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M. Supernak, **B. Świeczko-Żurek**
Technical University of Gdansk, Faculty of Mechanical Engineering, Narutowicza 11/12, 80-233 Gdansk, Poland, e-mail: milena@shl.pl

REACTIONS ON THE SURFACE OF THE IMPLANT UNDER THE INFLUENCE OF BIOFILM

ABSTRACT

The contact of a biomaterial with the biological environment in in vitro and in vivo tests leads to the production of a particular ecosystem in which the active roles perform both, the material surface and the extracellular matrix protein forming a biofilm. Proteins affect cell and bacteria adhesion processes, biological activity of cells and activation of inflammatory response.
The knowledge of the reaction mechanisms active on the surface of the material and the contacting tissues enables definite modification of the material surface layer eliminating their disadvantages, giving new, desired properties, affecting the biology of cells. Testing the biocompatibility of titanium materials emphasises their reactivity with proteins and cells and the possibility of modifying the biological reactivity of the surface by changing its properties by biochemical and surface engineering methods. This article presents results of previous studies on this problem.

***Key words**: biofilm, implants*

INTRODUCTION

Biofilm is a phenomenon which could happen every day. It occurs wherever there are microbes living in the aquatic environment. The formation of biofilm on the surface of an implant may lead to microbiological corrosion.
Biofilm is a multicellular creature made up of bacteria, fungi and other microscopic organisms [1-4]. It has a definite structure and adheres to the surface of organic and inorganic structures. In such compact structure, surrounded by mucus, there are conditions in which microbial cells are protected against adverse external factors [5]. Rising biofilm can be presented in the following phases (Fig. 1) [7]:

1. The reversible adhesion phase. Freely floating bacteria settle on the ground and attach themselves to it, forming clusters. Forces causing interactions between microorganisms and the surface are the electrical charges on the surface of bacterial cells, the impact of van der Waals and electrostatic attraction.

2. In the intermediates stage dominates the development of a sticky extracellular matrix structure (matrix), which main component is a polysaccharide cell wall(called EPS: Extracellular polymer substances), containing mannose and the glycoside residue. Changes in the environment cause a strong bound between bacteria and the surface;

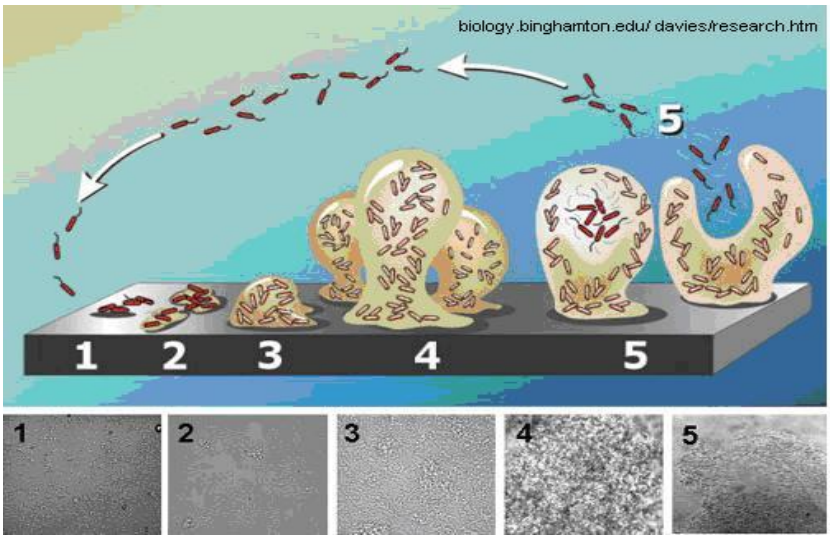

Fig. 1. Stages of biofilm formation [5]

3. During the phase of the maturation the biofilm structure follows further increase of extracellular substances, until totally surrounding it by rising colonies. At this stage in biofilm composition are included micro-organisms, dead cells, organic matter, precipitated minerals, etc. These structures bind another bacterial species.

4**.** The resulting chemical gradients allow the coexistence of different species of bacteria and are in various metabolic states. Bacteria sending themselves signals to stimulate them to multiply and create colonies;

5**.** Some cells leave the biofilm to create new clusters;when bacteria affix to the ground, creating a biofilm, they begin to produce hundreds of proteins not present in microbes leading free lifestyle. Some of these proteins are involved in the process called "bizarre reshuffle cells" immediately after the setting of bacteria on the ground, but before sticking to it [6,7].
The first outcome of introducing the biomaterial into the biological environment, both in vitro and in vivo, is appearing of the biofilm on the surface, Figure 2 [8].

