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# Comparison of Ti6Al4V titanium alloy membrane prototypes for bone defect repair made by laser sintering and electron beam melting

**Summary.** A comparative analysis of the microstructure and mechanical properties of guided bone regeneration frame membranes made from Ti6Al4V titanium alloy powder using 3D printing technology is presented in this paper. Two different methods were used to produce the samples: direct laser sintering of metals (DMLS) and electron beam melting (EBM). The plates, measuring 30×10×1 mm, were formed from layers 30 μm thick. The surface morphology of the samples was studied at both the micro and macro levels using scanning electron microscopy (SEM) and scanning impulse acoustic microscopy (SIAM). Biocompatibility was assessed both in vitro with mesenchymal stem cell (MSC) cultures and in vivo with laboratory animals. Mechanical properties were evaluated using a three-point bending test, which revealed differences in surface profile depth that was 100 and 150 μm for the DMLS and EBM correspondingly. Samples produced using DMLS technology demonstrated higher strength 2,180±20.7 MPa and elasticity 53,449±200 MPa than those produced by EBM strength 1500±26.1 and elasticity 25,633±125 MPa, according to the results of the mechanical tests. A more active proliferation of MSCs was observed in vitro in the DMLS samples, which was 70% higher compared to EMB and the control group. The bone tissue response to both types of titanium implants was good, with high levels of osseointegration, as confirmed by X-ray microtomography (μCT).

**Key words:** guided bone regeneration, titanium alloy, laser sintering of metals, Ti6Al4V titanium, dental implant, electron beam melting, porous microstructure

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# Сравнение прототипов мембран из титанового сплава Ti6Al4V для устранения дефектов кости, изготовленных методом лазерного спекания и электронно-лучевой плавки

**Аннотация.** В данной работе представлен сравнительный анализ микроструктуры и механических свойств мембран для направленной костой регенерации, изготовленных из порошкового титанового сплава Ti6Al4V с использованием технологии 3D-печати. Для изготовления образцов применялись два различных метода: прямое лазерное спекание металлов (DMLS) и электронно-лучевая плавка (EBM). Пластины размером 30×10×1 мм были сформированы из слоев толщиной 30 мкм. Морфология поверхности образцов была исследована

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на микро- и макроуровнях методом сканирующей электронной (SEM) и импульсной акустической (SIAM) микроскопии. Биосовместимость оценивали in vitro на культурах мезенхимальных стволовых клеток (МСК) и in vivo на лабораторных животных. Механические свойства оценивались методом трехточечного изгиба, что выявило различия в глубине профиля поверхности — 100 и 150 мкм для DMLS и EBM соответственно. Согласно результатам механических испытаний, изготовленные по технологии DMLS образцы продемонстрировали более высокую прочность (2180±20,7 МПа) и модуль упругости (53 449±200 МПа), чем EBM-образцы — 1500±26,1 и 25 632,6±125 МПа соответственно. Было отмечено, что более активная пролиферация мезенхимальных стволовых клеток наблюдалась in vitro в образцах DMLS. Реакция костной ткани на оба типа титановых имплантатов была хорошей с высоким уровнем остеоинтеграции, что было подтверждено рентгеновской микротомографией (µКТ).

**Ключевые слова:** направленная костная регенерация, титановый сплав, лазерное спекание металлов, титан Ti6Al4V, зубной имплантат, электронно-лучевая плавка, пористая микроструктура

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## INTRODUCTION

Due to the increasing life expectancy of humans, the development and improvement of dental implant technologies has become an area of growing scientific and clinical interest. Bone grafting is necessary for various reasons, such as odontogenic diseases, injuries to the maxillofacial region, and tumor conditions that lead to loss of jawbone tissue [1—3]. The most promising outcomes of bone grafting can be achieved through guided bone regeneration techniques (GBR) [4—6].

