *Polymers; Microbial Adhesion; Microbial Colonization; Biodestruction; Biocorrosion; Fluorocarbon Materials; Nanostructured Barrier Layers; Staphylococcus aureus; Scanning Electron Microscopy; Ion-Plasma Technology*

Introduction

Nowadays a very important task is to find ways to modify the surface of polymeric materials that may provide resistance to the microbial colonization. Adhesion of microorganisms is a key step in determining of the prospect of subsequent processes of biodegradation and biological corrosion of polymeric materials [1-3]. One of the most promising ways to fight with these effects is to form a barrier layer on the surface. From this point of view, a great interest is attracted by fluorocarbon materials, which are widely used in recent years in various fields of science and technology [3-8], as well as fluorocarbon films formed by ion-plasma technology [6,7], which can be the basis for the barrier layers. In [6,7] it was shown that by using the plasma-forming mixture of CF_4 + C_6H_{12} in ion stimulated chemical vapor deposition from the gas phase transient area was observed (transition from films deposition to their etching). Nanostructured fluorocarbon films which were formed in conditions of transient area have anti-adhesive properties in relation *S. aureus*. However, to understand the mechanism of this phenomenon and for further development of the proposed technological approach for formation of nanostructured fluorocarbon films it is necessary to expand the technology range and explore the anti-adhesive properties of the nanostructures, paying special attention to the process parameters that affect the behavior of the relief. Thus, the aim of this work is to study the interaction between *S. aureus* and nanostructured barrier layers based on fluorocarbon films formed by ion-plasma technology under different conditions.

Materials and Methods

The polytetrafluoroethylene (PTFE) was chosen as a model material, as one of the most widely used polymeric materials in various fields of science and technology.

S. aureus was chosen as the biodestructor microorganism. As it is known [1-4], it has possessed high destructive potential against certain polymeric materials. Test samples were incubated in the wells of the tablet in a liquid culture medium NB, containing *S. aureus* ATCC 29213 for 5 days at room temperature. Additional enrichment of culture medium during incubation was not carried out.

To control a biofilm formation these microorganisms for 5 days were incubated in parallel in the wells of the tablet, at the bottom of which glass coverslips were located.

After incubation, samples were washed three times with sterile water and fixed in 10% neutral aqueous solution of formalin during a day. Then the samples were removed from the medium, then were dried at a room temperature for 10 minutes and were mounted on the table of an electron microscope using carbon tape.

The samples were formed by the ion source operating in crossed electric and magnetic fields. The plasma-forming mixture of CF_4 + C_6H_{12}, which contains a component for film deposition (C_6H_{12}) and a component for etching (CF_4) was used.

Selection of samples was based on works [6,7], which show that at the content of the CF_4 in plasma-forming mixture in the range from 40% to 60% transient area is observed (from films deposition to their etching). The samples formed in the transient area have anti-adhesive properties in relation to *S. aureus*. In this paper, conditions of samples' formation were expanded. List of samples is shown in Table 1.

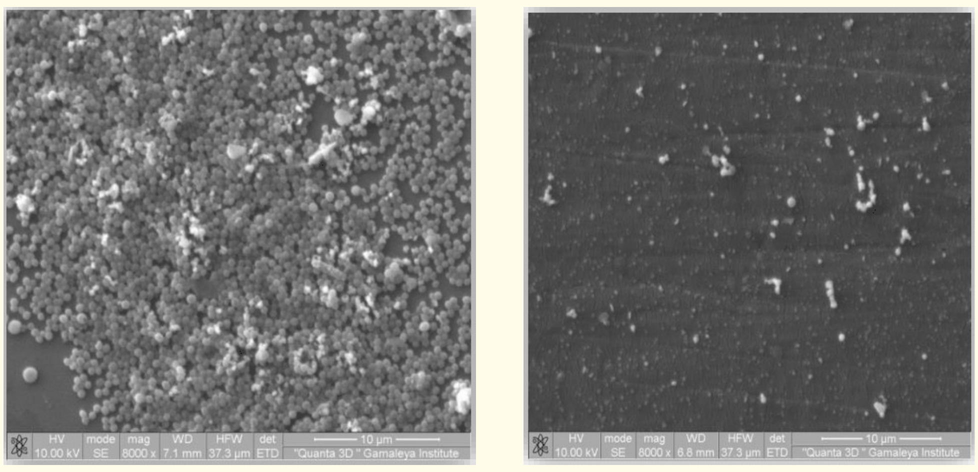
№	Conditions of sample forming
0	Glass coverslip (control 1)
1	PTFE (control 2)
2	PTFE, CF_4 treatment, 30 min
3	PTFE, CF_4 treatment, 30 min
	Deposition (C_6H_{12} + CF_4) (90% + 10%), 20 min
4	PTFE, CF_4 treatment, 30 min
	Deposition (C_6H_{12} + CF_4) (60% + 40%), 20 min
5	PTFE, CF_4 treatment, 30 min
	Deposition (C_6H_{12} + CF_4) (40% + 60%), 20 min
6	PTFE, CF_4 treatment, 20 min
7	PTFE, CF_4 treatment, 20 min
	Deposition (C_6H_{12} + CF_4) (90% + 10%), 20 min
8	PTFE, CF_4 treatment, 20 min
	Deposition (C_6H_{12} + CF_4) (60% + 40%), 20 min
9	PTFE, CF_4 treatment, 20 min
	Deposition (C_6H_{12} + CF_4) (40% + 60%), 20 min

