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Araştırma Makalesi **Research Article**

A Comparative Study of Densitometric Parameters of the Bone Tissue Under Transosseous Osteosynthesis by Using Experimental Implants Coated with Superhard Compounds

Galimzyan KABIROV[1], Faina SHAKIROVA[1], Jean Claude MANIRAMBONA[1], Ildar AKHTIAMOV[2,3], Elmira GATINA[2], Rustem ZAKIROV[3], Nailya MUNIROVA[3]*

[1]Kazan State Academy of Veterinary Medicine, Kazan, Russia
[2]Kazan State Medical University, Kazan, Russia
[3]Republican Clinical Hospital, Kazan, Russia

***Sorumlu Yazar / Corresponding Author:**

Jean Claude MANIRAMBONA
e-mail: manjeckad@yahoo.fr

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Abstract

By X-ray computed tomography (CT), density characteristics of the tibiae bones in 25 experimental adult male rats were studied after their tibiae bones have experimentally fractured and then repaired by osteosynthesis using different implants. According to the data that was obtained by computed tomography, it has been defined that bone densities depend on the type of the implant and the chemical composition of the coating which is applied on the surface of the implant. Thus, average values of the density of the tibiae bones in the experimental rats with implants coated with a combination of titanium and hafnium nitrides were not different from the density values of the intact bones. At the same time, the densities of the tibiae in rats with implants made of copper and implants coated with a combination of titanium and zirconium nitrides were 1.5-2 times lower than those of the intact bones. This leads us to conclude that implants coated with titanium and hafnium nitrides are biologically and chemically inert because less pathologic changes in the bone tissue were observed, in contrast to the copper-based implants and those where the coating contains zirconium.

Özet

Transosseöz Osteosentez Altındaki Kemik Dokusunun Dansitometrik Parametrelerinin Aşırı Sert Bileşiklerle Kaplanmış Deneysel İmplantlar Kullanarak Karşılaştırmalı Çalışması

Erişkin, erkek 25 deney sıçanının tibia kemikleri deneysel olarak kırılıp, sonra çeşitli implantlar kullanılarak osteosentez yoluyla düzeltilmesini takiben X-ışın bilgisayarlı tomografi (CT) kullanılarak tibia kemiklerinin dansite özellikleri araştırıldı. Bilgisayarlı tomografi ile elde edilen veriler ışığında, kemik yoğunluklarının implant tipine ve implantın yüzeyine uygulanan kaplamanın kimyasal kompozisyonuna bağlı olduğu tanımlandı. Böylelikle, titanyum ve hafniyum nitrit kombinasyonu ile kaplı implantları bulunan sıçanlardaki tibia kemikleri yoğunluklarının ortalama değerleri dokunulmamış kemiklerin yoğunluk değerlerinden farklı değildi. Aynı zamanda, bakırdan yapılmış implantlı ile titanyum ve zirkonyum nitrit kombinasyonu ile kaplanmış implantlı sıçanlardaki tibia yoğunlukları dokunulmamış kemiklerdekilerden 1,5-2 kat daha düşüktü. Bu da bizi, bakır-bazlı implantlara ve kaplamanın zirkonyum içerdiği implantlara oranla kemik dokusunda daha az patolojik değişiklikler gözlemlendiğinden titanyum ve hafniyum nitrit ile kaplanmış implantların biyolojik ve kimyasal olarak inert oldukları sonucuna varıldı.

Introduction

The relevance of this topic is due to the need to establish long-term and safe implants which can help to avoid several complications, such as allergic reaction to the alloys used in the manufacture of submersible medical instruments (Abdullin et al., 2004; Summer et al., 2007)

The serious disadvantage of metallic implants is that: they are prone to corrosion, due to which their mechanical strength is reduced, and the body is subjected to negative effects from the metal ions which pass into a solution. Membrane-like-coatings with a minimum thickness and biological resistance to a fairly aggressive biological environment are capable to create a protective barrier to the metal ions to access to the body fluids (Horowitz and Purdon, 1995; Steinemann, 1996; Zagorodnii et al., 2013).

In this regard, an important aspect is the use of methods to properly and reliably estimate their interaction with the surrounding tissues and the body as a whole (Amstutz et al., 1992).

Nowadays, a large number of metallic biomaterials composed of nontoxic and allergy-free elements are being developped. For orthopaedic implants, metals are required to have excellent toughness, rigidity, strenght, resistance to fracture and corrosion (Hermawan and Mantovani, 2009).

