Acta of Bioengineering and Biomechanics
Vol. 15, No. 1, 2013

Original paper
DOI: 10.5277/abb130109

The ability of *S.aureus* to form biofilm on the Ti-6Al-7Nb scaffolds produced by Selective Laser Melting and subjected to the different types of surface modifications

PATRYCJA SZYMCZYK[1]*, ADAM JUNKA[2], GRZEGORZ ZIÓŁKOWSKI[1], DANUTA SMUTNICKA[2], MARZENNA BARTOSZEWICZ[2], EDWARD CHLEBUS[1]

[1] Centre for Advanced Manufacturing Technologies, Wrocław University of Technology, Wrocław, Poland.
[2] Department of Microbiology of Wrocław Medical University, Wrocław, Poland.

The Gram-positive coccus, *Staphylococcus aureus*, is the leading etiologic agent of limb and life-threatening biofilm-related infections in the patients following the orthopaedic implantations. The aim of the present paper is to estimate the ability of *S. aureus* to form biofilm on titanium alloy (Ti-6Al-7Nb) scaffolds produced by Selective Laser Melting (SLM) and subjected to the different types of surface modifications, including ultrasonic cleaning and chemical polishing. The results obtained indicate significantly the decreased ability of *S.aureus* to form biofilm on the surface of scaffolds subjected to the chemical polishing in comparison to the scaffolds cleaned ultrasonically. The data provided can be useful for future applications of the SLM technology in production of Ti-6Al-7Nb medical implants.

Key words: *SLM, Micro-CT, scaffolds, Ti-6Al-7Nb alloy, orthopedics, S.aureus*

1. Introduction

The biofilm is a community of microorganisms adhered to the specific surface and coated with layers of extracellular slime protecting bacteria from unfavourable environmental factors, such as heat, cold, UV radiation and drying [1]. It is estimated that 99.9% of all bacterial biomass is organized in the biofilm form [2]. From the clinical point of view, the most important attributes of biofilm are extremely high resistance of this structure against antimicrobials (antibiotics, antiseptics) and immune system components (antibodies, macrophages) [3]. The rapid development of molecular and microscopic techniques observed in recent years made it possible to prove the existence and significance of the bacterial biofilms in infections of chronic wounds, bones, skin and soft tissues [1]. Biofilms are able to form not only on the biotic surfaces (tissues), but also on the abiotic surfaces of clinically used biomaterials, such as stents, catheters, orthopaedic implants, intubation tubes, etc. [4]. Although efforts are made to create biomaterials of antiadhesive properties or coated with antibiotics or other antimicrobials, such as silver, presently it seems that there is no biomaterial that could be described as "biofilm-proof". Biofilm related infections (BRI) make up 60% of all nosocomial infections [5] and concern mostly patients with compressed immune system (such as oncologic patients) or patients undergoing aggressive surgical procedures, for example, orthopaedic patients undergoing total joint or hip replacement. In fact, the frequency of musculoskeletal infections ranges from 0.5% to 10% depending on the type, length and scope of the operation, type of biomaterial used and perioperative antibiotic prophylaxis per-

* Corresponding author: Patrycja Szymczyk, Centre for Advanced Manufacturing Technologies, Wrocław University of Technology, ul. Łukasiewicza 5, 50-370 Wrocław, Poland. Tel.: +48-71-320-2074, fax: +48-71-321-1235, e-mail: patrycja.e.szymczyk@pwr.wroc.pl
Received: August 20th, 2012
Accepted for publication: November 18th, 2012

formed. Therefore, orthopaedic patients with implanted biomaterials are in the group of high risk of biofilm related infections, which can be devastating to the replaced limb and can be life-threatening [6]. The leading etiologic agent of orthopaedic implant infections is Gram-positive bacteria, *Staphylococcus aureus*, responsible for 33.8% of infections of such type [7]. However, virulence factors of *S.aureus*, responsible for biofilm formation and causing biomaterial-related infections are thoroughly investigated, the above mentioned bacteria still causes serious therapeutic problems, especially when it occurs in the biofilm form. One of the factors than can decrease the possibility of orthopaedic infection is the use of proper biomaterial for the implantation purposes [8].