Table 1: *List of the samples for the study of adhesive properties of PTFE.*

Evaluation of the surface structure of samples was made by a two-beam ion scanning electron microscope Quanta 200 3D (FEI Company, USA) in high vacuum mode with an accelerating voltage of 5 kV and 10 kV after deposition on the surface a thin (5 nm) gold film (999) using SPI -Module Sputter / Carbon Coater System (SPI Inc., USA).

Results and Discussion

On the glass surface the massive accumulation of microorganisms were formed, in some places even multilayered, partially covered by exomatrix (Figure 1a). On the PTFE control sample surface, dense clusters of bacterial cells were not detected (Figure 1b). They were located at a distance from each other, sometimes forming a chain of dividing cells.



a) b)

Figure 1: *The adhesion of microorganisms to the control surfaces of materials: a) glass coverslip (sample №0); b) PTFE (sample №1).*

On the surface of the sample №2 (only ions CF_4 flow treatment, 30 min) (Figure 2a) and №3 (ions CF_4 flow treatment + deposition of fluorocarbon film (10% CF_4) (Figure 2b) isolated separately located microbial cells were revealed, but signs of biofilm formation and cell division were not identified. Wherein on the surface of samples №4 (Figure 2c) and №5 (Figure 2d), the bacterial cells were not detected. It indicates a presence of anti-adhesive properties of the modified polymer surface.