Up to now, the three most used metals for implants are stainless steel, cobalt chromium (CoCr) alloys, titanium (Ti) alloys. The Nikel (Ni) (8-9.5%) containing in the medical stainless steel makes it stronger than steel and more resistant to corrosion. Further addition of molybdenum (Mo) has improved corrosion resistance (CoCrMo). Afterwards, the carbon (C) content has been reduced from 0.08 to 0.02% which improved its corrosion resistance (Brandes and Brook, 1992).

Promising are the coatings containing superhard metal nitrides, which are characterized by chemical and biological inertness. In previous publications a number of advantages of these coatings in assessing biomarkers reaction to them has been identified (Akhtyamov et al., 2012), but their effect on the organism of experimental animals in general has not been tracked (Akhtyamov et al., 2013).

The method of Computed tomography (CT) allows more objectively to interpret the parameters obtained by X-ray examination, to compare with each other and to form an idea of the spatial relationship of bones; especially this is significant during multislice computed tomography (MSCT) (Dyachkova et al., 2013). This type of survey has a high accuracy, reliability and informativeness in the two-dimensional and as in three-dimensional images (Dyachkova and Mitin, 2011).

The purpose of the present study was a comparative study of the effect of implants made of copper (Cu), medical steel 12X18H9T (alloy composition: C-0,2% ; Si-0,8% ; Mn-2% ; Ni-[8-9,5]% ; S-0,02% ; P-0,035% ; Cr-[17-19]% ; Cu-0,3% ; Fe-67%), steel 12X18H9T coated with titanium and hafnium nitrides (TiN + HfN), as well as, steel 12X18H9T coated with titanium and zirconium nitrides (TiN + ZrN) on density characteristics of the tibiae bone of experimental rats using the method of computed tomography.

Materials and Methods

The studies were conducted in accordance with ISO Standard 10993 (P) and were approved by the Local Ethics Committee of the Kazan State Medical University from the session protocol no 5 delivered on 25th June 2013. Experimental studies were carried out on male albino rats weighing 250-300 g. Implants are pins 8-10 mm long, 0.5 mm in diameter.

Characteristics of Implants and Coating Technology

Implants were manufactured at the “Scientific and manufacturing organization of Medical instruments "(Kazan, Russia) and consisted of wires 8-10 mm long, diameter Ø = 0.8 mm. The samples (steel 12X18H9T) were degreased with petrol B-70 and were dried in 96 ° ethylic alcohol. Afterwards, the samples were mounted inside the working chamber of an ion-plasma Sputter plant. The coating (titanium, hafnium, zirconium) was applied on the samples by ion bombardment under the following parameters: energy of metal ions: 0.8-1 keV, substrate temperature: 500 °C, Chamber pressure: 1.6 $x10^{-2}$ Pa, carousel rotational speed: 2 revolutions / min. Before applying the coating, the working chamber was supplied in gas (nitrogen) to form a nitride coating. The total coating thickness was 5µm.

Five experimental groups were set up, one of them contained intact rats (n = 5). Depending on the material from which the implants were made, the remaining animals were divided into four groups. Two comparison groups: no 1 – with implant made of steel 12X18H9T (n = 5) and no 2 - with implants made of copper Cu (n = 5), without coatings. Two experimental groups: no 3 - with implants made of steel coated with titanium and hafnium nitrides (TiN + HfN) (n = 5), no 4 - with implants made of steel coated with titanium and zirconium nitrides (TiN + ZrN) (n = 5).

Figure 1. Radiograph of the rat’s limb with an implant.

Şekil 1. İmplantlı bir sıçan uzvunun radyografisi.

Implantation was performed in aseptic and antiseptic conditions after dissection of soft tissues surrounding the tibia, under general potentiated anesthesia (Xylazine HCl: 10 мg/кg in IM, Tiletamine HCl + Zolazepam HCl 100: 10-15 мg/kg in IM). Implants were transosseously introduced into the middle third of the diaphysis of the tibia after preliminary drilling. The points of the implants were folded in the form of clips and immersed beneath the skin. The wound (muscles and skin) was sutured using uninterrupted spiral suture with non-absorbable polypropylene-based material (ETHICON PROLENE^{TM}).

To assess the impact of implants on the rats, densitometry of the rats' tibiae in the implantation zone on the 90^{th} days after implantation was performed. Computed tomographic scan was performed on rats by using a CT Scanner Brilliance 64 (Philips, Netherlands) in the transverse scanning mode (Edelson, 1984). Scan protocol: a native study was carried out with the following parameters: 120 kV, 139-158 mA, the helical pitch: 1 mm, slice thickness: 1 mm, the filter: Bone. Processing and image visualization were carried out on an additional workstation using a software package DICOM in MPR (MultiPlanar Reconstruction) mode in the axial, coronal and sagittal planes. Prior to the scan, implants were removed from rats.