Contemporary orthopaedics commonly uses various types of implants which replace damaged or malfunctioning parts of the osteoarticular system. The implants are manufactured using a number of construction materials fulfilling specific requirements. To numerous metallic materials belong austenitic steels, Co-Cr alloys, Ni-Cr alloys, and titanium alloys [9]. Presently, orthopaedic implants are made mostly from stainless steels and Co-Cr alloys, however titanium and its alloys exhibit the most suitable characteristic for biomedical applications. Two-phase Ti-6Al-7Nb alloy is one of the biomaterials of a new generation with regard to replacing the controversial vanadium by niobium in its chemical composition. Applications of titanium and its alloys in the most challenging fields of technology are based on the favorable combinations of characteristics such as strength and high corrosion resistance [12]. Additionally their high biological tolerance [13] and, relative to steel, two times lower density and elastic modulus are the cause of their use in bone surgery for implants to transfer static and dynamic mechanical loads.

The production of technologically advanced orthopaedic implants, which are often complex structures with small dimensions and complex geometry, requires advanced manufacturing technologies, such as Rapid Prototyping [14]. The big advantage of these methods is the ability to build accurate, finished parts based on geometric model CAD-3D or on virtual model obtained using Reverse Engineering, which is based on geometric reconstruction [15].

One of the representatives of Rapid Prototyping Technologies is Selective Laser Melting. Flexibility in creating the shape using this method allows the strength and porosity to be controlled [16], the parameters on which the regenerate bone growth depends, coupled also with the issue of "stress shielding" (i.e., bone loss) resulting from the elasticity (stiffness) of the scaffold structure, which enables production of an implant reflecting superseded tissue in terms of shape, strength or internal structure [17].

The aim of the following paper is to evaluate the ability of *S.aureus* to form biofilm on Ti-6Al-7Nb scaffolds produced by Selective Laser Melting and subjected to various surface modifications. Therefore, three groups of titanium scaffolds were fabricated using rapid manufacturing technology. The resulting structure was washed in alcohol and subjected to chemical treatment. Geometry characterization before and after surface modification of scaffolds was conducted using micro-CT technology. The samples were subjected to microbiological tests using Gram-positive *Staphylococcus aureus* bacteria cultures. The quantitative microbiological tests were confirmed by visualization of bacterial cells using electron microscopy.

2. Materials and methods

2.1. Fabrication of scaffolds

Based on studies evaluating the ability of generating structures [18], [19] with the SLM50 (Realizer) device, cylindrical samples were prepared in order to check the possibility of the formation of bacterial biofilms on scaffolds produced by generative technologies. Using the model developed, with unit cell size of 600 μm and 150 μm diameter beams, multiplied matrix cells were formed. Then, a cylinder with a diameter of 6.2 mm and a height of 6 mm was cut from the model designed. The next step was the selection of appropriate parameters and ensuring relevant conditions for the SLM process. Technological parameters of the process are shown in Table 1.

Table 1. The technological parameters used in the preparation of samples for microbiological tests

Laser power	25 W
Layer thickness	50 μm
Distance between scanning points	20 μm
Exposure time of single point	100 μs
Distance between the lines scan	50 μm
Scanning strategy	double *x–y*

2.2. Modifications of surface of scaffolds

The following types of scaffold surfaces were made: Group A – samples after SLM process, not sub-

jected to the surface modifications, served as a comparative group (control of impact of surface modification on the *S.aureus* biofim); Group B – samples washed in 99.7% isopropyl alcohol using an ultrasonic cleaner (MKD Ultrasonic) to clean the powder residue trapped between trabeculaes; Group C – samples precleaned in 99.7% isopropyl alcohol and subjected to chemical polishing using bath dedicated to titanium alloys: 200 ml H_2O + 5 ml HF 50% + 15 ml HNO_3 50% [20]. Chemical polishing time was 75 seconds.

Micrographs of the models generated before and after surface modifications were visualized using the Scanning Electron Microscope EVOMA25 (Zeiss).

2.3. X-ray computed microtomography (μCT)

To estimate the geometry of complex internal structures of samples made with SLM technology, computed micro-tomography methods (μCT) were used [21]. Scaffolds were scanned using μCT system (Metrotom 1500, Carl Zeiss, Oberkochen, Germany), which allowed information to be obtained on the actual surface of the trabeculae in each of the scaffolds [22]. The system consists of a flat panel detector with a resolution of 1024 × 1024 px (400 μm pixel size) and 16 bit gray scale, a rotary table and microfocus X-ray tube with max. accelerating voltage 225 kV and max. current 1000 μA. In order to achieve maximum resolution [23], the tube voltage was fixed at the level of 220 kV and the current 120 μA. The number of projections carried out during the 360° rotation of the sample was 800 with 1 s integration time for each. The result matched the parameters permitted to achieve a voxel size of 31 microns. The data obtained were analyzed using software VG Studio MAX (Volume Graphics GmbH, Heidelberg, Germany).