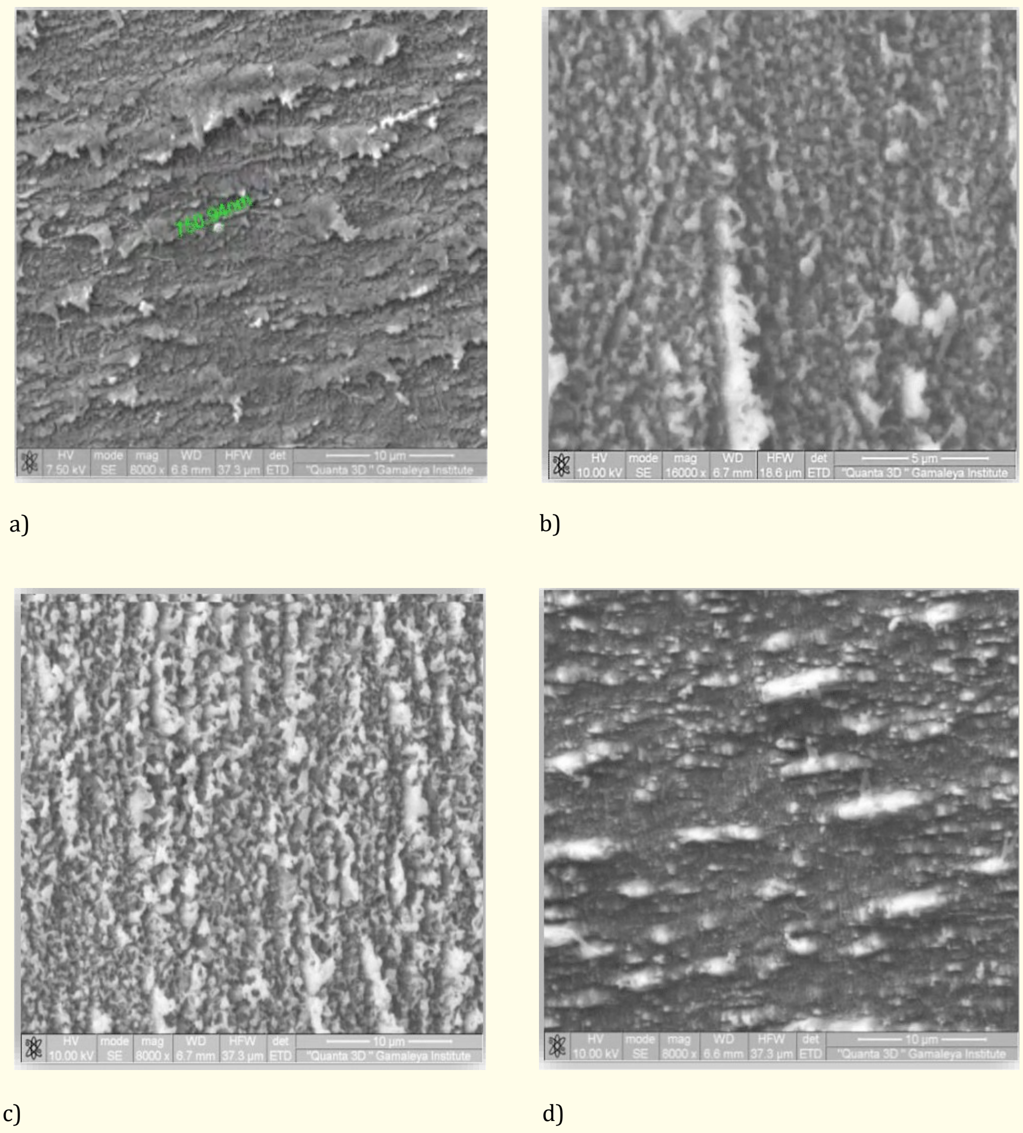
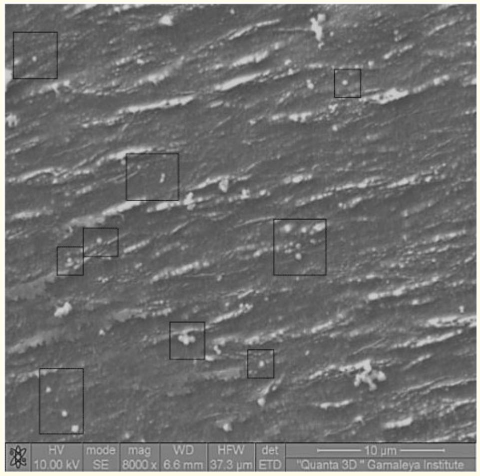
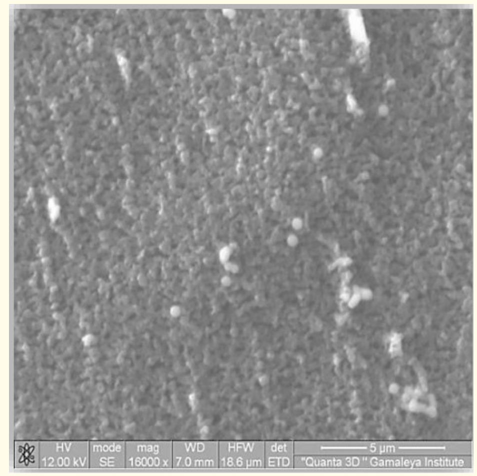


Figure 2: *Adhesion of microorganisms of PTFE samples' surface after 30 minutes treatment: a) sample №2; b) sample №3; c) sample №4; d) sample №5.*

