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In-vivo evaluation of new β(Zr-Ti)-alloys for dental implantation

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Summary.

Osteoinegration is a main factor of successful implant ingrowth. It is depends on quality of bone, lack of initial stability, excessive loading, loosening or fracture of screw and fracture of implants. Other factors that can affect osteoinegration are implant composition and futures of implant surface. There are a lot of research that show better results of implantation of combined alloy with Ti, Co, Ni and Zr. But it is still not clear how does alloy composition can influence on bone ingrowth around implant. Also a few research focused on Zr-based allow.

The **aim** of this research was the in-vivo evaluation of β(Zr-Ti)-alloy compare the traditional TiVT-6 dental implant.

In experiment we used 30 rabbits for implantation of β(Zr-Ti)-alloys (experimental) and TiVT6 dental implants (control) in distal epiphysis of the femur. In 1, 3 and 6 months after implantation we control bone/implant interface and of ion (Ca and P) distribution by Scanning Electron Microscope with X-Ray analysis REMMA-102 (Selmy, Ukraine).

After implantation of TiVT6-alloy we can see formation of connective tissue “cuff” that tightly connect with implant and does not turns to bone tissue. These processes lead to over mobility and over loud to implant and decrease mechanical forces to bone tissue. All above can lead implant failure and complications for surrounding bone tissue. At the same time β(Zr-Ti)-alloy with modified surface surrounds with bone tissue – woven in one month that turns into lamellar bone in three months after the implantation. To summarize our data we can suggest that β(Zr-Ti)-alloy is more suitable for implant material due to osteostimulatory potential

Key words. Dental implant, zirconia alloy, osteointegration, scanning electron microscopy.

Introduction.

There are a lot of metals and ceramics have used in dental surgery last 40 years. Dental implants have applied as a alternative treatment method for the prosthodontic restoration of partial or full edentulous patient [12]. But there are a lot of reports about implant failure that from 6% to 11% according different search [10, 19].We can suggest following factors that can lead implant failure such as poor quality of bone [10], lack of initial stability [2], excessive loading [18], loosening or fracture of screw [7] and fracture of implants. But the main reason of implant failure is disorders of osteointegration that depends on composition of implant material, surface structure and implant elasticity and strength [11].

Titanium is a good material for dental implants due it mechanical parameters, not-toxicity and bio-inert. But it has some disadvantages such as high Young Module, low elasticity and low bone integrity. To improve quality of dental implants we can modify titanium alloys by adding of other metal such as aluminum or zirconium [20]. The quality of the implant surface can be improved also by depositing bioactive materials onto the surface of the dental implant to induce osteoconductivity. Numerous studies have been shown the advantage of hydroxyapatite or calcium phosphate as a coating of dental implant [3, 5]. HA-coated implants have often been used in load bearing applications owing to their capability of bonding directly to the bone and improving new bone formation necessary for implant osseointegration [13].

The wide spread technic for better implant production is complex alloy with different metals as well as ceramics. Zirconia is a promising metal for this purpose. It is known that silicate group of biomaterials have the ability to release silicate ions at a definite concentration, which helps in the growth and differentiation of osteoblasts [25] thus lead into new bone formation. Milena R. Kaluđerović suggested that presence of zirconia on titanium surface has a higher beneficial effect on the osteoblast morphological changes and cell cluster formation [17]. Also some research show better osteointegration of submerged zirconia implant compare the titanium [21] as well as cell proliferation and mRNA expression [8]. One of the advantages of Zn-based materials is antibacterial properties. Al-Radha A. and other shown that zirconia material and titanium blasted with zirconia surface have superior effect to titanium material in reducing the adhesion of the bacteria [1]. Other research suggests decrease of bacteria proliferation (*S. aureus* and *A. actinomycetemcomitans)* on zirconia coating [9]. All above show significant advantages of zirconia for dental implant research and clinical application. But some research did not show privilege of pure zirconia compare the pure titanium. Martin Rosentritt did not find difference in failure resistance during fatigue testing between these materials [20]. And other research showed higher wear in Zirconia implant compare the titanium [22]. But addition of 1–3 vol% titanium leads to a significant decrease of zirconia destabilization and increase mechanical properties [4] as well as presence of zirconia on titanium surface has a higher beneficial effect on the osteoblast morphological changes and cell cluster formation [17]. Thus, as a result of previous studies, the combination of zirconia and titanium is a promising way for dental alloy production.

