In-vitro bioactivity evaluation of titanium layers manufactured on implants with LENS method

Ocena bioaktywności in-vitro warstw tytanowych wytwarzanych techniką LENS na implantach

BARTŁOMIEJ WYSOCKI
AGATA SUPEŁ
TOMASZ DUREJKO
WOJCIECH ŚWIĘSZKOWSKI *

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Laser Engineering Net Shaping (LENS), which is one of the 3DP techniques, allows for both, the fabrication and the modification of surface layer of the product previously prepared. In the current work, using LENS technique, highly developed bioactive layers, were prepared on the surface of the hip prosthesis. Both, fabricated layers and hip prosthesis, were fabricated from Ti-6Al-4V alloy. The tests were carried out using Simulated Body Fluid (SBF) solution, having all the necessary components on the inorganic human blood plasma. Scanning Electron Microscopy (SEM) showed the nucleation of calcium phosphates even after 2 days of immersion. Based on the results obtained in SEM and EDX tests, it can be claimed that Ti-6Al-4V layers manufactured using LENS technique exhibit high bioactivity in in-vitro, in the SBF solution.
KEYWORDS: LENS, SBF, in-vitro, bioactivity, calcium phosphates

One of the 3D printing techniques that allows for the implant surface modification is LENS (Laser Engineering Net Shaping). Increased implant surface development using LENS is achieved by applying a thin layer of powder fused in the laser focus. This process promotes osteoconductivity and eliminates the need for bone cement during implantation. Elimination of the use of bone cement in the hip endoplasty is particularly important because of the exothermic reaction that results in the death of surrounding tissues. Hip joint endoprosthesis coated with bioactive layer of Ti-6Al-4V alloy, made by LENS method, will provide good fixation of bone implant without the use of bone cement, which is the purpose of the experiment. The possibility of producing a permanent implant link at the implant border can be checked prior to clinical trials, e.g. by *in vitro* assays in SBF solution, during which calcium phosphates is formed, including hydroxyapatite, being the major component of human bone.

*Mgr inż. Bartłomiej Wysocki (bartlomiej.wysocki@wimpw.edu.pl), inż. Agata Supeł (agatasupel1@gmail.com) – Wydział Inżynierii Materiałowej Politechniki Warszawskiej, Materialscare Sp. z o.o.; dr inż. Tomasz Durejko (tomasz.durejko@wat.edu.pl) – Wojskowa Akademia Techniczna; prof. nadzw. dr hab. inż. Wojciech Święszkowski (wswieszk@inmat.pw.edu.pl) – Wydział Inżynierii Materiałowej Politechniki Warszawskiej

Materials and methods

Ti-6Al-4V layers were made on a solid substrate of the same alloy using the OPTOMEC MR-7 (fig. 1). The distance between the tracks was 0.5 mm and their height was 0.25 mm. After the manufacturing process, samples were purified from residual powder in water and ethanol with an ultrasonic scrubber. Prior to bioactivity testing, the samples were subjected to surface activation using 5M NaOH (24 h, 40 °C).

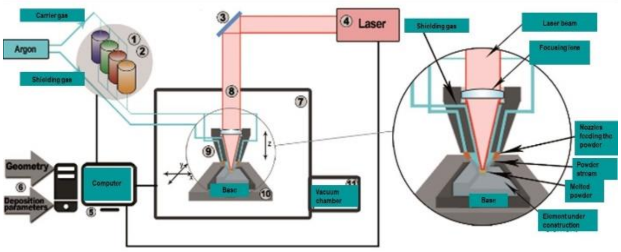

Fig. 1. Diagram of endoprosthesis modifications using the device

The study was conducted under conditions similar to those in the human body. For this purpose, a SBF solution of the composition given in Table [3] was prepared. It contains all necessary inorganic components of human blood plasma. The pH of the prepared solution also corresponds to the value in human body - 7.4. Incubation of samples was carried out at a temperature near the physiological body temperature of 37 °C, in sealed tubes. The maximum incubation period was 35 days.

TABLE. Comparison of ions concentration in plasma and prepared SBF solution

Ions	Ion concentration, mM	
	Plasma	SBF
Na^+	142,0	142,0
K^+	5,0	5,0
Mg^{2+}	1,5	1,5
Ca^{2+}	2,5	2,5
Cl^-	103,0	147,8
HCO_3^-	27,0	4,2
HPO_4^{2-}	1,0	1,0
SO_4^{2-}	0,5	0,5
pH	7,2–7,4	7,4

SEM observations

The formation of Ca-P nuclei on the surface of implants was observed after two days (Figure 2a). After a few days, the speed of formation and the number of nuclei are increasing. After four days the embryos begin to merge (fig. 2b) to start creating a continuous layer that is visible after six days (Figure 2c). After 35 days the indentations in the layers are covered with homogeneous Ca-P layers (fig. 2d).