An essential component of GBR is the use of a membrane, which acts as a tissue barrier and framework to restrict and protect the reconstruction site [7, 8]. This guided bone regeneration frame membrane provides mechanical support, maintains tissue volume, and ultimately ensures successful regeneration. Important mechanical properties that the frame should have include stiffness under load, strength, and fatigue resistance. When the stiffness of the frame exceeds that of natural bone, the concentration of stress on the surrounding bone can cause bone destruction. On the other hand, if the stiffness of the implant is less than that of natural bones, stress concentration in the implant may cause rejection and bone atrophy [9—11]. This mismatch in stiffness leads to uneven stress distribution between the implant and the bone, a phenomenon known as stress shielding [12, 13]. The effect of this has not been fully investigated in small titanium implants used for bone regeneration.

Titanium alloys are widely used in maxillofacial surgery, trauma, and orthopedics due to their unique combination of properties, including biocompatibility, resistance to corrosion, strength, and plasticity [14—16]. In 1952, Swedish orthopedic surgeon Per-Ingvar Brånemark discovered titanium's ability to bond with bone, a phenomenon known as osseointegration, which led to the development of the first titanium dental implant in 1965. Medical-grade titanium has since been used in fields such as orthopedics, craniofacial surgery, and heart valve replacement [17].

Two grades of medical titanium are most used: Grade 4 (VT-1.0 in Russia) and Grade 5 (Ti6Al4V). The gold standard for manufacturing medical implants is the use of computer-controlled milling machines to create blanks, but titanium alloys also offer the advantage of being suitable for 3D printing. For example, the direct laser fusion of titanium alloy powder allows for the creation of individualized skeletal structures, which provides an undeniable advantage in terms of successful implantation and longevity [18]. The intraoperative use of standard frame membranes is labor-intensive, as it is necessary to manually model its shape, which greatly increases the operation time. The individualization of the titanium membrane for guided bone regeneration leads to a reduction in the number of postoperative complications, such as membrane stripping and regeneration effusion.

In this paper, a comparative analysis of the microstructure and mechanical properties of prototypes of frame membranes made of Ti6Al4V titanium alloy powder by layer-by-layer 3D printing using direct laser sintering of metals (DMLS) technology and using an electron beam melting (EBM) is carried out, because these techniques are currently the most widely available and used in medical device manufacturing. The morphology of the surface of the samples was studied at the micro and macro levels using electron scanning and acoustic microscopy. Biocompatibility is assessed in vitro using a rat mesenchymal stem cell (MSC) culture and in vivo using laboratory animals. The mechanical properties were determined based on the results of three-point bending test.

**The aim** of this study was to conduct in vitro and in vivo studies of titanium plates produced by two different 3D printing techniques: laser sintering and electron beam melting. Investigation of their physico-chemical, strength and biological properties allows choosing the optimal prototyping method for further clinical use.

## MATERIALS AND METHODS

Titanium plates were produced in the company AB Universal (Moscow, Russia).

3D printing of frame membranes was done using finely dispersed titanium alloy powder Ti6Al4V, using the direct laser sintering of metals (DMLS) technology. The plates were created layer by layer using the ULS-125 laser system. The powder particles were spherical with a diameter of between 20 and 40 μm, which ensured good flowability and uniform distribution to fill the entire working volume of the machine. Argon was used to fill the chamber to avoid oxidation and powder ignition.

The features of the formation of frame membranes using 3D printing technology, specifically the process of layered fusion of titanium alloy powder using electron beam melting (EBM), are described in detail in [19]. For our work, we used Ti6Al4V powder with a particle size of 90—100 μm and the Arcam Q20 Plus electron beam gun, which operates in a vacuum chamber.

All products were manufactured in the form of flat plates measuring 30×10×1 mm. To further study the morphology of the plates, the surface of half the samples was ground using a carbide dental milling cutter to process metal frames, while half of the samples remained unchanged. The chosen approach is due to the existing standard of always grinding surfaces for clinical use, which leads to a change in the surface structure due to mechanical processing. The morphology of the sample surfaces was examined using a scanning electron microscope at an accelerating voltage of 10 kV and in the secondary electron detection mode.

The surface microstructure of the samples was examined by means of ultrasound [20]. A laboratory scanning pulse acoustic microscope (SIAM-2018) was used in reflection mode. The nominal frequency of the ultrasonic emitter was 50 MHz and the angular aperture of the lens was 30°. Distilled water was used for immersion. The focal spot diameter of the scanned beam was approximately 50 μm and the scanning step was in increments of 15 μm. Surface relief profiles were estimated from cross-sectional images (B scans) with an accuracy equal to the wavelength of sound in water, ±15 μm.