In our study we used β(Zr-Ti)-alloy which has low coefficient of elasticity and low magnetic susceptibility as well as high roentgen contrast range. And the **aim** of this research was the in-vivo evaluation of β(Zr-Ti)-alloy compare the traditional TiVT6 dental implant.

Materials and Methods.

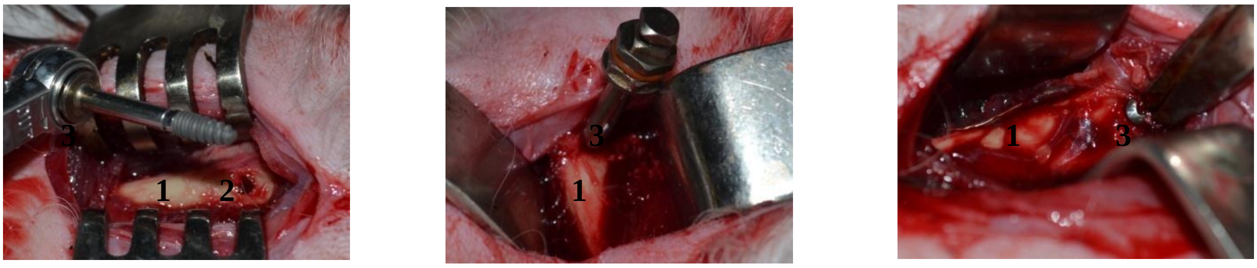
Materials for implantation.

β(Zr-Ti)-alloy was made by 10-fold electron-beam melting, followed by hot forging, cold rolling and strain. The surface of implants was modified by sand blasting and acid etching (SLA surface) that lead to formation of pores with size from 12 to 50 μm. TiVT6-alloy was a typical commercial dental implant (pic. 1).

Experimental protocol.

The experiment was conducted on the base of the "Center of Morphological Research", Sumy State University (Ukraine). In the experiment were used chinchilla rabbits aged 4-5 months, weighing 3 - 3.5 kg, obtained from the vivarium of the Medical Institute of Sumy State University. Animals and experiments were conducted under the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986) and approved by the Commission on Biomedical Ethics of Medical Institute (#7/12 on 11/06/2013).

Animals (30 rabbits) were randomized in two groups – experimental and control. Under general anesthesia (Ketamine 7 mg / kg and thiopental 10 mg / kg) was performed implantation of dental alloy in distal epiphysis of the femur with the load on the implant from 30 to 35 N (fig. 1). For the experimental group we used β(Zr-Ti)-alloys and for control - TiVT6 dental implants. After the operation the wound was sutured and as a prophylaxis of postoperative bacterial complications animals admitted antibiotics for 1 week.



A B C

Fig. 1.The procedure of the operation of implantation of β(Zr-Ti) and TiVT6 alloys to the femoral epiphysis. A – operative access and formation of bone defect; B – procedure of alloy implantation; C – general view after the implantation:

1 – femur; 2 – bone defect; 3 – dental implant.

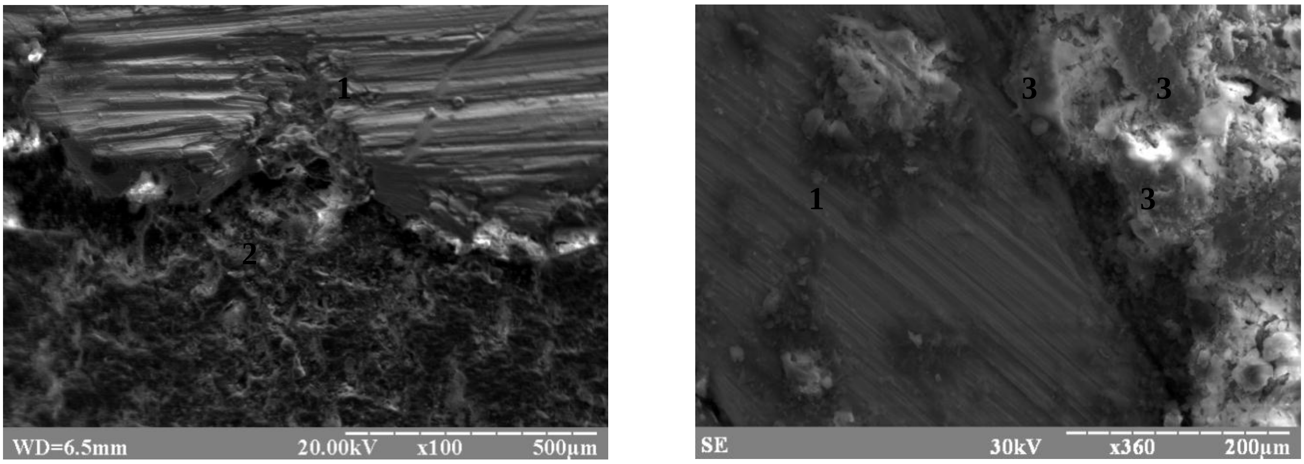
Data collection.

