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Biomechanical and Microtomographic Assessment of the Effectiveness of Osseointegration of Threaded Titanium Implants with Single- and Bilayer Bioceramic Coatings

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Threaded implants with bioactive coatings were studied. The implants under consideration were standard titanium screws modified with single- and bilayer coatings based on calcium phosphates and anatase. The effectiveness of their osseointegration was assessed in experiments on model animals using a biomechanical testing method and by computerized microtomography (micro-CT). The studies showed that the coatings not only stimulated the growth of bone tissue, but also increased the critical torque (to ~3.8-6.3 N·cm), at which point implant failure occurred.

Introduction

The mechanism of osseointegration of metal implants remains incompletely understood [1, 2]. Two main groups of relevant factors can be identified. The first is linked with biological aspects. The second involves the influences of various engineering factors in implant manufacture [3]. Among the possible methods for increasing osseointegration are the forced creation of surface roughness, formation of porous relief, etc. [3, 4]. In our view, the method based on application of special bioactive ceramic coatings to the surfaces of titanium implants holds more promise [5-7]. According to [8], the dominant method for assessing osseointegration is histomorphological analysis [9, 10]. In addition, assessment of the volume density of bone tissue by micro-CT is also used [10]. The first and second methods are qualitative. Analysis of the use of biocompatible coatings requires these methods to be supplemented with quantitative data, for example based on biomechanical testing [11]. One version of this approach consists of the torsion method [12, 13]. However, the literature contains only limited data on the biomechanical evaluation of the effectiveness of different coatings and these are primarily results of testing devices without coatings.

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Materials and Methods

Implants under study were standard surgical screws made of Grade 4 titanium, 11 mm long, with Sp1.2 × 0.7 threads of length 7 mm (OOO Konmet, Russia). The surfaces of these screws were modified by two methods. Single-layer calcium-phosphate coatings were applied by detonation spraying. Bilayer coatings were made as follows: microarc oxidation (MAO) was used to form a porous oxide coating [14]. This was then also sprayed with calcium phosphates by detonation [9, 15-17]. The starting material was commercial hydroxyapatite HAp (99.0%) with particle dispersion $d_p \leqslant 50$ μm (OOO Biteka, Russia). The surfaces of the starting screws were prepared by sandblasting.

Experimental studies were carried out using male Wistar rats (weight, 250-350 g; age, 2-4 months). Implantation was performed (see Fig. 1a) through a lateral incision in the knee (to model osseointegration into cancellous bone) and the mid-part of the femur (to model osseointegration into cortical bone). The contralateral limb was used for implantation of an uncoated control screw by the same procedure. Studies were run on a limited cohort of animals (eight individuals).

Mechanical testing of the osseointegration of implants integrated into bone tissue samples from experimental animals (see Fig. 1b) was carried out on an LFM-50 electromechanical universal test machine (Walter +

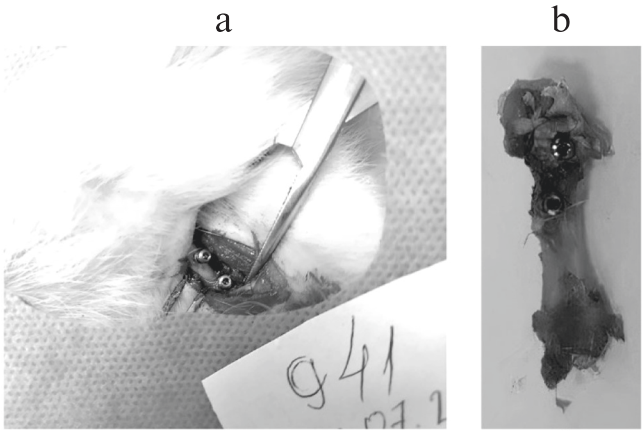

Fig. 1. Photographs of surgical incisions (a) in an animal's paw with implant, and autopsy specimen (b).

Bai AG, Switzerland). Before testing, a special rotating bit with a tetrahedral head was placed in the cylindrical screw head (see Fig. 2). Due to the rigid coupling of the screw slot 2 and bit 1, the bone tissue sample was suspended, and was rotated to come to rest with one of its ends against the angular displacement limiter. The sample was not rigidly fixed in space as this would require strict specification of the vertical movement of the screw head. The studies used a "floating sample" method. Angular displacement was 40-45° at a rotation rate of 5°/s. Load sensor 5 recorded the relationship between the torque developed and the angle.

