Joanna CZACH[1], Viktoria HOPPE[2], Patrycja SZYMCZYK[3], Adam JUNKA[4]

[1]Studenckie Koło Naukowe BioAddMed, Politechnika Wrocławska
[2]Studenckie Koło Naukowe Materiałoznawstwa im. doc. Rudolfa Haimanna, Politechnika Wrocławska
[3]Katedra Technologii Laserowych, Automatyzacji i Organizacji Produkcji, Centre for Advanced Manufacturing Technologies (CAMT/FPC), Politechnika Wrocławska
[4]Katedra Mikrobiologii Farmaceutycznej i Parazytologii, Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

MICROSTRUCTURE, HARDNESS MEASUREMENTS AND CYTOTOXICITY OF MEDICAL TITANIUM ALLOYS MANUFACTURED USING ADDITIVE MANUFACTURING

Summary: Additive Manufacturing (AM) is a rapidly developing technology that has many applications in the industry nowadays, as well as in medicine. That group of technologies have a significant advantage over traditional manufacturing processes as they enable fabrication of parts of almost any conceivable geometric shape and complex internal architecture. Electron Beam Melting (EBM) and Selective Laser Melting (SLM) are examples of Additive Manufacturing. Both use metallic powder as their building material, however energy sources used during the manufacturing process are different. First technology uses a concentrated electron beam and the second a high-energy laser. In this paper, cubic samples manufactured using EBM and SLM technologies from medical titanium alloys (Ti6Al4V and Ti6Al7Nb) were tested. Microstructure, hardness of samples and their cytotoxicity was determined. Due to very high gradients of temperature, during the AM processes, obtained microstructures are similar to multistage heat treatment of a conventionally manufactured titanium alloys. Hardness measurements show a great repeatability of results, with similar values regardless of building direction. They maintain at the level of 372 - 392 HV, which also suggests that heat treatment occurs during the process. For medical application, it is necessary that the used materials were characterized by low cytotoxicity. Due to their contact with human body, the possibility of harming cells must be eliminated. For this purpose, a biological analysis was performed under controlled conditions (37 ° C / 5% CO2) at 100% humidity, which confirmed the high purity of the materials.

Key Words: EBM, SLM, additive manufacturing, cytotoxicity test

1. INTRODUCTION

Additive manufacturing (*AM*) is a type of technology that builds three dimensional objects through joining very thin layers of material. A 3D digital model is divided in such layers via

computer software and then, during a process commonly know as “printing”, those sheets are connected, creating the element.

First norms that regulate nomenclature were published by American Society for Testing and Materials – ASTM International in 2012 in “ASTM International 2013. ASTM F2792-12a - Standard Terminology for Additive Manufacturing Technologies”. Additive manufacturing was divided into seven categories, with the method of connecting the material as the criterium. One of those categories is Powder Bed Fusion – connecting the particles that were earlier deposited into layers.

Examples of this are Electron Beam Melting (*EBM*) and SLM. Both uses metallic powder that is melted layers by layers using a concentrated electron beam or laser beam, respectively. As the electrons are the carriers of energy, inertia or strong reflections do not occur, which differentiates this kind of AM from those using photons (e.g. *SLM*) [1]. Very high temperature that transpires during the process (approximately 700 °C) allows to minimize both temperature gradients and local cooling rates, which is the reason for microstructures that resemble those after a multistage heat treatment [2–4].

The most common materials used in implants are titanium alloys, with Ti-6Al-4V and Ti-6Al-7Nb being the most standard. Both are examples of alpha-beta titanium alloy. They have high corrosion resistance, good biotolerance and mechanical properties that allow being used in implants. The additional advantage is their light weight [5–8].

2. THE PURPOSE OF THE STUDY

The purpose of this work was to compare microstructures and hardness of Ti-6Al-4V and Ti-6Al-7Nb cubic samples created using two different techniques of additive manufacturing - EBM and SLM, as well as to assess their compatibility with human cells.

