High fatigue strength and enhanced biocompatibility of UFG CP Ti for medical innovative applications

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Abstract. This study is focused on the fatigue properties of the UFG Ti Grade 4 and its biocompatibility. The UFG titanium was produced by ECAP-Conform in combination with subsequent drawing, which allows fabricating rods of up to 3 mm suitable for industrial applications. The endurance limit of smooth and notched samples of UFG Ti is considerably higher than in conventional Ti. The UFG Ti also demonstrates an increased capacity of human osteoblast-like U2OS cells to colonize and, therefore, better osseointegration. Torsional strength of standard Ti-6Al-7Nb products and UFG Ti Grade 4 products was evaluated. An advantage of the UFG titanium over the conventional coarse-grained material was shown.

1. Introduction
Titanium and its alloys are excellent biomedical materials and are widely used for the fabrication of implants [1]. As it is well known, reliability and durability of these products are ensured by a combination of high fatigue strength and long-term biocompatibility. Previously it was shown that the nanostructuring by severe plastic deformation (SPD) allows achieving in CP Ti record values of mechanical properties, and also increases resistance to high-cycle fatigue [2-4]. Moreover, this approach helps to improve biological properties associated with the formation of a specific surface relief [5-7]. These advantages of nanostructured titanium allowed developing a new concept of the dental implant with a reduced transverse section and improved survival in human tissues [7].

Our recent studies showed that modification of the equal-channel angular pressing (ECAP) into the ECAP-Conform (ECAP-C) technique in combination with subsequent drawing is a cost-effective way to produce long-length rods with an UFG structure [8-10]. Such rods are suitable for industrial applications, including as medical implants. The advanced technology of production of long-length titanium rods with an UFG structure also resulted in achieving high fatigue properties [11]. This paper is focused on the study of the innovation potential of UFG titanium produced by this technology for the use in dentistry and osteosynthesis. In particular, this includes fatigue strength on the notched samples, the influence of the UFG titanium surface relief on osteoblast cell growth, evaluation of fracture resistance when the article is screwed into the bone tissue.

Experimental material and procedures
The initial material was Grade 4 commercially pure (CP) Ti (manufactured by Dynamet Company, USA) in the form of hot-rolled 12 mm diameter rods with the chemical composition: Ti-base, 0.04%C, 0.14%Fe, 0.006%N, 0.36%O, 0.0015%H and with the average grain size of 25µm.

Processing of the UFG Ti Grade 4 rods included equal-channel angular pressing «Conform» (ECAP-C) and drawing. Ti billets of 15 mm in diameter were subjected to 8 ECAP-C passes at \(T_{def} = 450^\circ\text{C}\) in the die-set with a channel intersection angle \(\Phi\) of 120° along the route Bc. Then they were subjected to additional drawing with the accumulated strain of about 85%. By use of this treatment, it was possible to produce rods with a diameter of 6 mm and a length of more than 2 m. Subsequent reduction by drawing was performed at a temperature of \(T = 200^\circ\text{C}\) to form mostly equilibrium boundaries with low dislocation density inside the grains.
These microstructures enable a more homogeneous plastic flow and increase uniform elongation. The study of surface relief was conducted using atomic force microscopy (AFM). Microstructure analysis was performed using scanning (SEM) and transmission electron microscopy (TEM) to examine the transverse and longitudinal sections of deformation-processed rods. The surface of the samples for optical metallography was polished mechanically and etched in a solution of 4% hydrofluoric acid, 20% perchloric acid, and 76% distilled water. Blanks for foils were cut by electro sparking and mechanical thinned to 100µm. The resulting disks were subjected to electrolytic polishing using “Tenupol 5” machine. Polishing was performed at a temperature ranging from -20 to -35°C in a solution of 5% perchloric acid, 35% butanol, and 60% methyl alcohol. Microstructures within the polished foils were observed using a JEOL JEM 2100 microscope with an accelerating voltage of 200 kV.

Cylindrical (3 mm in diameter) samples for mechanical testing were prepared according to GOST 1497-84 (Russian standard). Room temperature tensile tests were performed using the “Instron” testing machine at a strain rate of $10^{-3}$ s$^{-1}$. For every condition, the tensile tests were repeated on a minimum of three samples. Rotating bend fatigue tests were performed up to $N = 10^7$ cycles using standard samples subject to constant stress amplitude and symmetrical loading (R = -1), at a frequency $f = 50$ Hz. To conduct the fatigue tests, the surface of gage section of the smooth cylindrical samples was ground and mechanically polished to a roughness $Ra$ of 0.63µm. Fig. 1 demonstrates the size and the shape of the channel of cylindrical notched samples. The notch dimensions were chosen to correspond with the standard sizes of the common screw threads. The theoretical elastic stress concentration factor ($k_t$) for notches with a radius of 0.2 was calculated as 3.9. The channel surface of the samples was polished to the roughness degree $Ra = 0.2µm$.

![Fig. 1. Notch shape and sizes of the tested samples](image)

To study the UFG Ti Grade 4 torsional strength, the test method for metallic medical bone screws was used according to ASTM F543: 2007. Tests were performed on the screws made of an UFG Ti Grade 4 rod, and also of a titanium Ti-6Al-7Nb alloy. Biocompatibility of the UFG CP Grade 4 Ti was evaluated using the example of proliferation of human osteoblast-like U2OS cells on the sample surfaces after different treatments. Cells growth was evaluated by XTT (2,3-bis [2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt) test in accordance with ASTM F543: 2007.

