Properties of biocomposites based on titanium scaffolds with a different porosity

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Abstract. Open-porous titanium scaffolds have been widely investigated for orthopaedic and dental applications because of their ability to form composites via bone ingrowth into pores and promote implant fixation with mother bone. In this work, porous titanium scaffolds coated with a diamond-like carbon were produced, and their ability to form biocomposites was evaluated through *in vivo* experiments. Three types of the open-porous scaffolds made of spongy titanium granules (porosity 0.3, 0.4 and 0.5, Young's modulus 4.4, 3.5 and 0.6 GPa) were implanted into a bone defect of sheep. Time dependences of the Young's modulus of titanium scaffold–bone tissue biocomposites were determined through the measurement of Young's modulus of the extracted scaffolds after 4, 8, 24 and 52 weeks of surgery. The Young's modulus of biocomposite is dependent not only on the time of composite formation but also on the porosity of scaffold.

Keywords. Porous titanium; osseointegration; biocomposite; mechanical properties.

1. Introduction

Pure titanium and its alloys belong to the biologically inert materials. They have a high strength-to-weight ratio and small elasticity coefficients as compared with other metals [1]. However, orthopaedic implants made of solid alloys have several disadvantages that may lead to their rejection. Due to the biomechanical mismatch between the metallic implant and the surrounding bone at the bone–implant interface, ‘stressor remodelling’ of the adjacent bone occurs. During this remodelling, new bone is destroyed more rapidly than it is formed because of the permanent contact between the surface and the solid metal implant. For the formation of a good connection between the bone tissue and an implant, the outer metallic layer should have quite definite geometry. To eliminate these problems, researchers have searched for a method to manufacture implants with elasticity similar to that of bone and with a structure similar to the structure of the trabecular bone [2]. Such materials are called open-cell porous metal, foamed metal or cellular metal and scaffold [3]. For osseointegration to occur, the porous materials should meet the following requirements. First, the pores should be open, be sufficiently large and form access channels that are connected to each other to allow for the following: the penetration of osteogenic progenitor cells into the pores, vascularization and the diffusion of nutrients [4,5].

During the long-term contact between porous materials and bone, a biocomposite is formed [6]. The overall mechanical properties of the biocomposite depend on the properties of its components—the porous scaffold and bone tissue [7,8]. The

mechanical characteristics of biometallic composites are not widely presented in the literature since experimental investigations in this field require a complicated, expensive and long-term process. This paper focuses on the experimental study of the biocomposites elasticity based on titanium scaffolds having a porosity of 0.3, 0.4 and 0.5. Sheep were used as model animals for biocomposites formation. Time of biocomposites formation was varied from 4 to 52 weeks.

2. Materials and methods

2.1 Scaffolds manufacturing

Detailed manufacturing techniques of porous titanium can be found in ref. [9]. Porous titanium with a different porosity (0.3–0.5) was made from granules (2–3 mm in size) of the titanium sponge (VSMPO AVISMA, Russia) by compaction in special moulds. Before compacting, the granules were cleaned by washing with distilled water in an ultrasonic bath with a subsequent triple drying in air, and then annealing in vacuum. The compact was finally annealed in vacuum ($P = 10^{-3}$ Pa) at 1100°C. The mechanical characteristics of porous titanium were measured on rectangular samples $1.0 \times 1.0 \times 1.5$ cm³ in size. Compression tests were conducted in a universal machine INSTRON at a strain rate of 0.3 mm min⁻¹.

For *in vivo* experiments, wedge-shaped scaffolds were designed and fabricated. Four planes of the scaffolds were shaped as an isosceles trapezoid, and two planes had the shape

of rectangles with the sizes 15×10 and 14×9 mm². To increase the similarity between the scaffolds and biological materials [10,11] the outer surface of scaffolds was covered with a diamond-like carbon (DLC) coating. The DLC coating (20–50 nm thick) was deposited using the method of pulsed arc sputtering of graphite. To deposit the coating onto the scaffolds, a rotary-type holder was used, providing the rotation not only of the holder, but also of scaffolds around its own axis. Preliminary ion etching (Ar⁺, $E = 4$ keV, $P = 6 \times 10^{-2}$ Pa, 30 min) of scaffold surface was carried out. Our earlier studies have shown that the DLC coating reduces the risk of dystrophy of the mother bone contacting with scaffold and accelerates the formation of a mechanical interlocking in the bone–implant interface [12].