Rabbits were taken from the experiment after 1, 3 and 6 months after implantation. Femur with implant were isolated and processes of osteointegration was studied by the Scanning Electron Microscopy.

Bones were fixed in 2% glutaraldehyde solution for 24 hours and then placed to the 1% solution of osmium tetroxide for 12 hours, dehydrated in alcohols of increasing concentration (50 - 70 - 80 - 90 and 100%) and poured into a mixture of resins "Epon-Araldite". To improve the surface of visualization samples were sprayed by silver in standard vacuum system VUP-5. For the visualization of bone/implant interface and control of ion (Ca and P) distribution we used Scanning Electron Microscope with X-Ray analysis REMMA-102 (Selmy, Ukraine).

Results and discussion.

One month after the implantation of TiVT6 alloy we determined zone of electron-dense substance around implant. There are no osteon-like structures and trabeculae around the implant. Tissue made of random fibers similar to coarse-fibered tissue with cells of fusiform and spherical shape similar to fibroblast (fig. 2 A). This zone is free of calcium and phosphorus which indicates the formation of a connective tissue "cuff" surround the implant. This data suggest the low osteoblast stimulatory effect of TiVT6 alloy that was shown in other research and our previous data [14]. In same time inert material surround by connective tissue that separate it from bone tissue. Non-fusion of implant with bone tissue may lead over mobility and implant failure.



A **B**

Fig. 2 Bone-implant interface between A - TiVT6 alloy (zoom 100) and B - β(Zr-Ti)-alloy (zoom 360) in one month after the implantation:

1 – implant; 2 - fibrous-like tissue with random fibers; 3 – random trabeculae of woven bone tiisue.

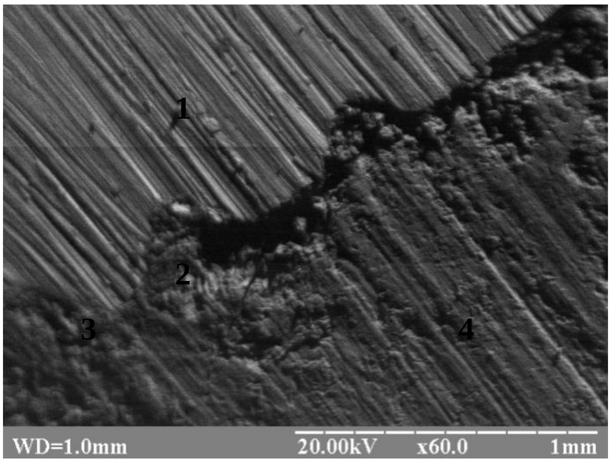
The next after the layer of connective "cuff" is defined bone formation, which is loosely fitted to the implant. It can be attributed as a woven bone due the random direction of bone trabeculae. The width of woven bone is 476±67 mcm and surrounds by the recipient's lamellar bone.

Implantation of β(Zr-Ti)-alloy leads bone formation around the implant that probably a result of osteoblast affinity to experimental alloy. Bone trabeculae joint with surface of implant and spread over the 319±52 mcm from alloy (fig. 2 B). Structure of this layer similar to woven bone due to random trabeculae orientation. There are some osteoblast cells on the trabeculae surfaces that suggest about bone formation and remodeling. X-ray analysis shown presence of calcium and phosphorus that is 37,2±2,1 wt% and 19,4±1,6 wt%. Just next to woven bone layer we can visualize lamellar of native bone. The key process in osteointegration is osteoblast attachment and some research show better cell attachment to zirconia compare to titanium. Also surface morphology can influence cell affinity and proliferation. Numerous research shown advantage of ruff surface with micro- and nanoscale roughness compare to smooth implants [23]. β(Zr-Ti) implant have modified surface that is possible mechanism of osteoblast attachment and further bone formation.