The mechanical properties of the samples were studied using a universal testing machine (Instron 5965), equipped with a load sensor of ±5000 N. The samples were tested in a three-point bending configuration at a constant loading rate of 1 mm/min, with a distance between supports of 10 mm and an initial pre-load of 1 N. Tests were conducted at a temperature of 23±2°C and relative humidity of 50±5%. Tensile strength $\sigma$ and corresponding fracture strain $\varepsilon$ were determined from the stress-strain curve, and modulus of elasticity E was calculated from the maximum slope of that curve.

Surface morphology studies, elemental composition and biocompatibility study of titanium plates were conducted at the North Caucasus Federal University (Stavropol, Russia).

Biocompatibility studies were conducted on mesenchymal stem cells (MSCs) derived from rat adipose tissue. The cells were cultured in a nutrient medium (Dulbecco's modified Eagle's medium, DMEM) with the addition of 10% fetal bovine serum (Thermo Fisher Scientific, USA) in 25-$cm^2$ culture vials under conditions of 37°C and 5% $CO_2$. The environment was changed every 3—4 days. Once the cells reached 80—90% confluency, they were harvested using 0.25% trypsin (Biolot) solution and counted using an automated cell counter Luna-FL (Logos Biosystems, Japan). The experiments used cells from the third passage.

The test samples were placed in a 24-well plate. A suspension of MSCs with a volume of 0.8 ml and a cell count of $2\times10^4$ were introduced into each well of a Corning tablet containing the samples. Cells from a well containing a milling smooth sample of Ti6Al4V served as negative control cells. The plate was incubated at 37°C and 5% $CO_2$ for 7 days.

The metabolic activity of the cells, which correlated with the number of viable cells, was evaluated in the experiment. After incubation, a sample of each type of cell was transferred to a 24-well plate containing 0.8 mL of fresh DMEM media. Then, 80 $\mu$L of activated EZ4U solution was added to each well, and the plate was incubated at 37°C and 5% $CO_2$ for 3.5 hours. After this, the samples were removed and the optical absorbance of the remaining solution was measured using a multifunctional imaging plate reader (Cytation 1, BioTek) at wavelengths of 450 and 620 nm. The absorbance is measured by a microplate-reader, set at 450 nm or 492 nm with 620 nm as a reference. Four wells were used per sample.

In vivo, an experimental study of osseointegration was carried out on titanium plates in the vivarium at the experimental station of the All-Russian Research Institute of Sheep and Goat Breeding (Stavropol, Russia). Eight mature North Caucasus sheep, aged 1.5—2 years and weighing 35—40 kg with fully formed skeletons were used in the study. The sheep were housed in individual cages and fed a standard diet.

All manipulations were performed under general anesthesia, and the animals were not fed the day before the surgery, but had free access to water. Tramadol 5% solution was administered at a dose of 2 mg/kg 15 min prior to the procedure. Then, premedication was administered intravenously: atropine sulfate 0.1% solution, 0.05 mg/kg; metamizole 50% solution, 0.5 ml/10 kg; diphenhydramine 1% solution — 0.5 ml/kg; ceftriaxone 500 mg/10 kg. General anesthesia was induced using intramuscular Telazole at 0.3 mg/kg. The effects of Telazol took effect within 5—7 min and lasted approximately 30 min, providing adequate anesthesia depth. The operation was performed using local anesthesia with sol. Ultracaine D-S 4% solution on the decorticated mandibular surface. Titanium microscrews ("KonMet", Russia), 1.2 mm in diameter and 5—8 mm long, were used to fix the plates on the bone surface (fig. 1).

The plate with a fragment of adjacent bone was removed after 6 months. The samples were sent for microtomographic examination. X-ray imaging of the processes of osseointegration of titanium plates and the study of the bone structure around the implants was carried out with the following scanning parameters: voltage on the X-ray tube of 90 kV; current in the tube of 270 μA; copper filter thickness of 0.1 mm; pixel size of 17.74 μm in the image; and tomographic mode with 360° rotation and a pitch of 0.2°, frame averaging of 4 frames. Layered images of the volumetric structures were reconstructed using the Bruker μCT NRecon software, with smoothing of 2 pixels, reduction of rings by 20%, beam hardening by 41%, and conversion of computer science (CS) to an image from −0.001 to +0.08. Post-processing, detailing, and analysis of these images were performed using the DataViewer program and the CT-Analyzer program (Bruker μCT).