2.4. Microbiological tests

2.4.1. Quantitative cultures

A reference *S.aureus* ATCC6538 strain, cultured on the stable CA medium (Biocorp), was transferred to the liquid BC medium and incubated at 37 °C for 24 hours in the aerobic conditions. After incubation, density of bacterial suspension was measured using densitometer (Biomerieux) and diluted to reach 3×10^8 cells/ml. Subsequently, SLM samples from groups A, B, C (see Section 2.2) were incubated with bacterial suspensions at 37 °C for 24 hours. After incubation, samples were thoroughly rinsed using physiological saline to remove non-adhered bacteria and to leave only biofilm forming microorganisms. Subsequently, samples were transferred to 1 ml of mild detergent (0.5% saponine, Sigma-Aldrich) and vortexed vigorously for 1 minute to free bacterial cells from biofilm extracellular layers. After vortexing, bacterial suspensions obtained were diluted 10–1000.1000 times. 100 μl of each dilution was cultured on the appropriate stable medium and incubated at 37 °C for 24 hours. After this time, bacterial colonies were counted and the number of bacterial cells forming biofilm on the implants was assessed. All measures were repeated three times. All experiments were performed in triplicate to calculate the average value. Additionally, the numbers of cfu are normalized to the sample surface by the equation: cfu per mm^2 surface = cfu per sample/surface area mm^2.

2.4.2. Confirmation of *S.aureus* ATTC6538 strain to form biofilm on the biomaterials tested using scanning electron microscopy

Results of quantitative microbiological tests (see Section 2.3) were additionally confirmed by visualization of bacterial cells using electron microscopy. The microscopy procedure was the following: The samples with *S.aureus* biofilm on it were fixed using 3% glutarate (POCH) for 15 min at room temperature. Thereafter, the samples were rinsed twice with phosphate buffer (Sigma-Aldrich) for the purpose of fixative elimination. The next step consisted in dehydratation of ethanol in increasing concentrations (25, 60, 95, 100%) for 5 minutes in every solution. After rinsing off the ethanol, the samples were dried. Finally, the samples were covered with Au/Pd (60:40, sputter current: 40 mA, sputter time: 50 sec) using QUORUM machine and examined on Scanning Electron Microscope Zeiss EVO MA25.

3. Results

3.1. Scaffold fabrications

Scaffolds were made of titanium alloy (Ti-6Al-7Nb) powder (TIMETAL®367) in the range of particle size to 63 microns. The results of the building of scaffolds produced by SLM are shown in Fig. 1.