3. METHODS AND RESULTS

Tests were conducted in order to determine microstructure, hardness and compressive strength of samples manufactured via EBM and SLM technologies, as well as cytotoxicity. Using an optical microscope NIKON ECLIPSE MA200 and NIS Elements BR software pictures of samples, both non-etched and etched with Kroll’s reagent, were taken in 200x, 500x and 1000x magnification. Microscopic examinations were performed on appropriately prepared metallographic specimens. For this purpose, samples were cut along the XZ and XY plane. Samples’ hardness HV10 was tested using Zwick/Roell ZHU hardness tester. Cytotoxicity was measured according to standard PN-EN ISO 10993-5. In vitro test was using human osteoblasts from collection ATCC CRL-11372 (American Type Culture Collection). Scaffolds were subjected to steam sterilization in 120 °C (2,2 bar, 10 minutes) according to ISO 10993 standard. Five specimens of both materials were put in a sterilized medium with foetal serum used for cells’ cultivation and left there for 24 hours in a controlled environment (37 °C / 5% CO2 / full humidity). After that time the medium was used in cell culture, cultivated in a controlled environment (37 °C / 5% CO2 / full humidity) for 24 or 48 hours in 96-spaces plate. Subsequently the plates were cleared with saline and every space was filled with 100 µl NR solution. They were incubated for 3 hours in controlled environment (37 °C / 5% CO2 / full humidity) and after clearing with PBS buffer, they remained until desiccation. Afterwards, every space was filled with 100 µl solution used for extraction of red light and the plate was inserted into a shaker for 25 minutes in the absence of light. The samples were analyzed using spectrometer UVM-340 with wavelength of 540 nm.

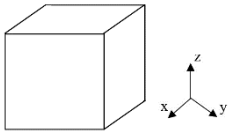
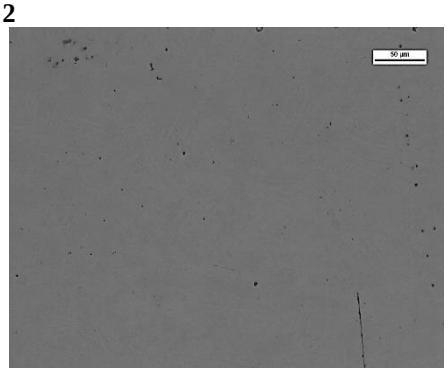


Fig. 1 Sample shape

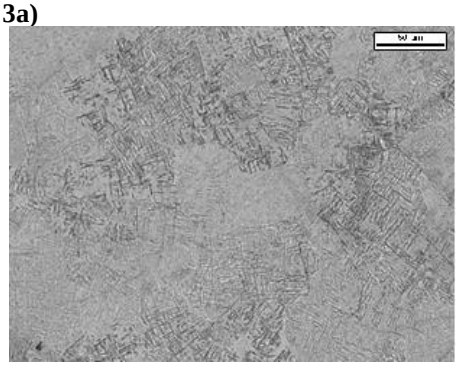
3.1. Microstructure

Additive manufacturing creates a very distinctive morphology - α' + β or α + β. EBM technology causes β grains grow homoepitaxially (with the same orientation), causing gradual solidification of layers. It results in grains that are elongated in the direction of thermal gradient. During cooling, α lamellae grow in β grains [9]. SLM induces occurrence of lamellar α + β microstructure [10]. Mechanical properties of titanium alloys, especially two phase ones, are morphology dependant [11].

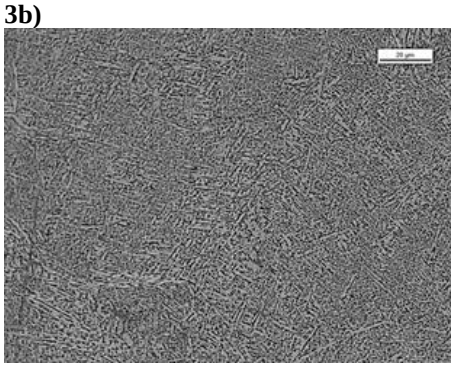
2



3a)



3b)



3c)