2. Results and discussion

The conditions used for processing of CG Ti Grade 4 by ECAP-C with subsequent drawing produced a three-meter rod with an UFG structure and an average grain size of 200nm. Fig. 2 shows typical microstructures of UFG titanium obtained by this method. Tensile strength of the samples cut from this rod reached a record value of 1400MPa. Elongation of the samples was no less than 10%.
Fatigue tests on smooth UFG Ti Grade 4 samples by the symmetric scheme of rotational bending show increase in the fatigue limit $\sigma_{-1}$ to 620MPa on the base of $10^7$ cycles (Fig. 3). This is a 50% addition to the same material in the coarse-grained structural condition ($\sigma_{-1}$ = 320 MPa).

Notching on the surface of the samples decreases their fatigue strength, which is due to the stress concentration during the tests. Fig. 3 indicates that endurance limit of notched samples ($k_t = 3.9$) is $\sigma_{-1}$ = 150MPa.

![Fig. 2](image1.png)

**Fig. 2.** TEM-images of UFG Ti Grade 4 structure obtained by ECAP-C with drawing in cross section: (a) bright field, (b) dark field

Similar results were obtained on UFG Ti Grade 4 samples that are described in the paper [12], in which the effect of the notch size and shape on the material endurance limit and sensitivity to stress concentration was investigated. We should note that these characteristics of resistance to fatigue fracture are as good as the ones for titanium Ti-6Al-4V and Ti-6Al-7Nb alloys, which are the most popular materials for the manufacture of screw articles in osteosynthesis [1, 12].
Fig. 4 presents standard medical bone screws made of the UFG Ti Grade 4 and Ti-6Al-7Nb alloy.

![Fig. 4. Machined screw type HA3.5](image)

Evaluation of torsional strength of the produced product samples demonstrated that the screws of UFG Ti Grade 4 have the best results compared to the standard products. They are presented in Table 1.

| Samples material       | Rupture torque, (N·m) | Rupture deformation Angle, (°) | Torque at 2°, (N·m) | Maximum Torque, (N·m) |
|------------------------|-----------------------|--------------------------------|---------------------|----------------------|
| CG Ti-6Al-7Nb          | 2.7                   | 120.2                          | 2.2                 | 2.8                  |
| UFG Ti Grade 4         | 3.8                   | 143.2                          | 2.7                 | 3.8                  |

Another important criterion of the implant material is its survival in human tissues, which depends not only on composition, but also on the state of material surface. In particular, the formation of an ultrafine-grained structure in titanium creates conditions for the fabrication of a specific relief. In this paper the tests on metabolic activity of human osteoblast-like U2OS cells on the CG and UFG Ti Grade 4 surface were conducted. The samples surface was mechanically polished (Fig. 5a, b), and then etched in an acid mixture (Fig. 5c, d).

![Fig. 5. The surface relief of the CG (a, c) and UFG (b, d) CP Ti Grade 4 after polishing (a, b) and after etching in a mixture of HF and HNO3 for 20 minutes (c, d)](image)
In Fig. 5 we can observe a more developed relief on the UFG titanium surface in comparison with the CG state after mechanical polishing and etching. The effect of the surface microroughness increase and formation of a specific relief in UFG titanium has been shown previously in the works of other researchers [5, 13, 14].

The results on kinetics of the cell metabolic activity are shown in Fig. 6. The figure displays that after 3 days of seeding of cells the greatest activity was observed on the surface after etching compared to the polished surface of UFG and CG samples. On the etched samples surface the difference in cell activity in UFG and CG titanium was small, as opposed to the polished surface, when the cell growth in UFG samples was significantly higher than in the CG condition. Upon 7 days since the seeding of cells their activity on the etched surface remained very high, while on the polished surface it reduced considerably in both states. We should also note a higher cell concentration on the UFG titanium surface compared with the CG one. The obtained regularities of cell growth confirm the increased capacity of the UFG titanium surface for proliferation of human osteoblast-like U2OS cells and, therefore, better osseointegration. These results are consistent with in-vitro test results obtained by different researchers using fibroblast and osteoblast cells [5, 7].

![Fig. 6. Metabolic activity of human osteoblast-like U2OS cells in a 3 and 7 days after seeding on the CG and UFG Ti Grade 4 samples with various surface treatments](image)

Thus, the results of this study once again demonstrated the benefits of nanostructured titanium for the manufacture of advanced implant for medical purposes with enhanced functional properties and biocompatibility. Development of a new, more efficient combined technology, including ECAP-C and drawing, creates conditions for the commercial application of nanostructured titanium rods. The range of their application may be expanded, in particular, in dentistry and osteosynthesis.

### 3. Conclusions

It was shown that the UFG titanium Grade 4 produced through a combination of ECAP-C and drawing demonstrates the record characteristics of strength (1400MPa), endurance limit (620MPa), improved fatigue strength of the notched samples, and a higher capacity for osseointegration in comparison with the conventional coarse-grained titanium.

The articles in the form of bone screws produced from UFG Ti demonstrated high torsional strength compared to the titanium Ti-6Al-7Nb alloy.
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