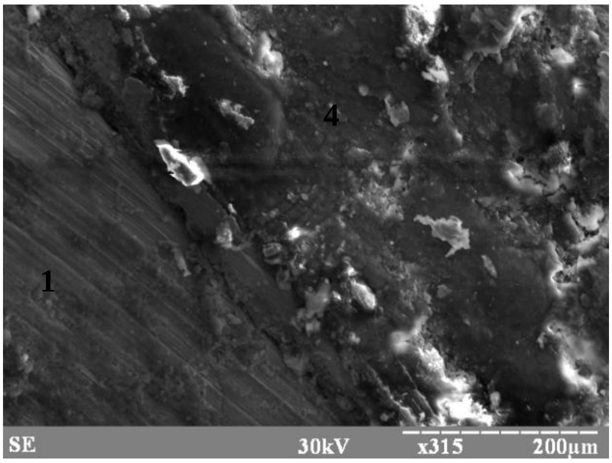
We did not see any morphological differences in connective "cuff" around the TiVT6 implant but identify its calcification by the X-ray analysis in three months after implantation. The level of calcium and phosphorus is 19,34±1,31 wt% and 6,43±0,25 wt% respectively. This data does not suggest about hydroxyapatite formation but more similar for amorphouscalcium phosphate [16]. Woven bone that was present in last time-point turned into the lamellar. In some cases new formed bone contact with implant but contact area is not large. There are no random fibers and new tissue made of lamellar bone trabeculae similar the native bone tissue. The difference between new and native is level of calcification. New formed bone has less level of calcium and phosphorus - 31,8±4,9 wt% and 16,3±1,4 wt%. Border between new and native bone is a cement line that have higher electron density compare the surrounding tissue.

Random bone trabeculae surround β(Zr-Ti)-alloy transformed to lamellar bone in three months after the implantation. We can see tight connection between new formed bone tissue and dental implant, that prove high osteointegration potential of β(Zr-Ti)-alloy. Level of calcium and phosphorus on the surface of new bone is 51,9±6,4wt% and 32,5±2,7wt% and does not differ from native bone.

The structure of surrounding tissue does not different in six month after the implantation compare to three months in control and experimental groups. After implantation of TiVT6-alloy we can see formation of connective tissue "cuff" that tightly connect with implant and does not turns to bone tissue. These processes lead to over mobility and over loud to implant and decrease mechanical forces to bone tissue [6]. All above can lead implant failure and complications for surrounding bone tissue [24]. At the same time β(Zr-Ti)-alloy with modified surface surrounds with bone tissue – woven in one month that turns into lamellar bone in three months after the implantation.



A



B

Fig. 3 Bone-implant interface between A - TiVT6 alloy (zoom 60) and B - β(Zr-Ti)-alloy (zoom 315) in three months after the implantation:

1 – implant; 2 –connective tissue "cuff"; 3 – bone attachment to implant; 4 - bone tissue.

There are some evidences about advantages for Zr-based alloys for osteoblast attachment and proliferation. Majumdar P. and other shown better protein absorption and cells adhesion on Ti-13Zr-13Nb-alloy compare the pure Ti material [15] as well as LefaixH. suggested about better sorption of fibronectin on surface of $Ti_{45}Zr_{38}Ni_{17}$ alloy [14].Experiment on fibroblast cells (L-929) and osteoblast-like cells (MG 63) show better sells proliferation on Zr–2.5Nb alloy compare to pure Ti and Ti–6Al–4V alloy [26]. Thus, zirconia alloy has significant advantages for cell adhesion and proliferation compare to pure titanium ones. In first stage after the implantation better cells adhesion, predominantly osteoblasts, leads formation of bone tissue surround the β(Zr-Ti)-alloy compare the TiVT6 ones that edged by "cuff" of connective tissue.

Conclusions.

1. The integration of dental implants finish in three months after the implantation and characterize by formation of connective tissue "cuff" around the TiVT6 alloy and bone tissue – round to β(Zr-Ti)-alloy.
2. β(Zr-Ti)-alloyis more suitable for implant material due to osteostimulatory potential.

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