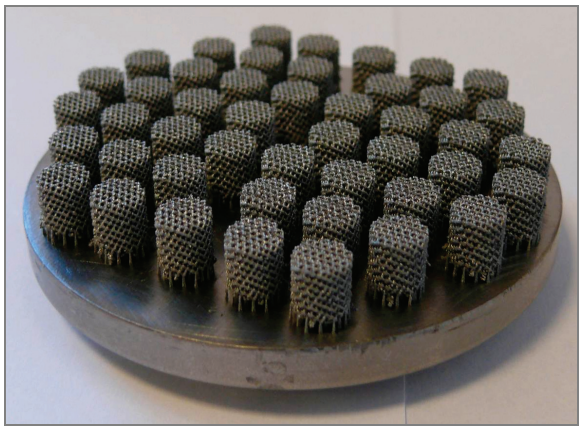


Fig. 1. Scaffolds produced by Selective Laser Melting

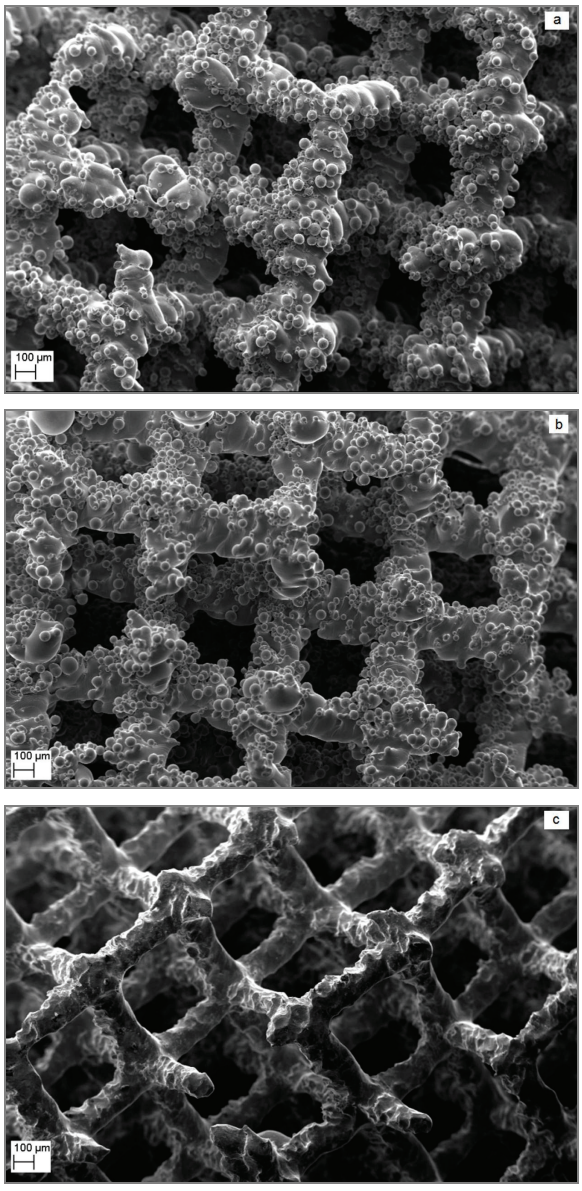


Fig. 2. Scaffold surfaces: (a) after the SLM process – GROUP A, (b) after the washing in isopropyl alcohol – GROUP B, (c) after the chemical polishing – GROUP C; Scanning Electron Microscope, resolution 100 µm

Chemical composition (in wt.%) of the scaffolds manufactured, determined using spectroscopic method was: 5.5% Al, 6.8% Nb, 0.25% Fe, 0.31% Ta, and Ti as the remainder [18]. All of the elements determined were in agreement with the ISO 5832-11 Standard. Before incubation with reference *S.aureus* 6538 ATTC strain, samples of groups B and C were subjected to surface modifications as was described in Section 2.2, whereas samples of group A were not subjected to surface modifications and served as a comparative group. Electron microscope micrographs of the samples from the above three groups are presented in Fig. 2. The analysis of scaffold surfaces presented in Fig. 2a and Fig. 2b showed no significant differences between samples of groups A and B. This is due to the fact that the process of cleaning in an ultrasonic cleaner to remove permitted only partly related to the powder particles and to remove the powder trapped between trabeculae in the SLM. Contrary to the above-mentioned two groups, the surface of scaffolds after chemical polishing (group C) displayed different characteristics (Fig. 2c).

Modification of the Ti-6Al-7Nb scaffold surfaces introduced significant changes of the strut and node surface morphology. Non-melted or partly related powder grains, attached to the surface have been removed. The chemical polishing conducted allowed the quality of the surface of the scaffolds to be improved.

3.2. X-ray computed microtomography (µCT)

To obtain quantitative data concerning actual surface of the trabeculae on each of the scaffolds, samples were scanned using a µCT system.

The first step was the separation of the background and material volume. An indispensable tool for this purpose is thresholding which is based on the segmentation of gray scale. This process was conducted automatically and the results of segmentation on the 2D slice are shown in Fig. 3a. The yellow line represents the surface of the object analyzed and each point on the line is known in 3D space (Fig. 3b). Based on this it is possible to estimate the surface on the whole volume of the scaffolds. The surface area was measured automatically using the VG Studio MAX software. Results of this study are presented in Table 2.

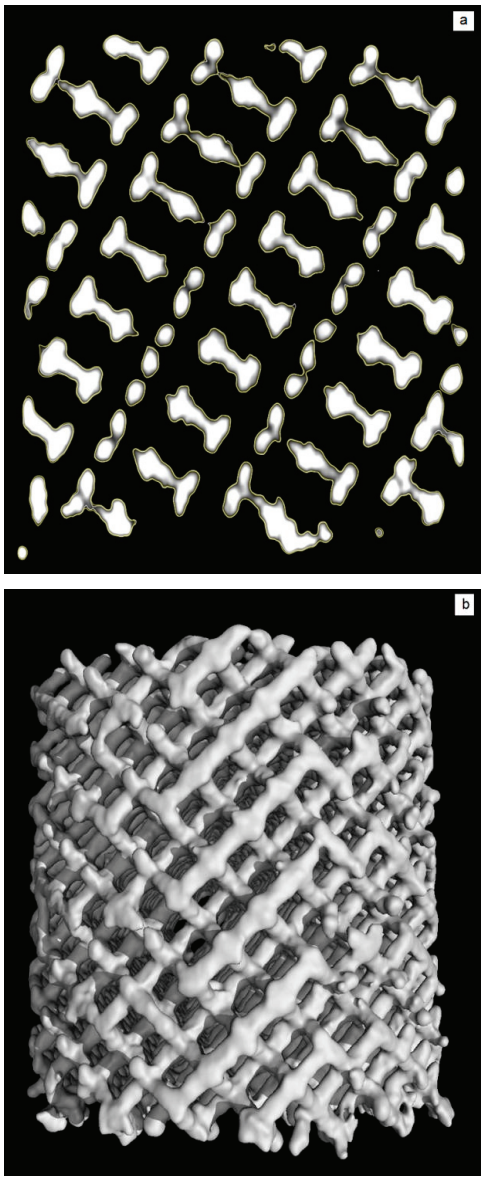


Fig. 3. (a) Yellow line represents the surface on 2D slices, (b) 3D reconstruction after segmentation