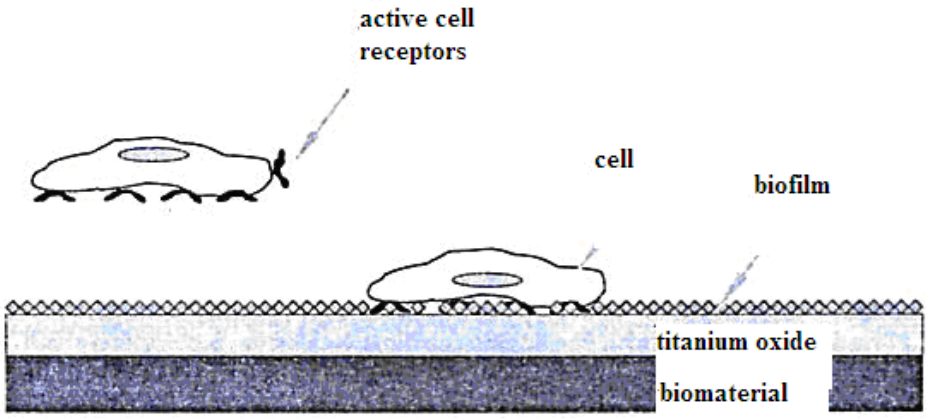

Fig. 2. Topography of the ecosystem on the surface of biomaterial [8]

Location and thickness of the biofilm depends on surface properties, especially such as chemical composition, topography, surface energy and electric charge [6]. It may constitute a layer of homogeneous and evenly spaced, irregular network, or so called islands. Growth of the biofilm layer is possible owing to forming connections between the side chains of proteins [6].
Biofilm produced in vitro is made of proteins present in the culture medium and in vivo, of the substance of the extracellular matrix proteins and body fluids. Proteins which are synthesized by cells adjacent to the biomaterial and ions present in the tissue environment are embodied into the biofilm. Composition, distribution and thickness of the biofilm change during the time contact of material with the cells / tissues [9,10].
In case of metallic biomaterials biofilm connects to the oxide layer of the implant creating a reacting zone with the surrounding ions. The thickness of this zone is of the order of 1 to 10 nm and depends on the chemical composition, surface layer of material, method of its preparation, cleaning and sterilization [6,9].

MATERIALS AND METHODS

The investigated material was a titanium alloy Ti6Al4V with the chemical composition: Ti, 4.08% V, 6.39% Al, 0.17% Fe, 0.015% C, 0.185% O, 0.005% N, 0.0035% H and microstructure as shown in Fig. 3.

Fig. 3. Microstructure of the two (α + β) phase Ti6Al4V alloy

Tests were carried out at the Department of Microbiology, Local Hospital in Kościerzyna. Samples in the form of discs of titanium alloy Ti6Al4V (Fig. 4) were soaked for a period of 6 months in bacterial solutions - Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter cloacae. Next step was the viewing area for degradation on scanning electron microscope - Philips XL30. Final examination included observation of the biological colonies of bacteria on implants removed from the human body (Fig.5-7) with the biological microscope.

Fig. 4. Samples in the form of discs of the Ti6Al4V alloy

RESEARCH RESULTS AND DISCUSSION

Initial tests included microscopic observation of samples after 6-month exposure in a solution of bacteria - *Staphylococcus aureus* (Fig.4), *Staphylococcus epidermidis* (Fig.5), *Enterobacter cloacae* (Fig. 6).

Fig. 5. Appearance of alloy surface after an exposure in *Staphylococcus aureus* solution

Fig. 6. Appearance of alloy surface after an exposure in *Staphylococcus epidermidis* solution

Fig. 7. Appearance of alloy surface after an exposure in *Enterobacter cloacae* solution

Microscopic views of the surface of tested samples are shown in Figures 8-10.

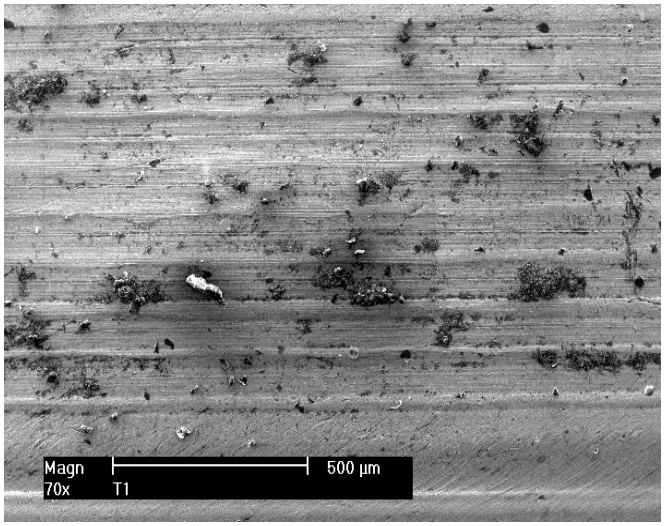

Fig. 8. The sample surface after removal from the bacteria *Staphylococcus aureus*