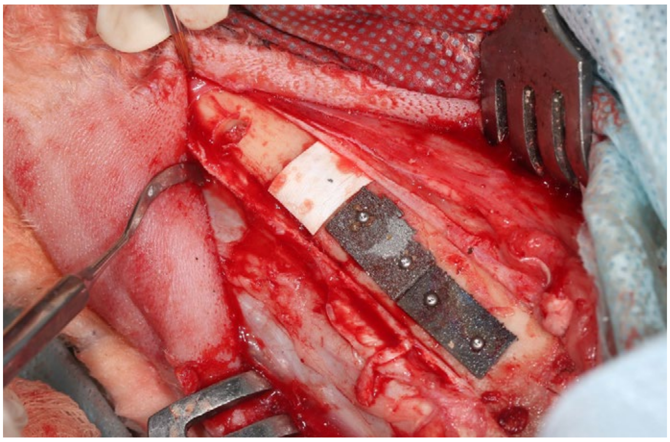

*Fig. 1. Titanium plates are attached with titanium micro-screws to the bone*

## EXPERIMENTAL RESULTS

The micrographs of the samples at different magnifications are shown in Figure 2. Analysis shows that the surfaces of the studied samples, regardless of DMLS technology are porous and consist mainly of spherical formations with smooth surfaces (fig. 2, A and C). These DMLS samples have spherical formations that vary in size from 10 to 150 μm, with cracks and small particles in the range of 0.1 to 1 μm observed on their surfaces. Samples produced using EBM have particles ranging in size between 30 and 120 μm without any cracks on their surface (fig. 2, B and D).

Figure 3 shows acoustic images of the structure of DMLS and EBM frame membrane samples. The surface images reveal differences in the porous structure. The DMLS structure of titanium coating is denser and more compact, which may be due to the smaller size of powder particles, as indicated by electron microscopy (fig. 2).

Acoustic images (fig. 3, A and B) reveal differences in porosity between the two materials. Samples produced with EBM exhibit a looser structure with smaller, but deeper pores compared to those produced by DMLS. To obtain quantitative porosity data, we analyzed ultrasound images