The surfaces of the scaffolds of groups A and B did not differ in any significant manner (756.26 mm^2 vs. 726.34 mm^2, respectively), which suggests low ability of post-SLM ultrasonic cleaning to remove the powder residue strapped between surface trabeculaes, as is presented in Fig. 4.

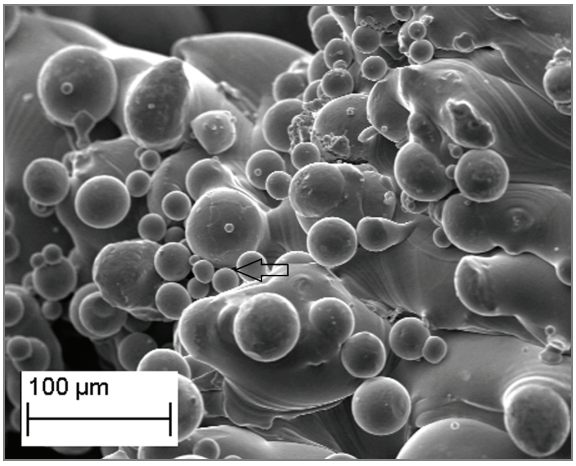


Fig. 4. Scaffold surface of sample from group A (after SLM process). Trapped particles of the powder are marked with black arrows), SEM

By contrast, chemical bath used for group C of the samples resulted in a significant decrease of the scaffold surface (Table 2) and gives evidence of the highest, of the methods tested, ability of removing powder residues.

3.3. Microbiological tests

As can be seen from Table 3, the *Staphylococcus aureus* ATTC 6538 strain investigated was able to

Table 2. Results of measurements of the scaffold surfaces [mm^2] performed with VG Studio MAX

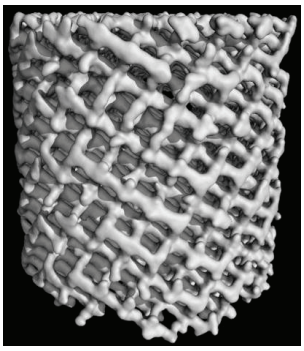
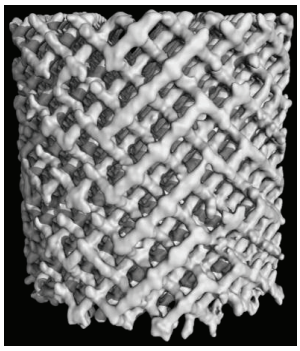
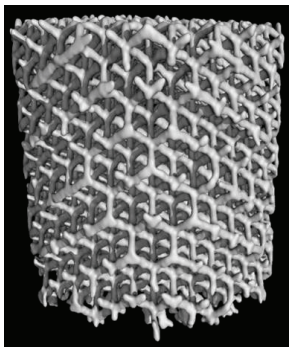
Group A (after SLM process)	Group B (after washing in alcohol)	Group C (after chemical polishing)
		
Surface [mm^2]		
756.26	726.34	519.18

Table 3. Ability of *S.aureus* ATCC 6538 to form biofilm on the investigated scaffolds

Scaffolds		Number of bacterial cells/implant [cfu/implant]			Number of bacterial cells/mm^2 of scaffold	
Type	Surface area [mm^2]	*Staphylococcus aureus* ATCC 6538				
		Results of counts of individual samples			average value	
Group A	756.26	2.43×10^9	2.27×10^9	2.29×10^9	2.33×10^9	3 080 950
Group B	726.34	2.2×10^9	2.49×10^9	2.12×10^9	2.27×10^9	3 125 258
Group C	519.18	8.7×10^6	8.3×10^6	8.5×10^6	8.5×10^6	16 372

form biofilm on Ti-6Al-7Nb scaffolds, however the number of cells adhered to the implants was dependent on the type of surface modification.