Osseointegration dynamics were additionally assessed by micro-CT. These studies were carried out on a SkySkan 1178 microtomograph (Bruker BioSpin, Germany). Visualization and 3D reconstruction were performed using VSG Avizo software (Thermo Fisher Scientific, USA). The density of bone/cartilage regenerate at the bone/implant boundary was assessed using the Hounsfield scale on days 30, 60, and 90 after implantation (without consideration of the density of the metal implant). SkySkan CTan software (Bruker BioSpin, Germany) was used for this purpose.

Results and Discussion

As demonstrated in our previous studies [9, 14, 16], the methods used here to create bioactive coatings provide deposition of rather thick layers (Ca-P thickness of ~100 μm). The composition of the coatings is defined by the presence of only biocompatible phases (HAp, different tricalcium phosphate phases, and anatases). The crystallinity of the coating was ~23% with a crystallite size of ~22.2 nm. Histomorphological studies of these coatings provided evidence of the stimulation of chondrogenic osteogenesis at the contact zone between implant and bone [9]. It can be noted that other gas-thermal methods (particularly plasma spraying [18]), as compared with detonation, are characterized by large heat flows in coatings, which leads to the formation of unwanted compounds (CaO). As noted above, there are virtually no published data on biomechanical testing of samples with coatings.

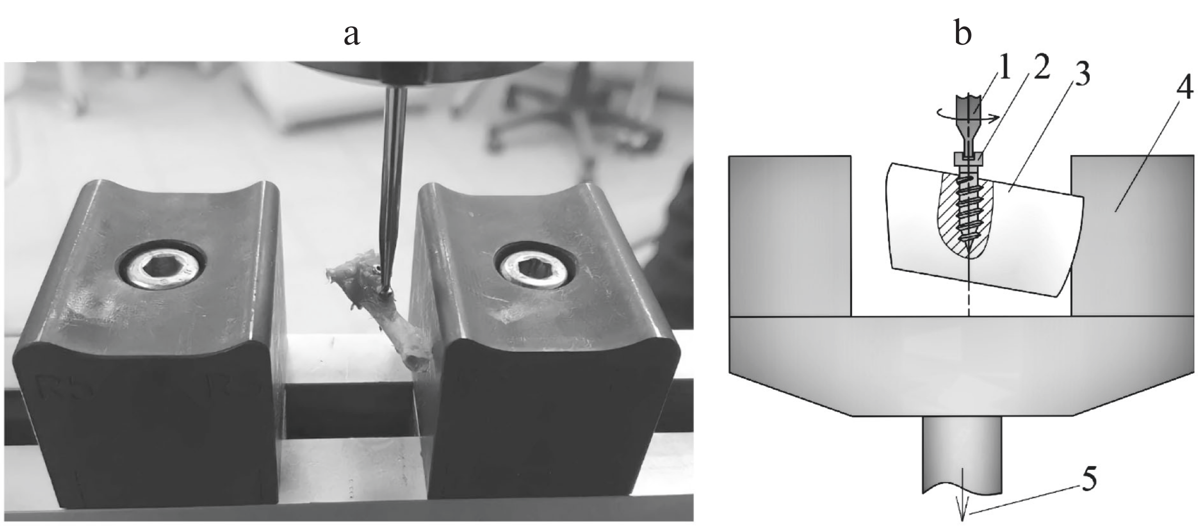

Fig. 2. Photographs of a specimen in the test machine cell (a) and diagram of the test (b): 1) bit; 2) implant; 3) bone tissue specimen; 4) angular rotation limiter; 5) to load sensor.