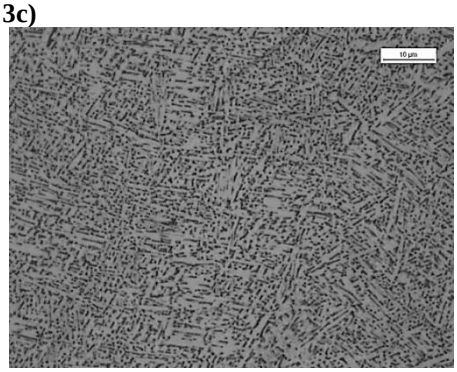
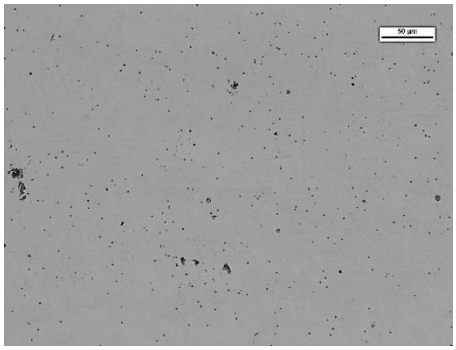


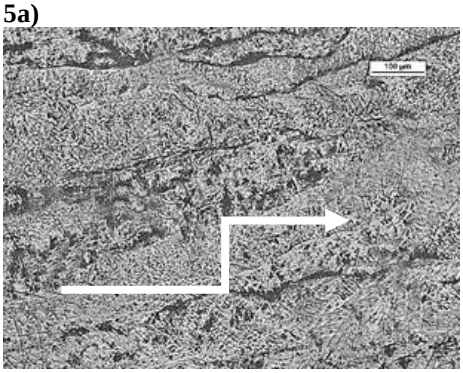
Fig. 2 Ti-6Al-4V alloy, EBM-processed, transverse microsection. Line of small, spherical porosities. Unetched. Light microscopy. Mag. 200x

Fig. 3 Ti-6Al-4V alloy, EBM- processed, transverse microsection. Changing direction of thin lamellar α grains. Phase β is dark, between lamellae. Intertwining basket-like structure. Etched with Kroll's reagent. Light microscopy. a) Mag. 200x b) Mag. 500x c) Mag. 1000x

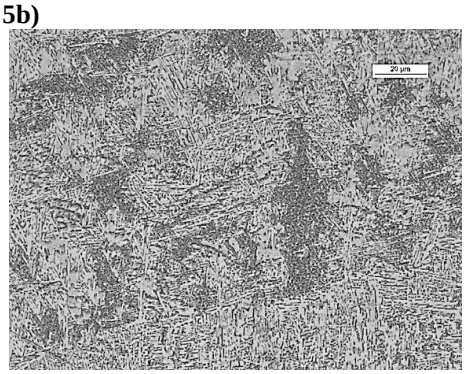
4



5a)



5b)



5c)

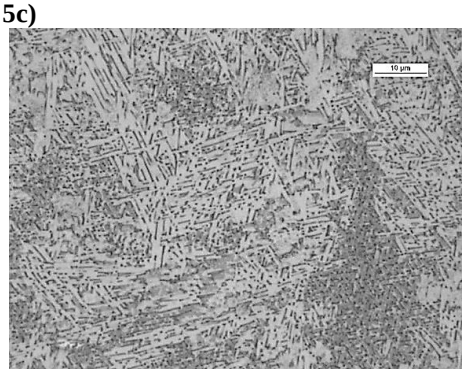
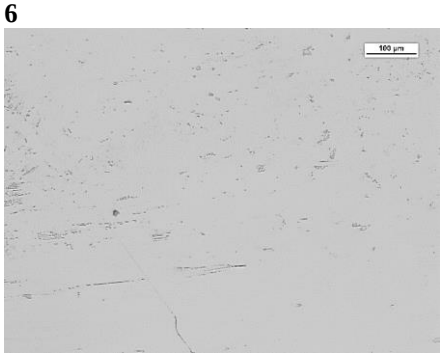


Fig. 4 Ti-6Al-4V alloy, EBM-processed, longitudinal microsection. Multiples of small, spherical porosities. Unetched. Light microscopy. Mag. 200x