The resulting digital data was processed statistically using the software packages SPSS v.13.0. Student's t-test with a Bonferroni correction was used for all statistical analyses. Differences were considered significant at a significance level of P<0.05.

Results

The results showed that the density of intact tibia (non-operated rats) on the diaphysis level equalled 1181.4 ± 67.6 HU (Hounsfield Units) (Figure 2).

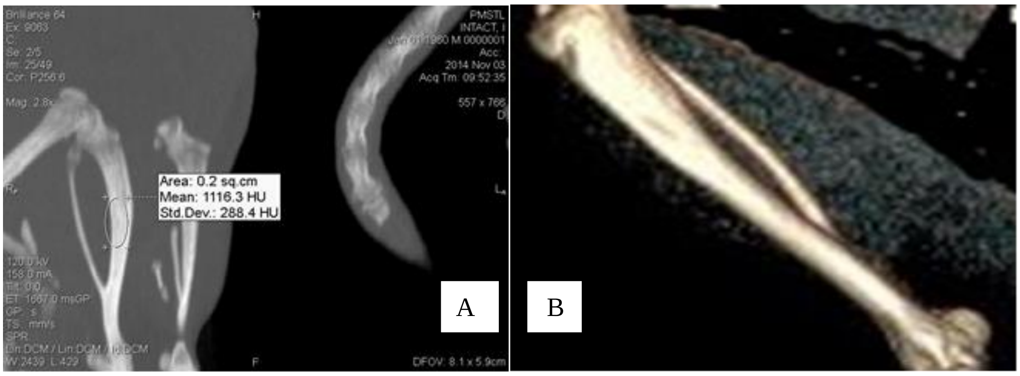

Figure 2. Tomographic image showing: A. the density of the tibia in the MPR mode, B. 3-D modeling of the tibia in the group of intact rats.

Şekil 2. A. MPR modunda tibia yoğunluğunu, B. dokunulmamış sıçan gruplarında tibianın 3-D modellemesini gösteren tomografik görüntü.

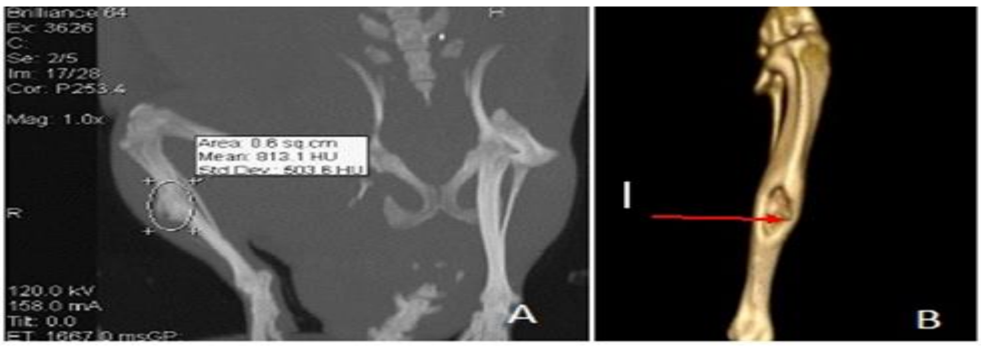

Figure 3. Sectional image showing: A. the density of the tibia in the MPR mode, B. 3-D modeling of the tibia of rats. Bone defect (arrow) in the group of rats with copper-based implants.

Şekil 3. A. MPR modunda tibia yoğunluğunu, B. Sıçanların tibialarının 3-D modellemesini gösteren kesitsel görüntü. Bakır-bazlı implantlı sıçan grubunda kemik bozukluğu (ok).

The results of computed tomography of experimental rats with implants made of copper (Cu) showed an extensive defect in the implantation zone. In some cases, the implant could fall under the skin, after having lost contact with the bone. Bone density in the area of direct contact was 1.5 times lower than the density of the intact bone, which equaled 803.5 ± 36 HU (Figure 3).

In groups with implants made of steel coated with TiN+ZrN combination, non significant bone defects with distinct signs of bone depression were observed. The bone density in the contact area was 797.3 ± 26.5 HU (Figure 4).