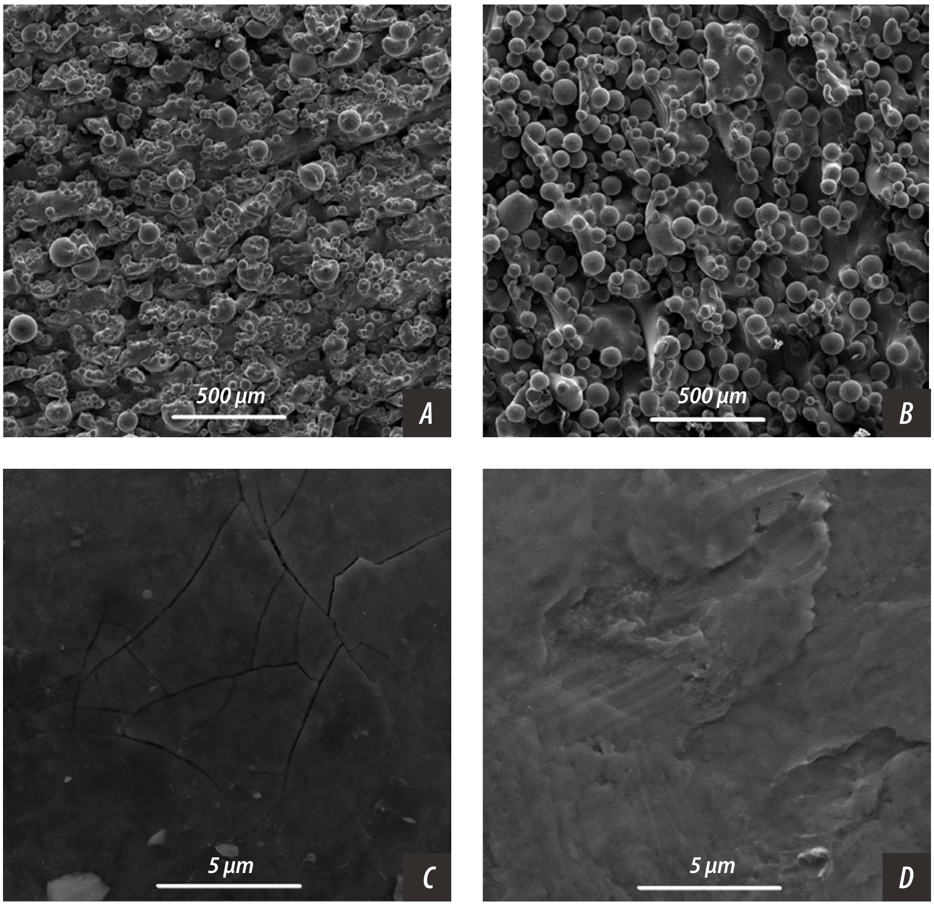


*Fig. 2. Electronic scanning microscopy of titanium alloy plates obtained using the technology: A and C — DMLS; B and D — EBM*

of 3×3 mm size at a depth of 50 µm using ImageJ software (fig. 3, C and D) [21]. For titanium produced by the DMLS process, the percentage of area covered by pores was 23.2±3%. For EBM samples, it was 11.7±2%.The analysis of the cross-sectional view of the samples allows us to determine the depth of the surface profile (fig. 3, E and F). We measured the average distance between the maximum and minimum points of relief from 10 different profiles of 8 mm diameter sample, and found that it was 100 µm and 150 µm for samples produced using DMLS and EBM technology, respectively.

The mechanical properties of titanium specimens were studied based on three-point bending tests. The ultimate strength, fracture strain, and elastic modulus were determined. The results of the measurements are shown in table 1. Linear sections of the loading curve were chosen for measurements,

**Table 1. Geometric and mechanical characteristics of the samples under the bending test. Average values using 5 samples**

| Moulding technology | DMLS | EBM |
|---|---|---|
| **Distance between supports, mm** | 10.00 | 10.00 |
| **Width, mm** | 8.20±0.18 | 8.40±0.10 |
| **Thickness, mm** | 1.15±0.04 | 1.05±0.03 |
| **Strength, MPa** | 2,180±20.7 | 1,500±26.1 |
| **Fracture strain, %** | 10.6±0.1 | 10±0.2 |
| **Modulus of elasticity, MPa** | 53,449±200 | 25,633±125 |
| **Speed of sound, m/s** | 6,200±15 | 6,000±20 |

and the effect of sample slippage in the clamps of the testing machine was minimized as much as possible. As can be seen from the data obtained, there is a significant difference between the values of modulus of elasticity and ultimate strength for samples produced using different technologies. Titanium plates produced by the DMLS process have a significantly higher elastic modulus 53,449 MPa as well as a bending strength 2,180 MPa that is one and a half times higher than that of EBM samples.