Before introduction into the bone tissue, the scaffolds were sterilized through autoclaving and saturated with an adhering fraction of autologous bone marrow cells via incubation of scaffolds in complete culture medium under standard conditions for 14 days [12].

2.2 Experiment in vivo

For the *in vivo* experiments, 6- to 9-month-old Romanov adult sheep weighing 20–35 kg were used. The animals were kept in standard conditions stipulated by the ‘Rules of the work using experimental animals’. These animals were chosen because sheep are the standard model for mechanical studies in the field of bone tissue regeneration. The mechanical load on the tibia of a sheep is approximately half the corresponding load in humans [13], and the shape and size of the metaphysis of tibias and femurs are sufficient for the formation of a rectangular defect that has the necessary size. Incomplete defects are preferred because additional fixation of the injured segment is not required. In addition, after the surgery, the animals quickly return to their usual functional activity.

To introduce a scaffold, a bone defect with a size of 14.5×9.5 mm² was formed, making it possible to use a scaffold with a smaller butt end for better contact with the bone walls

without injury to the bone tissue. In all, 48 scaffolds were introduced into the condyles of tibias and femurs of 12 sheep.

2.3 Mechanical test

After 4, 8, 24 and 52 weeks of the surgery, three sheep were euthanized. The porous titanium scaffolds filled into new bone tissue (biocomposites) were extracted from the bone and subjected to a mechanical treatment that smoothed the edges. A compression test was performed using the INSTRON 2580 universal test machine with a loading rate of 0.3 mm min^{-1} . The software in the test machine calculated the Young’s modulus for each sample using the measured stress–strain curve.

2.4 Study of new bone tissue

For the study of new bone tissue, extracted biocomposite was cut into two parts. Then each part was cut into half. Thus, using the obtained cross-section of the biocomposite, we were able to analyse the new bone formed in the peripheral and the internal pores of the scaffold.

Before analysing the surface of the cross-section, it was polished using diamond paste, cleaned in an ultrasonic bath (distilled water) and dried at room temperature. During polishing, titanium layer inevitably adheres to certain areas with pores. As a result, only part of the pores with ingrown bone tissue remained available for analysis. The composition of the new bone formed in the pores was examined using a QUANTA 200 scanning electron microscope equipped with an EDAX analyser. To characterize the new bone, the Ca/P ratio was used. The Ca/P ratio was determined on the surface of the scaffolds in the areas with enhanced calcium content (figure 1).

3. Results and discussion

In the present work, we performed *in vivo* experiments. The main goal of *in vivo* experiments is to obtain biocomposites,

Figure 1. Scanning electron microscopy images of the biocomposites surface after cutting and polishing.

Table 1. Average values of the Young's modulus of scaffolds and biocomposites.

	Scaffold	Biocomposite formation time			
		4 weeks	8 weeks	24 weeks	52 weeks
$E^{0.3}_{BC}$	4.7 ± 0.3	4.7 ± 0.3	6.0 ± 1.0	8.2 ± 1.7	9.6 ± 2.00
$E^{0.4}_{BC}$	3.5 ± 0.1	3.5 ± 0.2	3.9 ± 0.5	4.4 ± 0.7	6.9 ± 1.3
$E^{0.5}_{BC}$	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.9 ± 0.2	1.5 ± 0.3

Figure 2. Young's modulus of the titanium scaffold–bone tissue biocomposite vs. biocomposite formation time. Squares, circles and triangles—biocomposites based on scaffold having a porosity of 0.3, 0.4 and 0.5, respectively.

for which the Young's modulus can be measured. Sheep are used for this purpose, and scaffolds of sufficient size are introduced to the sheep. After 24 weeks of composite formation, the scaffolds are extracted only by cutting a part of the bone. The Young's modulus is measured after the edges of the composites are mechanically smoothed. It is worth noting that a solid bone layer remains on the surface of some scaffolds after 24 and 52 weeks even after mechanical polishing.