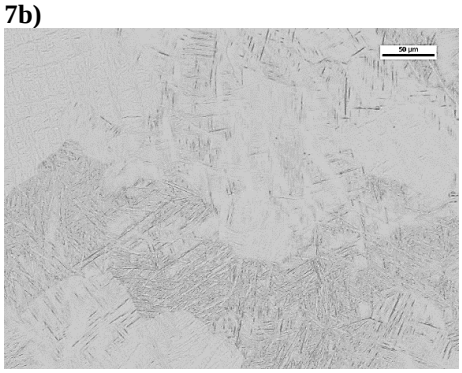
Fig. 5 Ti-6Al-4V alloy, EBM- processed, longitudinal microsection. Changing direction of thin lamellar α′ grains. Phase β is dark, between lamellae. Some globular α grains. Lots of porosities. Direction of accretion shown with arrow. Etched with Kroll's reagent. Light microscopy. a) Mag. 200x b) Mag. 500x c) Mag. 1000x

6



7a)

7b)



7c)

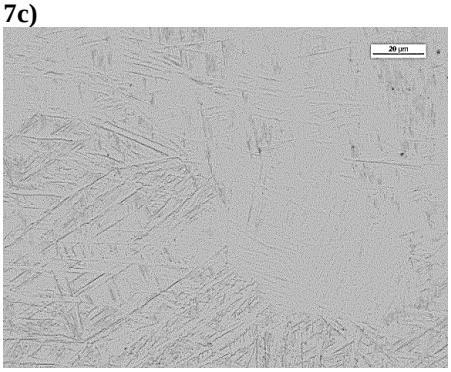


Fig. 6 Ti-6Al-4V alloy, SLM-processed, transverse microsection. Small porosities. Unetched. Light microscopy. Mag. 100x

Fig. 7 Ti-6Al-4V alloy, SLM- processed, transverse microsection. Thin lamellar α grains are spread radially. Phase β is small and grey, between lamellae. β-grains' boundaries are visible. Etched with Kroll's reagent. Light microscopy. a) Mag. 200x b) Mag. 500x c) Mag. 1000x.

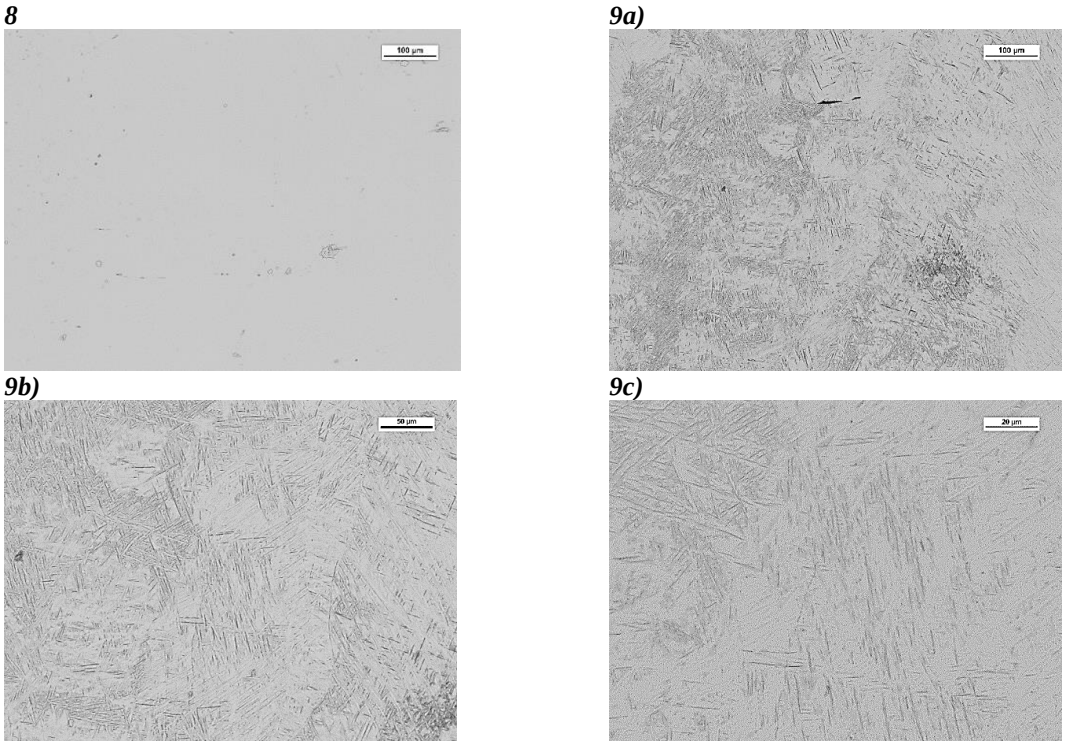


Fig. 8 Ti-6Al-4V alloy, SLM-processed, longitudinal microsection. Multiples of small, spherical porosities. Unetched. Light microscopy. Mag. 200x