In animals with implants made of steel 12X18H9T, a hole with clear, smooth edges, with no signs of destructive changes could be visualized. Density in the area of direct contact with the bone was 997.1 ± 63 HU (Figure 5).

In rats with implants made of steel coated with titanium and hafnium nitrides, as in the group with implants made of steel without a coating, bone integrity was saved, the edges of the hole around the implant were smooth and clear. The bone density in the contact area was 1126.4 ± 94.9 HU (Figure 6) which corresponds to the density of the intact bone.

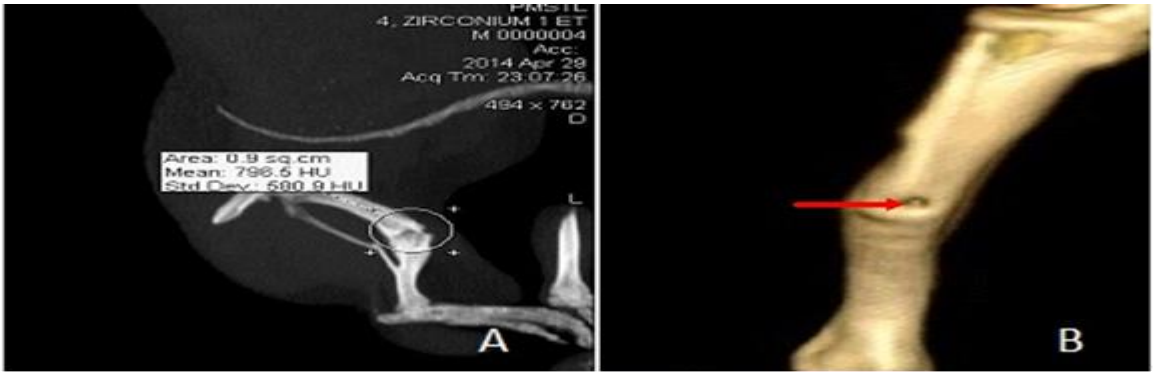

Figure 4. Tomographic image showing: A. the density of the tibia in the MPR mode, B. 3-D modeling of the rat tibia in the group of rats with implants made of steel coated with titanium and zirconium nitrides (TiN + ZrN) with bone defect (arrow).

Şekil 4. A. Tibia yoğunluğunu MPR modunda, B. Kemik bozukluğu (ok) olan, titanyum ve zirkonyum nitrit (TiN+ZrN) ile kaplı çelikten yapılmış implantlı sıçanlar grubunda sıçan tibiasının 3-D modellemesini gösteren tomografik görüntü.

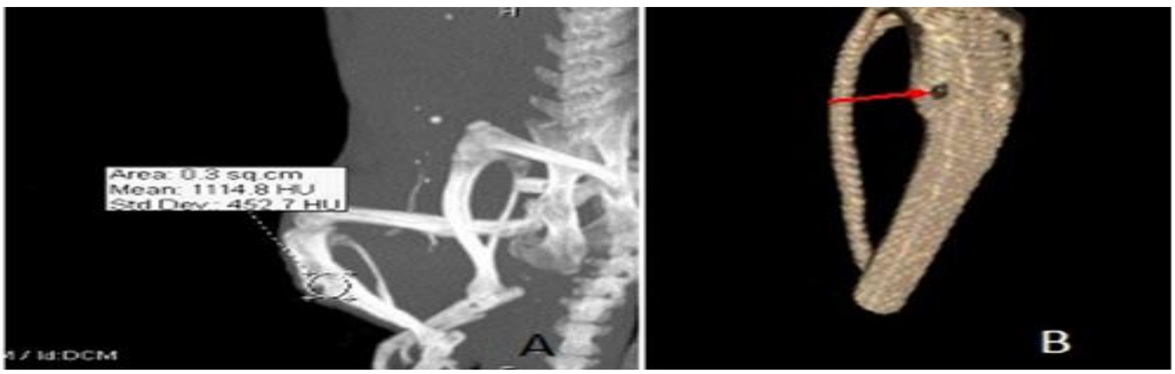

Figure 5. Tomographic image showing: A.the density of the tibia in the MPR mode, B. 3-D modeling of the rat tibia in the group with implants made of steel 12X18H9T without appreciable local pathological changes (arrow).

Şekil 5. A. MPR modunda tibia yoğunluğunu, B. Ölçülebilir lokal patolojik değişiklik (ok) bulunmayan, 12X18H9T çelikten yapılmış implantlı grupta sıçan tibiasının 3-D modellemesini gösteren tomografik görüntü.