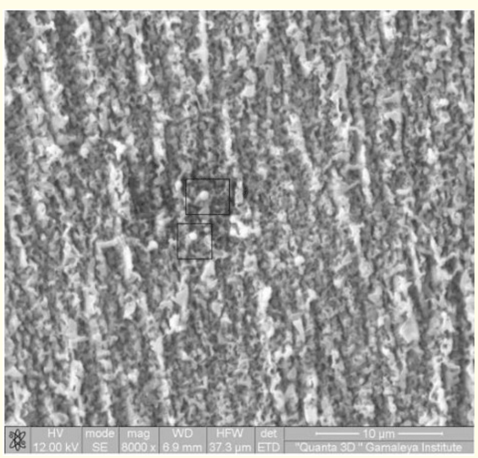
After 20 minutes treatment of PTFE surface and all subsequent fluorocarbon coatings deposited after this treatment signs of the division of bacterial cells and small clusters of them were observed. Thus, when using only the surface treatment (sample №6) (Figure 3a) in each field of view 8 - 15 bacterial cells were recorded, the biofilm was not found, but there were pairs of dividing cells and small clusters of 3 to 5 cells. For sample №7 (Figure 3b-3d) in each field of view several cells of microorganisms ware recorded, but clear signs of division and formation of biofilm was not revealed.



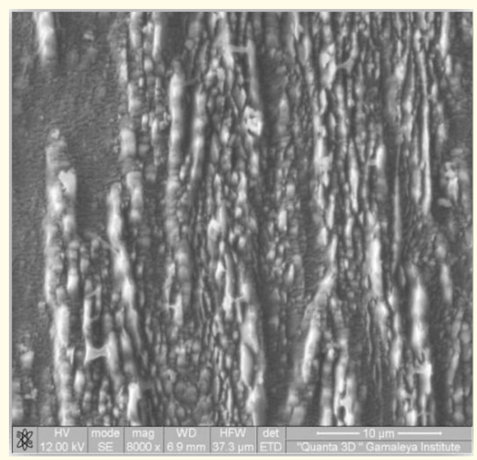
a)



b)



c)



d)

Figure 3: *Adhesion of microorganisms PTFE samples' surface after 20 minutes treatment: a) sample №6; b) sample №7; c) sample №8; d) sample №9.*

For samples №8 and №9 in rare fields of view individual bacteria's were recorded, which indicates the importance of the primary polymer treatment time (surface topography) and weak anti-adhesive properties of the polymer samples, regardless of the conditions of fluorocarbon films formation.

Conclusion

According to the results of studies on the possibilities of representatives of gram-positive microflora (S. aureus) adhesion, colonization and biofilm formation on the PTFE surface (control and modified) the following conclusions may be drawn:

1. The strain *S. aureus* ATCC 29213 studied during the experiment showed the ability to adhesion, division and formation on the glass (control) multilayer cluster tightly adjacent to each other microbial cells, sometimes covered by exomatrix. These features correspond to morphologic criteria of biofilm structure.

2. The ability to adhere to the studied materials depends on the type of surface modification. A major role is played by the primary surface treatment time (surface nanostructuring time), i.e. parameters of the surface topography. The microorganisms on the surfaces of the samples treated with CF_4 for 30 minutes with further deposition of the fluorocarbon film with a content of CF_4 in the plasma-forming mixture from 40% to 60% were not detected.

3. Structures that are identical to the biofilm were not formed on the studied materials.

Bibliography

1. Shah AA., *et al*. "Biological degradation of plastics: A comprehensive review". *Biotechnology Advances* 26.3 (2008): 246-265.

2. Pemraj R and Doble M. "Biodegradation of polymers". Indian Journal of Biotechnology 4 (2005): 186-193.

3. Donlan RM. "Biofilms: Microbial life on surface". *Emerging Infectious Disease* 8.9 (2002): 881-890.

4. LV Didenko., *et al*. "Study of the processes for colonization and persistence of microorganisms on artificial materials of medical appointment". *Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii* 5 (2015): 64-69.

5. Zaichenko O Yu., *et al*. "Testing of acrylic plastics for resistance to artificial biodestruction in imitation model with the use of microbial association". *Russian Stomatologicheskii Journal* 3 (2002): 19-24.

6. Elinson VM., *et al*. "Colonization by Staphylococcus aureus of Nano-Structured Fluorinated surfaces, Formed by Different Methods of Ion-Plasma Technology". *Bulletin of Experimental Biology and Medicine* 162.1 (2016): 71-74.

7. Elinson VM., *et al*. "The chapter "Nanostructured fluorine-containing surfaces: physicochemical properties and resistance to biodestruction". Polymer science: research advances, practical applications and educational asppects. Formatex Research Center, Madrid, Spain (2016): 342-347.

8. Yanglood JP., *et al*. "New materialsfor marine biofoiling resistance and release: semi-flurinated and pegylated block copolymer bilay coating". *Polymer Materials Science and Engineering* 88 (2003): 608-609.

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