Fig. 9 Ti-6Al-4V alloy, SLM- processed, longitudinal microsection. Thin lamellar α grains are crossing themselves. Phase β is small and grey, between lamellae. β-grains' boundaries are visible. Etched with Kroll's reagent. Light microscopy. a) Mag. 200x b) Mag. 500x c) Mag. 1000x

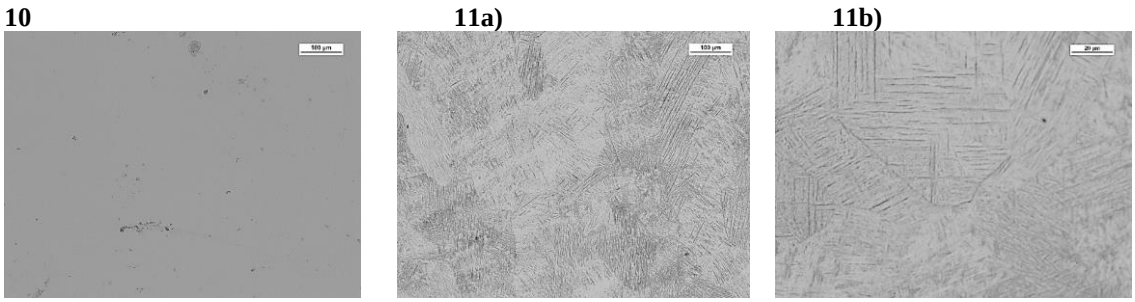


Fig. 10 Ti-6Al-7Nb alloy, SLM-processed, transverse microsection. Small porosities. Unetched. Light microscopy. Mag. 100x.

Fig. 11 Ti-6Al-7Nb alloy, SLM- processed, transverse microsection. Thin lamellar α grains are parallely packed . Phase β is small and grey, between lamellae. β-grains' boundaries are visible. Etched with Kroll's reagent. Light microscopy. a) Mag. 200x b) Mag. 500x c) Mag. 1000x

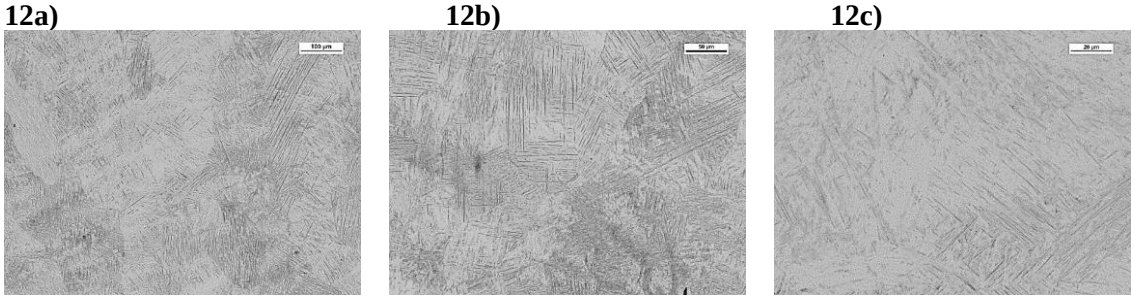


Fig. 12 Ti-6Al-7Nb alloy, SLM- processed, longitudinal microsection. Thin lamellar α grains are needle-like, both parallely packed and crossing themselves, creating intertwining basket-like structure. Phase β is small and grey, between lamellae. β-grains' boundaries are visible. Etched with Kroll's reagent. Light microscopy. a) Mag. 200x b) Mag. 500x c) Mag. 1000x

3.2. Hardness

Tests of hardness show results in range 372 – 392 HV (Fig. 13), which is similar to conventionally processed alloys [12,13], though with a slight rise for the alloy with niobium. Standard deviation is below 5% for EBM processes and 10% for SLM processes, with the exception of Ti-6Al-4V alloy cut longitudinally. Hardness of both Ti-6Al-7Nb samples was higher than Ti-6Al-4V samples created using both SLM and EBM.