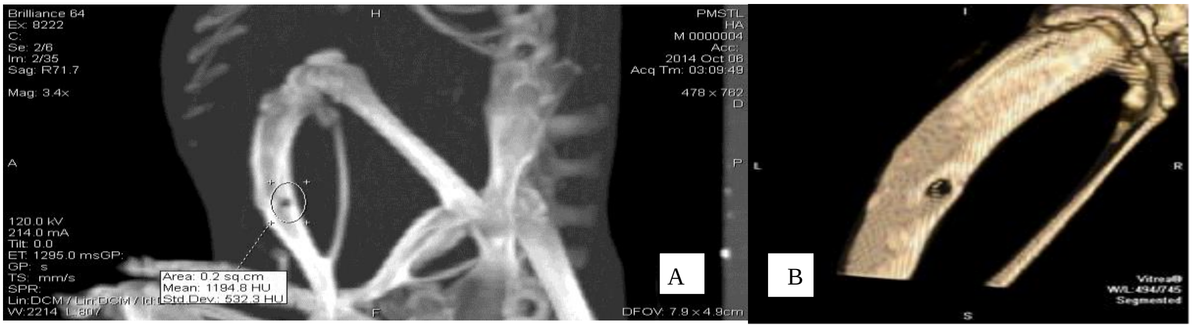

Figure 6. Tomograpic image showing: A. the density of the tibia in the MPR mode, B. 3-D modeling of the rat tibia in the group of rats with implants made of steel coated with titanium and hafnium nitrides (TiN + HfN).

Şekil 6. A. MPR modunda tibia yoğunluğunu, B. Titanyum ve hafniyum nitrit (TiN+HfN) ile kaplı çelikten yapılmış implantlı sıçan grubunda sıçan tibiasının 3-D modellemesini gösteren tomografik görüntü.

Discussion

Titanium is featured by its light weight. Its density is only 3.8g/cm^3 compared to 7.9g/ cm^3 for stainless steel and 8.3 g/cm^3 for cast CoCrMo alloys (Brandes and Brook, 1992). Ti and its alloys, i.e. TiA614V are known for their excellent tensile strength and pitting corrosion resistance (Brandes and Brook, 1992).

A variety of ceramic coatings has been tested and used for improving the metal surface and enhancing bone ingrowth (Cooke, 1992; Davidson, 1993). Lately, there has been an increasing interest in using titanium nitride (TiN) coating to improve the wear properties of TiA614V. TiN coating is biologically inert and has been approved for use in titanium and the components of titanium alloy implants. In several experiments in vitro, TiN-coated implants were shown to have decreased polyethylene and metal wear when compared with other materials used for prostheses (Pappas et al., 1995; Peterson et al., 1988).

From the physical properties of pure hafnium (Hf) and its nitrides (HfN), it has been shown its resistance to corrosion, unless it was under high temperature and pressure, especially in aggressive body fluids (Lidin et al., 2000).

Thus, by combining TiN and HfN coatings on the surface of metal implants, in the present study we have obtained the required properties for biomaterials that are used in orthopedics such as the strengthened positive effect in terms of resistance to corrosion, high toughness and inertness. The results of the present study of density characteristics of bone tissue by computed tomography are in accordance and correlated with the results of the clinical and hematological studies that we have conducted before on these same rats: 'the less pronounced negative local and systemic changes were observed in the groups with rats with implants made of steel with the titanium and hafnium (TiN+HfN) coatings (Akhtyamov et al., 2014).

Copper is among the more frequently reported metals with which patients are poisoned, and routinely ranks third (behind lead and arsenic) in non-medicinal metal exposures reported to US Poison Control Centers (Lewis et al., 2002). Copper-based implants in this study have been reported to cause bone depression, i.e. to reduce bone matter or to cause demineralization of the bone tissue which was expressed by the diminution in the bone density's value.

Although we didn't find any report in the literature concerning the clinical negative impact of the chemical element zirconium (Zr) on the living cells and tissues, the obtained picture in the groups with implants coated with nitrides of titanium and zirconium showed the lowest value of the bone density on tomography compared with other studied implants.

Conclusion

According to Computed Tomographic research in this present study, implants made of copper and steel coated with titanium and zirconium nitrides lead to the bone depression (decrease of bone matter or demineralization) of bone tissue. Their further presence in bone tissue may be accompanied by pathological fracture.

After using implants made of steel coated with titanium and hafnium nitrides (TiN+NHf) bone density is not different from that of the intact bone which indicates that this coating is bioinert and should be recommended for clinical use.

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