The staphylococcal cells formed biofilm easily and in a similar number on the unprocessed samples (group A) and on the samples washed in isopropyl alcohol (group B). Differences in the number of bacterial cells, observed between samples of groups A and B, were not statistically significant (3 080 950 vs. 3 125 258 cfu/mm^2, respectively). On the contrary, chemical polishing performed for the samples of group C, correlated with a statistically significant decrease in the number of adhered staphylococcal cells in comparison to the number of biofilm forming cells on the samples of groups A and B.

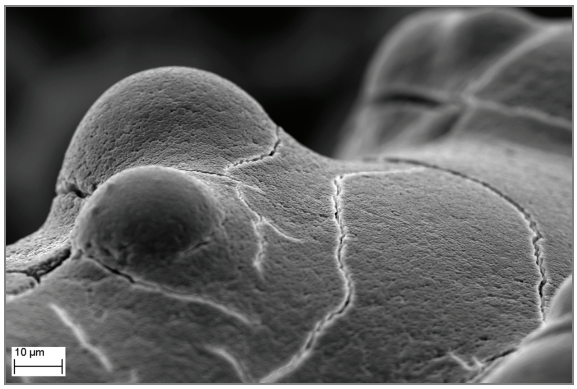


Fig. 5. *Staphylococcus aureus* ATCC 6538 forms biofilm layer on the surface of Ti-6Al-7Nb scaffold in initial state (after SLM process)

Additionally, scanning electron microscopy of biofilm formed on the samples was performed to confirm results obtained using quantitative cultures described above. As can be seen in Figs. 5 and 6, surfaces of samples after SLM process and after ultrasonic polishing are covered with dense layer of cells, whose contours are partially hidden as a result of extracellular slime production. In effect of chemical polishing of implants (Fig. 7), microbiologically intact surfaces of biomaterial are visible, bacterial cells are less numerous and they form rather small clusters than a dense cell-to-cell layer.

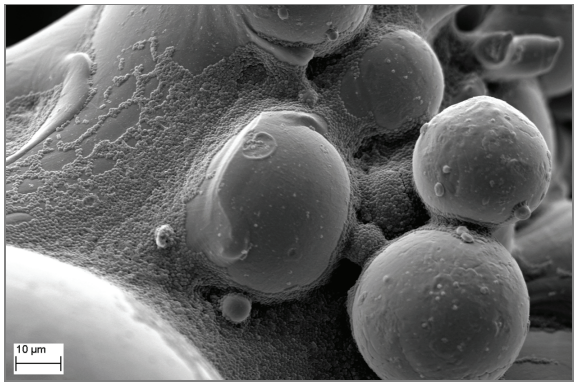
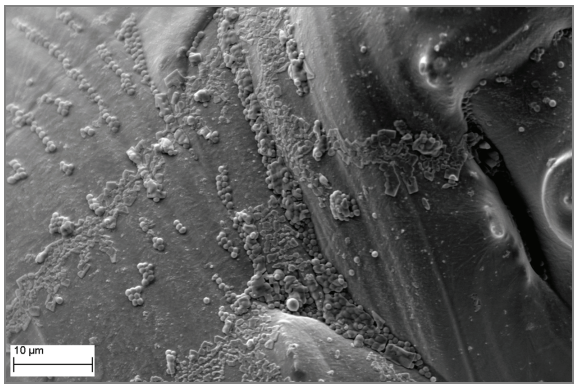


Fig. 6. *Staphylococcus aureus* ATCC 6538 forms biofilm layer on the surface of Ti-6Al-7Nb scaffold – Group B (after ultrasonic cleaning process)



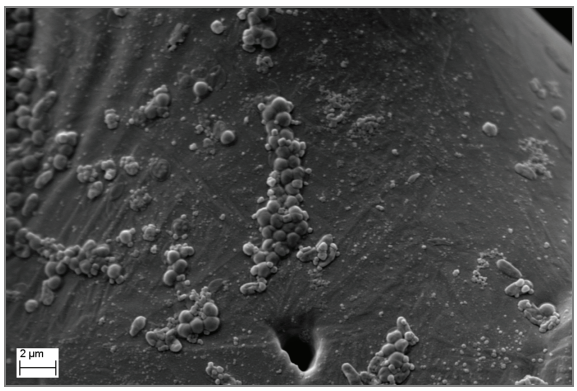


Fig. 7. Cells of *Staphylococcus aureus* ATCC 6538 adhered to the surface of Ti-6Al-7Nb scaffold after chemical polishing. The number of cells is visibly smaller in comparison to the number of cells adhered to the control scaffold after SLM process (Table 3, Fig. 4)

4. Discussion

Implant infections still remain the major complication in clinical use of the biomaterials [10], therefore it is obvious that new therapeutic and preventive strategies have to be developed and introduced. During the long evolutionary journey, microorganisms had enough time to master their mechanisms allowing them to adhere and persist on practically every type of surface. As was mentioned, there is presently no completely "biofilm-proof" biomaterial [24]. It is known that even the use of the antibiotic-loaded biomaterials, which seemed to be a very promising strategy, needs significant improvements. The main challenges that have to be solved are the kinetics of antibiotics in the biomaterials, possible alteration of the physicochemical structure and, often observed, a decrease of the biomaterial biocompatibility in result of antibiotic incorporation [11].