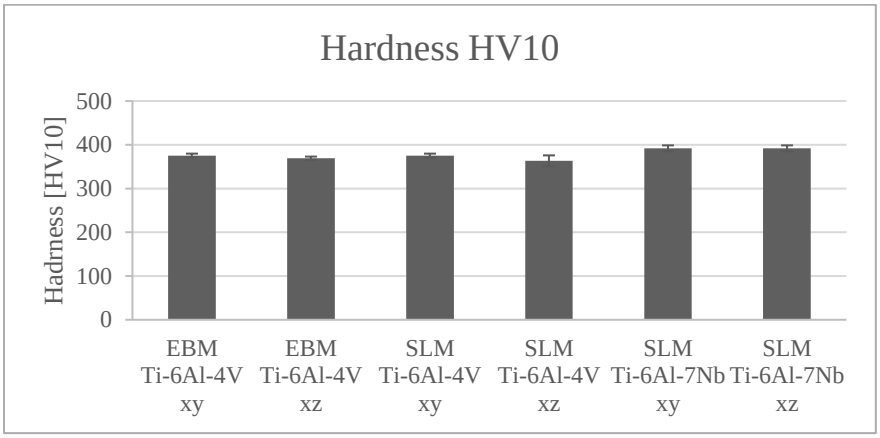


Fig. 13 Hardness of samples

3.3. Cytotoxicity

Cytotoxicity was tested according to norm PN-EN ISO 10993-5: after sterilization in 120 °C, samples were inserted into a medium used for cells growth and remained there for 24 and 48 hours. After that time, samples were extracted and the liquid was used for cells growth. Results of the test are shown in Table 1.

Table 1. Results of cytotoxicity test

Type of implant	Osteoblasts' survival rate; 24h.	Assessment of cytotoxicity 24h	Osteoblasts' survival rate; 48h.	Assessment of cytotoxicity 48h
Ti-6Al-4V	93,70 [%]	Low cytotoxicity, high survival rate	77,95 [%]	Moderate cytotoxicity, small reduction in life expectancy
Ti-6Al-7Nb	75,39 [%]	Moderate cytotoxicity, small reduction in life expectancy	90,50 [%]	Low cytotoxicity, high survival rate

The cytotoxicity of Ti-6Al-4V samples was low after 24h (survival rate of osteoblasts was 93,70%), but it changed after 48h, becoming moderate (73,39%). The Ti-6Al-4Nb samples acted inversely, having a moderate cytotoxicity after 24h (75,39%), but low after 48h (90,50%). It suggests that Ti-6Al-7Nb is ultimately less toxic for cells and should be chosen over Ti-6Al-4V if possible. To lower the cytotoxicity, a different form of sterilization might be considered.

4. CONCLUSION

During EBM and SLM processes heat treatment occurs, causing different microstructures due to the distinction in temperatures. Porosities occur due to the nature of the processes; however, they can be minimized with adequate parameters or hot isostatic pressing (HIP) process after manufacturing. Results of achieved hardness of samples are similar to those acquired from conventional implants, with a slight rise for the Ti6Al7Nb alloy, which was the highest achieved in the entirety of the study. All results are repeatable, with smaller (less than 5%) standard deviations for the EBM-manufactured cubic samples. Although the microstructure is different than that standard processed medical alloys, cytotoxicity was assessed as low - both metals after those processes are safe to use in medicine, allowing for the growth of cells.