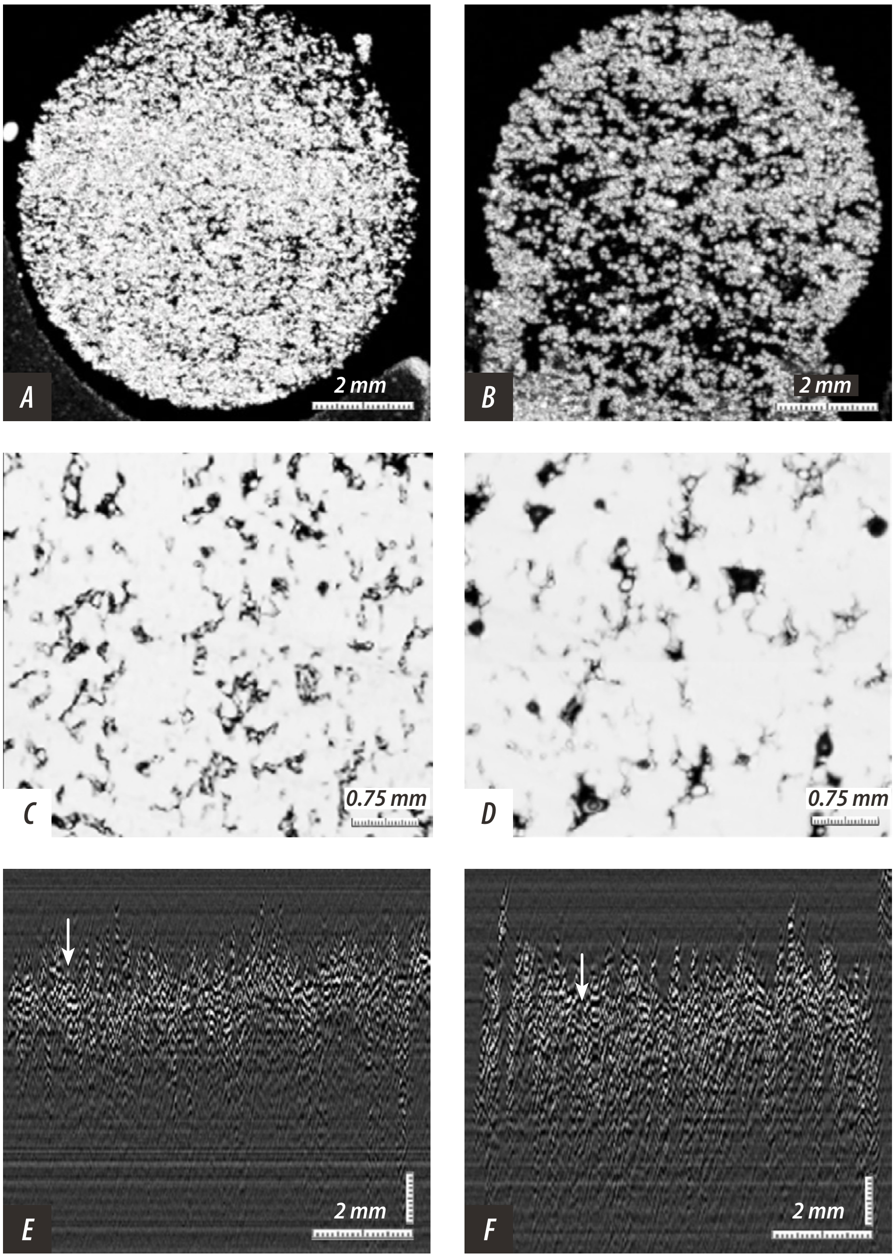


*Fig. 3. Acoustic images of the microstructure of Ti6Al4V titanium alloy samples obtained using DMLS (left) and EBM (right) technologies: A and B — C-scan; C and D — subsurface C-scan of 0.2 mm depth; E and F — profile B-scan, arrow is difference in profile 0.1 (E) and 0.15 (F) mm*

## RESULTS OF THE IN VITRO STUDY

A statistically significant increase in cell proliferative activity was observed in samples produced by the direct laser sintering process (DMLS). The value was 171.28±22.43% ($t$=2.792, $p$<0.05). In samples produced using the electron beam melting (EBM) method, the proliferation index was 94.26±19.89%. This value did not significantly differ from that of the control group, which had a proliferation index of 100.00±12.19%.

### Implantation of titanium plates in vivo

Figure 4 shows an image of the results from a study on osteointegrated titanium plates (Ti6Al4V) that were made using the EMB and DMLS techniques, on the surface of the bone of an animal, as obtained through computerized microtomography.

The study of samples obtained using both technologies (DMLS and EMB) showed differences in the level of bone tissue regeneration in the areas of defects, without signs of a pronounced inflammatory reaction. The analysis of the bone defect regeneration under the plates revealed no differences. The level of maturity of the bone tissue, as well as the area of its contact with the regenerate, did not show statistically significant differences in both study groups, which indicates a high level of osseointegration in both cases (table 2).

It is also worth noting the subperiostal bone formation on the surface of both types of plates and their "overgrowth" with bone. Initially, the plates were raised above the surface of the implantation site and in contact only with the periosteum.