The average values of the Young's modulus of scaffolds and biocomposite are presented in table 1. Figure 2 shows the time dependences $E_{BC}^{0.3}(t)$, $E_{BC}^{0.4}(t)$, $E_{BC}^{0.5}(t)$, where E_{BC} is Young's modulus of the biocomposite, and the superscript indicates porosity of the scaffold. The mechanical characteristics of scaffolds are determined by sticking strength of granules at the contact areas. The number and length of the contact areas of granules decrease with increasing porosity, which reduces the Young's modulus. At $\theta = 0.5$ a sharper reduction in the Young's modulus is observed. At this porosity a system of interconnected macrochannels is created, which is a result of using 2–3 mm granules as the material for scaffold manufacture. This can be accompanied by discontinuity

Figure 3. (a) Relative change in the Young's modulus of the biocomposite vs. the time of biocomposite formation. Squares, circles and triangles—biocomposites based on scaffold having a porosity of 0.3, 0.4 and 0.5, respectively. (b) The rate of relative change of Young's modulus vs. porosity in the range 24–52 weeks of biocomposite formation.

of the solid phase and, consequently, by reduction of elastic properties.

After 4 weeks of composite formation and given the margin of the measurement error, the average values of the Young's modulus of the biocomposites do not differ from the initial values of Young's modulus of the scaffold. After 24 weeks, a composite is formed with elastic properties that differ from the properties of scaffold.

For a comparative analysis of biocomposites based on titanium scaffold with different porosity, the relative changes in the Young's modulus of biocomposite were calculated as $\Delta E_{BC} = E_{BC} - E_{SC}/E_{SC}$, where E_{BC} and E_{SC} are Young's modulus of the biocomposite and scaffold, respectively. The ΔE_{BC} is calculated after 4, 8, 24 and 52 weeks of biocomposite formation (figure 3a).

The bone formed in the pores has little effect on elastic properties of biocomposites based on scaffolds having a porosity of 0.4 and 0.5 up to 8 weeks of composite formation. At 24 weeks, the relative change of Young's modulus of

Table 2. Ca/P ratio of mother bone and bone tissue formed in the pore of scaffold having different porosity.

Mother bone			
Cortical bone		Cancellous bone	
Ca/P (wt%)			
2.2–2.4		1.7–1.9	
Ca/P (wt%)			
Porosity of scaffold	Peripheral pores	Pores located between the peripheral and the central pores (~1/4 of scaffold length)	Central pores
<i>New bone tissue after 24 weeks of surgery</i>			
0.3	2.20 ± 0.13	1.96 ± 0.20	1.76 ± 0.10
0.4	2.17 ± 0.12	1.54 ± 0.57	1.18 ± 0.11
0.5	2.12 ± 0.10	1.65 ± 0.30	1.32 ± 0.28
<i>New bone tissue after 52 weeks of surgery</i>			
0.3	2.00 ± 0.18	2.04 ± 0.10	1.74 ± 0.16
0.4	2.04 ± 0.16	1.89 ± 0.14	1.79 ± 0.11
0.5	2.04 ± 0.11	1.90 ± 0.12	1.83 ± 0.13

biocomposite based on scaffolds having a porosity of 0.3, $\Delta E_{BC}^{0.3}$, is higher than $\Delta E_{BC}^{0.4}$ and $\Delta E_{BC}^{0.5}$: $\Delta E_{BC}^{0.3} > \Delta E_{BC}^{0.5} > \Delta E_{BC}^{0.4}$.

The osseointegration of scaffolds having a porosity of 0.3 and 0.4 during 52 weeks results in the formation of biocomposite having the Young's modulus two times higher as compared with Young's modulus of the scaffold. At the same time of the osseointegration, the Young's modulus $E_{BC}^{0.5}$ increases by more than 2.5 times.

After 24 weeks, only a slight increase of $\Delta E_{BC}^{0.3}$ is observed. When osseointegration scaffold has a porosity of 0.4 and 0.5, there is a significant increase in the $\Delta E_{BC}^{0.4}$ and $\Delta E_{BC}^{0.5}$.

The rate of relative change of Young's modulus, calculated as $\partial \Delta E / \partial t$, in the range of 24–52 weeks depends on porosity (figure 3b). The zero value of $\partial \Delta E / \partial t$ corresponds to a porosity of 0.15. The scaffold having porosity less than 0.16 does not form a biocomposite because the pores do not form a continuous cluster according to the percolation theory [14].