BIBLIOGRAPHY

[1] Körner C.: Additive manufacturing of metallic components by selective electron beam melting — a review. International Materials Reviews, vol. 61(5), 2016, p. 1–17.
[2] Klingbeil N.W., Beuth J.L., Chin R.K., Amon C.H.: Residual stress-induced warping in direct metal solid freeform fabrication. International Journal of Mechanical Sciences, vol. 44(1), 2002, p. 57–77.
[3] Moussaoui K., Mousseigne M., Senatore J., Chieragatti R.: The effect of roughness and residual stresses on fatigue life time of an alloy of titanium. The International Journal of Advanced Manufacturing Technology, vol. 78(1-4), 2015, p. 557–563.
[4] Weiwei H., Wenpeng J., Haiyan L., Huiping T., Xinting K., Yu H.: Research on Preheating of Titanium Alloy Powder in Electron Beam Melting Technology. Rare Metal Materials Engineering, vol. 40(12), 2011, p. 2072–2075.
[5] Dobrzański L.A.: Materiały inżynierskie i projektowanie materiałowe. Podstawy nauki o materiałach i metaloznawstwo. Wydanie II. WNT, Warszawa 2006.
[6] Chlebus E., Kuźnicka B., Kurzynowski T., Dybała B.: Microstructure and mechanical behaviour of Ti-6Al-7Nb alloy produced by selective laser melting. Material Characterization, vol. 62(5), 2011, p. 488–495.
[7] Lütjering G., Williams J.C.: Titanium (Engineering Materials and Processes). vol. 1. Springer, New York 2007.
[8] de Formanoir C., Michotte S., Rigo O., Germain L., Godet S.: Electron beam melted Ti-6Al-4V: Microstructure, texture and mechanical behavior of the as-built and heat-treated material. Material Science Engineering: A, vol. 652, 2016, p. 105–119.
[9] Szymczyk, P.; Ziółkowski, G.; Junka, A.; Chlebus, E.: Application of Ti6Al7Nb Alloy for the Manufacture of Biomechanical Functional Structures (BFS) for Custom-Made Bone Implants. Materials 2018, 11, 971.
[10] Uhlmann E., Kersting R., Klein T.B., Cruz M.F., Borille A.V.: Additive Manufacturing of Titanium Alloy for Aircraft Components. Procedia CIRP, vol. 35, 2015, p. 55–60.
[11] Sieniawski J., Ziaja W., Kubiak K., Motyka M.: Microstructure and Mechanical Properties of High Strength Two-Phase Titanium Alloys. Titan Alloys Advances in Properties Procedia CIRP, vol. 35, 2015, p. 55–60.
[12] Niinomi M.: Mechanical properties of biomedical titanium alloys. Material Science Engineering: A, vol. 243(1-2), 1998, p. 231–236.
[13] Semlitsch M.F., Weber H., Streicher R.M., Schön R.: Joint replacement components made of hot-forged and surface-treated Ti-6Al-7Nb alloy. Biomaterials, vol. 13(11), 1992, p. 781–788.

MIKROSTRUKTURA, POMIARY TWARDOŚCI ORAZ CYTOTOKSYCZNOŚĆ MEDYCZNYCH STOPÓW TYTANU WYPRODUKOWANYCH PRZY UŻYCIU WYTWARZANIA PRZYROSTOWEGO

Abstract: Wytwarzanie przyrostowe to szybko rozwijające się technologie mająca wiele zastosowań, zarówno w przemyśle, jak i medycynie. Charakteryzują się one wyraźną przewagą nad tradycyjnymi sposobami produkcji, gdyż pozwalają na wytwarzanie każdego geometrycznego kształtu, a także skomplikowaną architekturę wewnętrzną. Przetapianie Wiązką Elektronów (EBM, ang. *Electron Beam Melting)* oraz Selektywne Przetapianie Laserowe (SLM, ang. *Selective Laser Sintering*) są przykładami wytwarzania przyrostowego. Oba używają proszku metalowego jako materiału, jednakże źródła energii wykorzystywane w czasie produkcji są różne. Pierwszy używa skoncentrowanej wiązki elektronów, a drugi wysokoenergetycznego lasera. Podczas badania wyznaczono mikrostrukturę, twardość i cytotoksyczność próbek wykonanych metodami EBM i SLM z medycznych stopów tytanu (Ti6Al4V i Ti6Al7Nb).W związku z wysokimi gradientami temperaturowymi, mikrostruktury otrzymane podczas wytwarzania przyrostowego przypominają te, które daje konwencjonalna, wieloetapowa obróbka cieplna. Pomiary twardości wykazały powtarzalność wyników, z podobnymi wartościami niezależnie od kierunku budowy próbki. Znajdują się one w zakresie 372 – 392 HV, co sugeruje zachodzenie obróbki cieplnej podczas samego procesu. Użytkowanie materiału w medycynie wymaga niskiej cytotoksyczności, ze względu na kontakt z ludzkim ciałem. Próbki poddano biologicznej analizie w kontrolowanych warunkach (37 ° C / 5% CO2) w wilgotności równej 100%, co potwierdziło wysoką czystość materiałów.