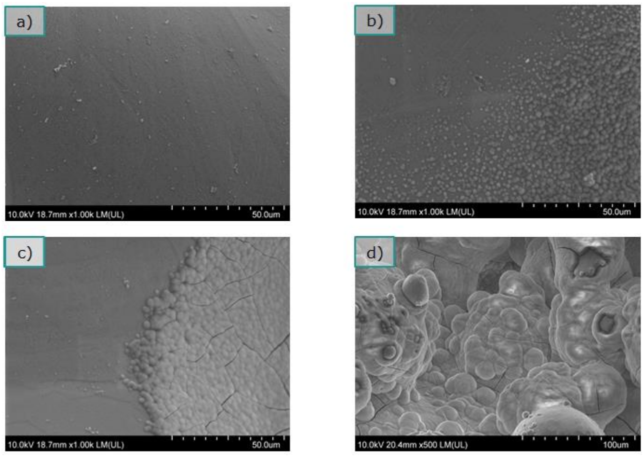

Fig.2. Surface of samples after: a) 2 days, b) 4 days, c) 6 days, d) 35 days

Analysis of chemical composition

Reconstruction of the generated layer prior to the immersion process is shown in Figure 3. The EDS analysis (Figure 4) confirms that Ca-P layers are first formed in the recesses of the coatings produced. After 35 days, the calcium phosphate layer was homogeneous, uniform and at least 1-2 μm thick. The EDS analysis showed that the Ca/P ratio in the resulting layers was approximately 1.33, suggesting the formation of calcium phosphate (OCP) [3].

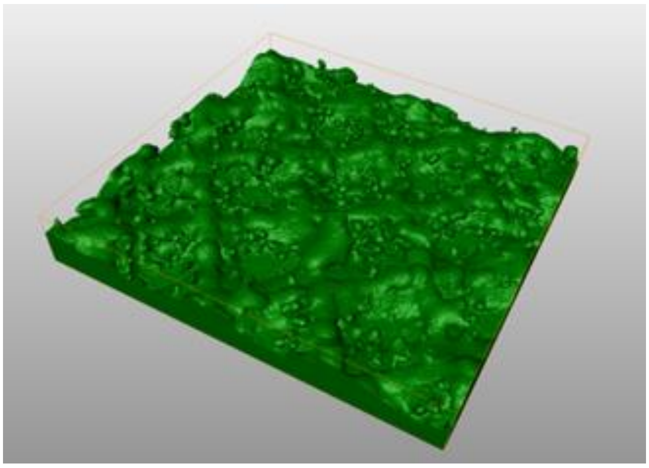

Fig. 3. Reconstruction of the produced surface (CT analysis)

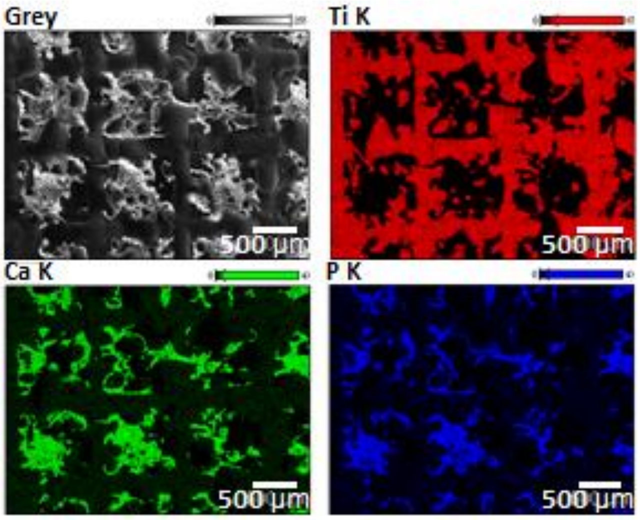

Fig. 4. Analysis of the chemical composition of the sample surface (35 days) after immersion in SBF

Conclusions

Based on the studies conducted, it can be concluded that the layers made of Ti-6Al-4V alloy by means of LENS method exhibit high bioactivity under SBF *in vitro* conditions. The formation of homogeneous Ca-P layers on implants demonstrates the possibility of providing an ostheointegration between the implant and the surrounding *in vivo* bone tissue. The results obtained are particularly important for the predicted cell and clinical studies.

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