The scaffold contacts the bone tissue at the biocomposite, which forms a heterogeneous structure whose mechanical properties are dependent not only on the time of composite formation but also on porosity of the scaffold. The metal matrix is a basic component of the composite, and its properties do not depend on the duration of the biocomposite formation. The biological component changes in the process of filling the pores.

Obtained results are in good agreement with the measurements of the mineral component composition of new bone tissue (Ca/P ratio) in peripheral pores and in the inner volume of scaffolds inserted into the bone of sheep. After 8 weeks, bone tissue with various degrees of maturity is formed in the pores of the scaffold. This bone tissue fills the volume of the pores. In the peripheral pores, the composition of the

bone tissue is close to the composition of a cancellous bone, whereas in the bulk, we mostly observe immature bone tissue with a low Ca/P ratio (about 1.3). At approximately 24 weeks after surgery, the bone tissue grows through the whole scaffold depth, and the bone composition in the peripheral pores is close to the composition of the solid (compact) bone. We also observe that the Ca/P ratio decreases along the direction from the scaffold edge to its centre in all types of scaffolds (table 2). However, it should be noted that the compact and cancellous bone fills the peripheral and inner pores in case of the scaffold having a porosity of 0.3 only.

After 52 weeks, the composition of the bone mineral component in peripheral and inner pores is closer to that of the compact and cancellous bone, but the pore composition does not become equivalent to the composition of the intact compact bone until the end of the observation period. At this time, the compact and cancellous bone fills the peripheral and inner pores of scaffolds having a porosity of 0.3, 0.4 and 0.5.

A change in the biocomposite structure fraction of titanium from 0.7 to 0.5 that has a higher rigidity as compared with the bone tissue results in a change in the conditions of bone formation. According to the obtained experimental data, the scaffold having a porosity of 0.3 (Ti fraction 0.7) creates the most favourable conditions for the formation of mature bone up to 24 weeks after surgery (table 2).

The scaffold structure is not perfect. When the structure is pressed, an additional system of pores is formed between the 2 and 3 mm spongy titanium granules. These additional pores are interconnected with the pores inside the granules. The feature of such porous system inevitably influences the effective mechanical properties of both the scaffold and the biocomposite, on which it is based. When the porosity is more than 0.5, a system that is largely interconnected is created.

This is accompanied by reduction of Young's modulus of the scaffold ($E_{SC}^{0.5} = 0.55$ GPa, table 1). On the other hand, the Young's modulus of scaffold having a porosity of 0.5 is close to that of cancellous bone [15]. Perhaps, in conditions where the elasticity of scaffold is in close agreement with the elasticity of new bone that contacts the titanium walls, a large cluster of mature bone fills the interconnected macro-channels. This can lead to a more rapid change in Young's modulus $\Delta E_{BC}^{0.5}$ after 24 weeks.

4. Conclusion

During long-term contact between porous titanium scaffold and bone tissue, new bone fills the pores, and the porous scaffold–new bone tissue biocomposite is formed. The scaffold contacts the bone tissue at the biocomposite, which forms a heterogeneous structure whose mechanical properties are dependent on the time of composite formation and the porosity of scaffold. After 4 weeks of biocomposite formation, the average value of the Young's modulus of the biocomposite does not differ from the initial value of Young's modulus of the scaffold. After 24 weeks, a composite is formed with elastic properties that differ from the properties of scaffold irrespective of its porosity. At 24 weeks after surgery, the bone tissue grows through the whole scaffold depth, and the bone composition (Ca/P ratio) in the peripheral pores is close to the composition of the compact bone. The Ca/P ratio decreases along the direction from the scaffold edge to its centre in all types of scaffolds. At this time of the biocomposite formation, the compact and cancellous bone fills the peripheral and inner pores. Elastic properties of the scaffold affect bone formation in the pores. The use of scaffold having an effective Young's modulus close to the Young's modulus of compact bone and sizes through channels of about 100 μm creates favourable conditions for the formation of the mature bone in the inner volume of scaffold in the period up to 24 weeks of osseointegration (in our case the scaffold having a porosity of 0.3). In the range of (24–52) weeks of composite formation, the relative change of Young's modulus depends

on porosity: $\Delta E_{BC}^{0.5} > \Delta E_{BC}^{0.4} > \Delta E_{BC}^{0.3}$. The surgeons should take into consideration the obtained results before using the porous scaffold for repair of bone defect.

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