Various materials used in biomedical applications may be grouped into metals, ceramics, polymers, and composites made from the above groups [25]. Among different materials used in orthopaedics, titanium and titanium alloys display favourable attributes in the aspect of physical attributes (strength and corrosion resistance) and good biocompatibility. The application of metallic materials, among which titanium or its alloys is most suitable, seems to be justified because of their high fatigue strength as well as well-known features of titanium as biomaterial [26]. Selective Laser Melting (SLM) technology, used for fabrication of Ti-6Al-7Nb scaffolds described in this work, allows implants to be designed that can precisely fill the bone loss or replace affected joint. Therefore, it can be predicted that this technology is going to play more and more important part in orthopedics of the future.

Our results indicate the ability of *S.aureus*, the main etiological factor of orthopedic implant infections, to adhere and to form biofilm on the Ti-6Al-7Nb scaffolds produced by SLM. It is well known that more than half of prosthesis-associated infections are caused by *S. epidermidis* and *S. aureus* [27] with biofilm formation representing a major step in their pathogenesis. In the *in vitro* study of Gad et al. [28], be ability of biofilm's forming *S.aureus* strains to adhere to investigated biomaterials used in orthopedic surgery was assessed as high as 90.9%. In our study, we achieved a significant decrease in the number of adhered cells/mm^2 of scaffold using post-SLM chemical bathing technique; however, the question we have to answer is whether this reduction may have clinical value, in other words, whether this reduction of *S.aureus* adhesion potential would be enough to help the immune system components and antimicrobials to eradicate this microorganism from implant before it organizes in the mature biofilm form and becomes highly resistant. In our opinion, the results obtained are of paramount value, however to further answer the above question, further experiments are required to be performed on the animal model.

5. Conclusions

The data obtained indicate that the *S.aureus* strain tested is able to adhere on the Ti-6Al-7Nb scaffolds produced by Selective Laser Melting; however, the type of post-SLM surface modifications influences the ability of this microorganism to form biofilm on the titanium scaffold. Use of chemical bathing proved to be a significantly more favourable type of modification than ultrasonification and correlates with a decreased number of staphyloccocal cells on the samples, which was proven using quantitative cultures and scanning electron microscopy. Use of μCT and electron microscopic methods allowed us to explain possible reason of this phenomenon showing higher ability of chemical bathing to remove residue particles from the trabeculae of scaffold.

References

[1] BJARNSHOLT T., *Biofilm Infections*, Springer Science, 2011, ISBN 978-1-4419-6083-2.

[2] BLACK C.E, COSTERTON W., *Current concepts regarding the effect of wound microbial ecology and biofilms on wound healing*, The Surgical Clinics of North America. 2010, 6, 1147–1160.

[3] FLEMMING H., WINGENDER J., SZEWCZYK U., *Biofilm Highlights*, Springer Series on Biofilm 2008, Vol. 5, ISBN 978-3-642-19939-4.

[4] SHUNMUGAPERUMAL T., *Biofilm eradication and prevention: a Pharmaceutical Approach to Medical Device Infections*, John Wiley & Sons, New York, 2010.

[5] FUX C. et al., *Bacterial biofilms: diagnostic and therapeutic challenge*, Expert Rev. Anti. Infect. Ther., 2003, 4, 667–683.

[6] MURDOCH D.R., ROBERTS S.A., FOWLER V.G., *Infection of orthopaedic prostheses after Staphylococcus aureusbacteremia*, Clin. Infect. Dis., 2001, 32, 647–649.

[7] KRZEMIŃSKI M., BARTOSZEWICZ M., CZARNIAK E., GREGOROWICZ-WARPAS D., MĄCZYŃSKA B., JUNKA A., *The use of octenidine dihydrochloride in the treatment of musculoskeletal infections*, Adv. Clin. Exp. Med., 2010, 19, 631–636.

[8] AN Y.H., FRIEDMAN R.J., *Concise review of mechanisms of bacterial adhesion to biomaterial surfaces*, J. Biomed. Mater. Res., 1998, 43, 338–348.