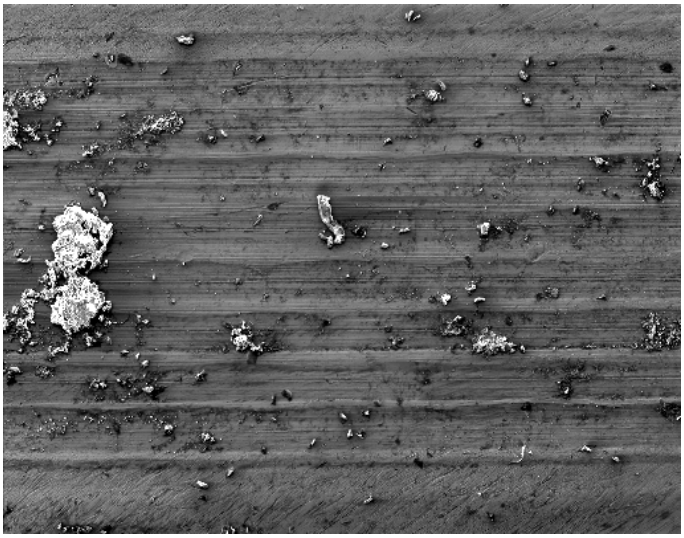

Fig. 9. The sample surface after removal from the bacteria *Staphylococcus epidermidis*

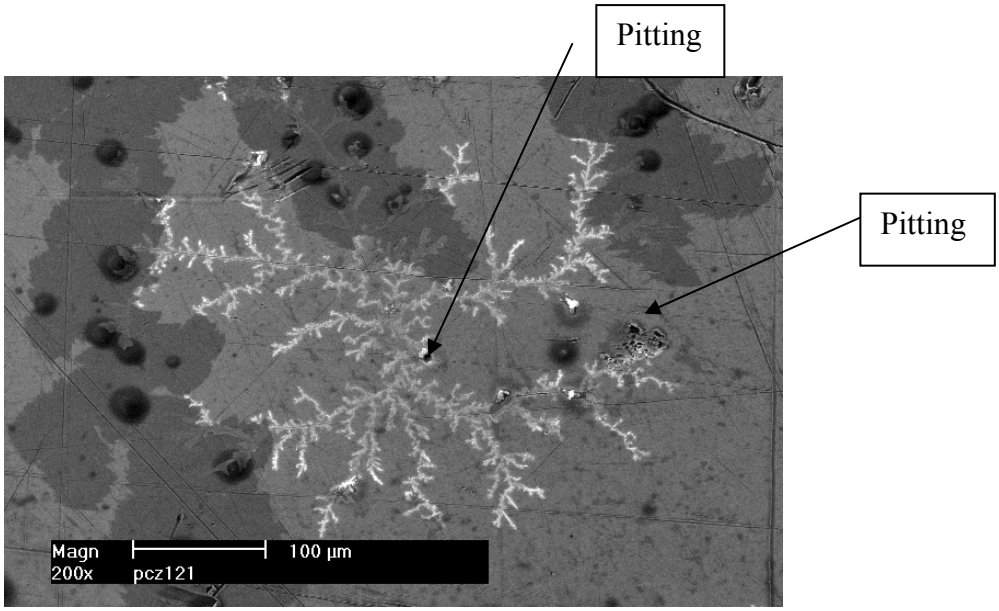

Fig. 10. The sample surface after removal from the bacteria *Enterobacter cloacae*

All the bacteria mentioned as components of the human microflora may be agents of hospital infections.
Lifelong man lives in a very close view of many germs that more or less permanently settle their respective niches. Given the persistence of this compound, the human microflora can be divided into several categories. The first form organisms, which with some breaks accompany man throughout his life. Create a second category for organisms of the transitory flora, which may come from another individual or the environment. There is also a third category, consisting of transitory residents.

Hospital strains of bacteria characterize with many features, which do not have traditional bacteria. Among other things, they can break the resistance of the environment and settle the existing bacteria to crowd out .
Research shows that the most aggressive bacterium proved to be intestinal bacterium Enterobacter cloacae. During 6 months, it caused the greatest damage to the material - deep pitting.
Observation of the implants with the biological microscope showed bacteria colony, which seated on the material in the form of a biofilm.

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