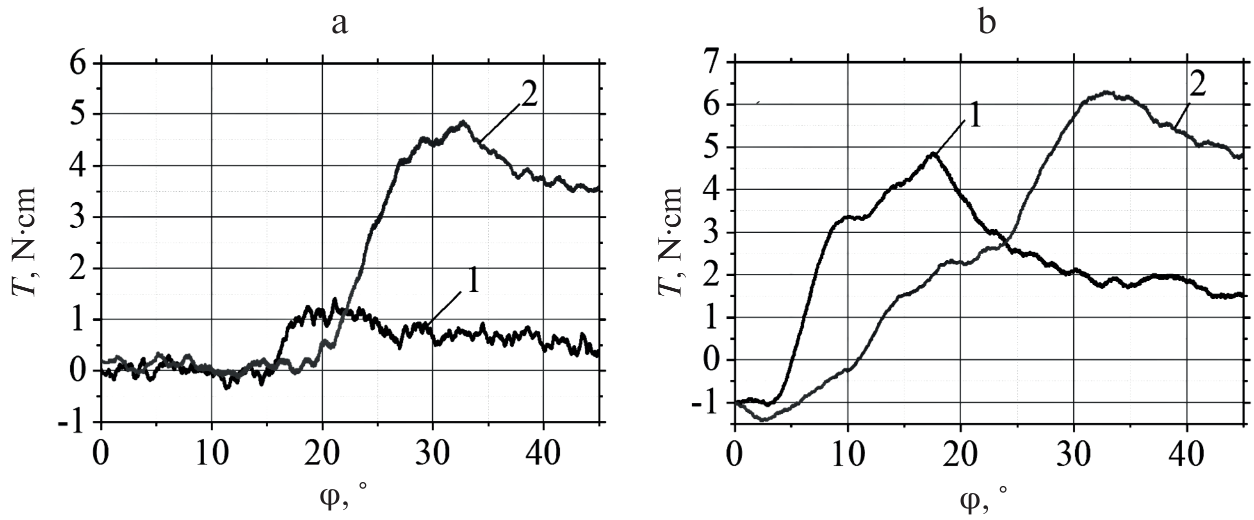

Fig. 3. Characteristic curves of implant loading on torsion of titanium implants with Ca-P coating (a) and with TiO_2/Ca-P coating (b): 1) control; 2) experiment.

It is therefore difficult to give comparisons with data from other authors at this time.

Figure 3 shows the relationship found between changes in the torque and the rotation angle for titanium implants with single-layer Ca-P and bilayer TiO_2/Ca-P coatings. In the case of cancellous bone, reproducibility of results was not achieved in the study cohort, so the data presented below apply to implants in cortical bone. At the first stage, corresponding to movement of the bone to the support, the signal was the static noise of the sensor. Then, after making contact with the clamp jaws, there was a quite sharp increase in the torque to critical value T_{max}. When coatings were applied, implant failure occurred at larger rotation angles ($\Delta\varphi$ = 15-25°) than in the case of screws without coatings, where $\Delta\varphi$ = 5-15°. Loss of contact at the bone/implant interface took place at this point, with failure of the device. The torque then decreased. The gradual nature of the reduction in torque, rather than a sharp drop to ~0°, was due to the presence of fine bony concretions, metal fragments, etc. in the screw thread. It is important to note that application of coatings led to increases (with T_{max} = 3.0 ± 1.8 N.cm) in the critical torque by factors of 1.5-4.5. Greater values (T_{max} = 6.3 ± 1.3 N·cm) were seen for bilayer coatings. For single-layer Ca-P coatings, torques were T_{max} = 4.0 ± 1.0 N·cm. Table 1 shows critical torques.

TABLE 1. Quantitative Characteristics of the Osseointegration Effectiveness of Titanium Implants with Different Coatings (60 Days Post-implantation)

	No coating	Single-layer Ca-P coating	Bilayer TiO_2/ Ca-P coating
T_{max}, N·cm	3.0 ± 1.8	4.0 ± 1.0	6.3 ± 1.3
X_b, %	45.3 ± 19.3	81.2 ± 4.3	56.1 ± 38.8
X_c, %	31.4 ± 7.2	7.6 ± 2.2	10.8 ± 6.5

Micro-CT studies showed that the main effects became apparent 60 days after implantation. Comparison of the characteristics of the bone/implant interface showed that tissue density was generally greater around samples with coatings. Table 1 shows data computed from these investigations: the percentage contents of bone X_b and cartilage X_c tissues by weight. The calcium-phosphate coatings studied here were found to stimulate implant osseointegration by increasing the content of bone tissue. Bilayer (TiO_2/Ca-P) coatings may have greater potential from this point of view, as they gave the greatest values for X_b (up to ~95%). The cartilage tissue content was also slightly greater in this case (up to ~10.8%). It should also be noted that these data provide evidence that osseointegration effectiveness is greater for coated samples than for implants with uncoated roughened surfaces (control screws).

The obtained data provide the basis for refining knowledge of the characteristics of osseointegration of metal screw implants with bioactive ceramic coatings. Quantitative data were obtained from in vivo tests.

Conclusions

Quantitative data were obtained on the effectiveness of the osseointegration of standard titanium screws modified with single- and bilayer coatings. Application of coatings was found to increase the critical torque at which failure of the bone/implant interface occurred by factors of 1.5-4.5. Micro-CT studies showed that bone tissue formation on application of coatings occurred for periods of 60 days. The data provide the basis for refining our picture of the processes of the osseointegration of this type of implant with bioactive coatings.

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