[9] KRZAK-ROŚ J., FILIPIAK J., PEZOWICZ C., BASZCZUK A., MILLER M., KOWALSKI M., BĘDZIŃSKI R., *The effect of substrate roughness on the surface structure of TiO_2, SiO_2, and doped thin films prepared by the sol-gel method*, Acta of Bioengineering and Biomechanics, 2009, Vol. 11, No. 2.

[10] MONTARO L., SPEZIALE P., CAMPOCCIA D., *Scenery of Staphylococcus implant infections*, Future Microbiol., 2011, 6, 1329–1349.

[11] DUNNE N.J., HILL J., MCAFFE P., *Incorporation of large amounts of gentamicin sulphate into acrylic bone cement: effect on handling and mechanical properties, antibiotic release and biofilm formation*, Proc. Inst. Mech. Eng. H., 2008, 222, 355–365.

[12] LIU X., CHU P.-K., DING C., *Surface modification of titanium, titanium alloys, and related materials for biomedical application*, Materials Science and Engineering, 2004, R47, 49–121.

[13] LÓPEZ M.-F., GUTIÉRREZ A., JIMÉNEZ J.-A., *In vitro corrosion behavior of titanium alloys without vanadium*, Electrochimica Acta, 2002, 47, 1359–1364.

[14] VAN BEAL S., CHAI Y.C., TRUSCELLO S., *The effect of pore geometry on the in vitro biological behavior of human periosteum-derived cells seeded on selective laser-melted Ti6Al4V bone scaffolds*, Acta Biomaterialia, 2012, 8, 2824–2834.

[15] YADROITSEV I., BERTRAND P.H., SMUROV I., *Parametric analysis of the selective laser melting process*, Applied Surface Science, 2007, 8064–8069.

[16] DEEPAK K., PATTANAYAK, FUKUDA A., MATSUSHITA T., TAKEMOTO M., FUJIBAYASHI S., SASAKI K., NISHIDA N., NAKAMURA T., KOKUBO T., *Bioactive Ti metal analogous to human cancellous bone: Fabrication by selective laser melting and chemical treatments*, Acta Biomaterialia, 2011, 7, 1398–1406.

[17] HOLLISTER S.J. et al., *Engineering craniofacial scaffolds*, Orthodontics & Craniofacial Research, 2005, 8, 162–173.

[18] CHLEBUS E., KUŹNICKA, B., KURZYNOWSKI T., DYBAŁA B., *Microstructure and mechanical behaviour of Ti-6Al-7Nb alloy produced by selective laser melting*, Materials Characterization, 2011, 62, 488–495.

[19] KURZYNOWSKI T., CHLEBUS E., KUŹNICKA B., REINER J., *Parameters in selective laser melting for processing metallic powders*, Proceedings of SPIE – The International Society for Optical Engineering 8239, San Francisco, 2012; art. no. 823914.

[20] GARRETT E.R., ABHAY S.P., DIMITRIOS P.A., *Porous titanium scaffolds fabricated using a rapid prototyping and powder metallurgy technique*, Biomaterials, 2008, 3625–3635.

[21] HO SAEY TUAN, HUTMACHER D.W., *Application of micro CT and computation modeling in bone tissue engineering*, Computer-Aided Design, 2005, 37, 1151–1161.

[22] MORRIS D.E., MATHER M.L., *Time-optimized X-ray micro-CT imaging of polymer based scaffolds*, Appl. Biomater., 2012, 100B, 360–367.

[23] YE TAN, KIM K., *Material Dependent Thresholding for Dimensional X-ray Computed Tomography*, International Symposium on Digital Industrial Radiology and Computed Tomography, Berlin, Germany, 2011.

[24] SHUNMUGAPERUMAL T., *Biofilm eradication and prevention*, John Wiley & Sons, ISBN 978-0470-47996, 2010.

[25] CHANDRAMOHAN D., MARIMUTHU K., *Characterization of natural fibers and their application in bone grafting substitutes*, Acta of Bioengineering and Biomechanics, 2011, Vol. 13, No. 1.

[26] SŁOWIŃSKI J., *Procedure of generating the individually matched bone scaffolds*, Acta of Bioengineering and Biomechanics, 2011, Vol. 13, No. 3.

[27] ZIMMERLI W., OCHSNER P.E., *Management of infection associated with prosthetic joints*, Infection, 2003, 31(2), 99–108.

[28] MAHMOUD GAD G.F., ABDEL AZIZ A.A., IBRAHEM R.A., *In-vitro adhesion of Staphylococcus spp. to certain orthopedic biomaterials and expression of adhesion genes*, J. App. Pharm. Sci., 2012, 02 (06), 2012, 145–149.