## DISCUSSION

Individualized titanium frame membranes are successfully used to perform targeted bone regeneration in the alveolar area. Two main methods are used to create these personalized titanium alloy frames: machining titanium blanks with numerically controlled machines and layer-by-layer fusing of fine titanium powder, also known as additive manufacturing. The 3D printing technique offers numerous benefits for creating custom-made, perforated titanium frames in various shapes and size.

The rate of osseointegration for titanium dental implants depends on their chemical composition and surface roughness. Implants with a rough surface provide more reliable bone fixation and biomechanical stability. In the literature, one can find works [22] that explore different methods of post-processing titanium implantable products for the early stages of osseointegration. Since the effect of these methods on the morphology of the implant surface is still being studied, we used untreated surfaces in our work to evaluate the relief of implants formed naturally during the formation of titanium products using two different 3D printing technologies: direct laser sintering of metals (DMLS) and using an electron beam melting (EBM).

It was found that the size of the spherical formations on samples produced by DMLS varied within a slightly wider range (10—150 μm) compared to those produced by EMB (30—120 μm). Additionally, small cracks and particles measuring 0.1—1 μm were observed on the surfaces of the spheres produced by DMLS.

Acoustic microscopy has shown that the structure of the EMB plates is looser. The differences in the structure of DMLS and EMB samples can be explained by the use of powders with different particle sizes. It is likely that this looser structure causes the lower strength and modulus of elasticity of EMB compared to DMLS, as shown in table 1. In both cases the modulus values for titanium Ti6Al4V samples are significantly higher than those for human mandibular bone, which has a modulus of 1000—5000 MPa [23].

In this case, differences in cell proliferation on the implant surfaces, depending on the indicated sizes and number of pores, occur only at the initial stages of the cell activity, as confirmed by our in vitro tests. Despite some differences in the surface morphology of the two types of samples our in vivo experiments did not reveal significant differences in the results of the implantation of titanium plates produced by the two specified methods, DMLS and EMB. We found tight contact between the plates and bone tissue as well as subperiostal bone formation on the surfaces of both types of plate.

In vitro and in vivo studies show the following advantages of titanium plates made by direct laser metal alloy technology compared to plates made by electron-beam fusion: mechanical strength is 680 MPa higher (2180 MPa and 1500 MPa respectively); biocompatibility (percentage of living cells) is 77% higher (171.28 and 94.26% respectively); contact area with bone tissue is 1.23% greater (99.2 and 98.0%).

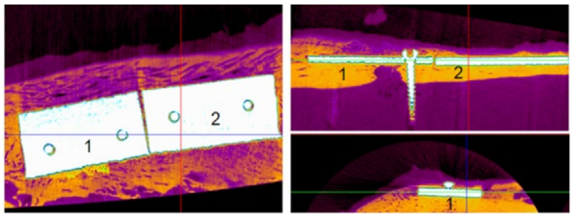


*Fig. 4. Multiplane reconstruction of tomographic image of titanium (Ti6Al4V) implants on the surface of sheep bone; samples are made using DMLS (1) and EBM (2) methods. The plates size of 30×10×1 mm*

**Table 2. Parameters of bone tissue in the area of titanium implant after 6 months**

| Moulding technology | DMLS | EBM |
|---|---|---|
| **The volume occupied by newly formed bone tissue surrounding the plate (≈ 1 mm from the boundary across the entire area of the plate), %** | 35.6 | 38.57 |
| **Bone-implant contact of titanium plate, %** | 40.23 | 40.0 |

## CONCLUSIONS

Studying titanium plates produced by two different 3D printing technologies — laser sintering and electron beam melting, was conducted in vitro and in vivo studies. Our experiments showed that DMLS based on Ti6Al4V medical titanium alloy proved to be more successful in terms of structure, strength, and biological properties. The products manufactured by DMLS were 23.7% stronger than those grown by EMB, according to the results of in vitro tests. A more active proliferation of MSCs was observed on DMLS samples, which was more than 70% higher compared to EMB and the control group. In vivo, bone tissue reacted well to both types of titanium samples, with high levels of osseointegration, due to their developed surface with a profile depth of 100 and 150 μm for DMLS and EMB technology, respectively. Data are base for choosing the optimal prototyping method for future clinical use.

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