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Araştırma Makalesi

Research Article

Morphological Studies of Local Influence of Implants with Coatings Based on Superhard Compounds on Bone Tissue under Conditions of Induced Trauma

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Abstract

In this paper we analyze the response of bone tissue to a transosseous introduction of implants made of copper (Cu), medical steel 12X18H9T, steel with nitrides of titanium and hafnium coatings (TiN + HfN), as well as steel coated with titanium and zirconium nitrides (TiN + ZrN) into the diaphysis of the tibia of experimental rats. The obtained results showed that the restoration of the injured bone and bone marrow in groups with implants made of steel 12X18H9T occurred without the participation of the granulation and cartilaginous tissues, but with implants made of steel coated with titanium and hafnium nitrides (TiN + HfN), this bone recovery also took place in the early term. At the same time, in groups, where the implants were made of copper (Cu), implants were made of steel coated with titanium and zirconium nitrides (TiN + ZrN) were used, such phenomena as necrosis, lysis and destruction of the bone were registered and the bone tissue repair went through formation of the cartilaginous tissue.

Özet

Aşırı Sert Bileşiklerle Kaplı İmplantların İndüklenmiş Travma Altında Kemik Dokusu Üzerine Lokal Etkilerinin Morfolojik Yönden Araştırılması

Bu çalışmada, deney grubundaki sıçanların tibialarının diafizleri içine bakır (Cu), medikal çelik 12X18H9T, titanium ve hafnium nitrit kaplı çelik (TiN + HfN) ve titanium ve zirkonium nitrit (TiN + ZrN) kaplı çelikten yapılmış implantların transosseoz uygulamalarına kemik dokusunun verdiği cevap araştırılmıştır. Elde edilen sonuçlar 12X18H9Tçelikten yapılmış implantlı gruplarda hasarlı kemik ve kemik iliği onarımının granülasyon ve kartilajlı dokuların ayrılması olmadan gerçekleştiğini göstermektedir; öte yandan titanium ve hafrium nitrit (TiN + HfN) ile kaplı çeliklerle yapılmış implantlarda da bu kemik iyileşmesi erken evrede oluşmuştur. Aynı zamanda, bakırdan (Cu) yapılan implantlı gruplar ile titanium ve zirkonium nitrit (TiN + ZrN) ile kaplı çelikten yapılmış implant kullanılan gruplarda kemik nekrozu, dağılması ve bozulması gibi durumlar belirlenmiş ve kemik doku onarımı kartilaj dokusunun oluşumu ile meydana gelmiştir.

Introduction

The incidence of complications (Ivanov et al., 2008) that occur after the use of implantable materials is associated with the formation of false joints, bone deformities due to violation of the principle of immobilization. Migration of metal ions from the used implants causes inflammation and destabilization of the implant, followed by an implant rejection, and allergic reactions in the patient's body.

These factors delay the osteoregeneration process and induce undesirable complications. The cost of treatment of the patient upraises. In this regard, the development of chemically and biologically inert and hypoallergenic coatings for implantable materials remains an important issue in traumatology and orthopedics (Berchenko, 2001; Berchenko, 2002).

Studies on the histological level of the bone reaction to the introduction 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 implants made of steel coated with titanium and zirconium nitrides (TiN + ZrN) in the diaphysis of tibia of experimental rats is the final critical step in the evaluation of local and systemic impact of implants on the body of experimental rats, which was the aim of this work.

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 20 male albino rats weighing 250-300 g. Implants were pins 8-10 mm long, 0.5 mm in diameter.

Implantation was performed under aseptic and antiseptic after dissection of soft tissues surrounding the tibia, under general potentiated anesthesia (Xylazine HCl: 10 mg/kg in IM, Tiletamine HCl + Zolazepam HCl 100: 10-15 mg/kg in IM). Implants were transosseously introduced into the middle thirds of the diaphysis of the tibia after preliminary drilling. The ends of the studs were folded in form of clips and immersed beneath the skin. The wound was sutured tightly.

Depending on the material from which the implants were made, rats were divided into four groups. Two comparison groups: №1 – with implant made of steel 12X18H9T (n=5) and №2 - with implants made of copper Cu (n=5), without coverage. Two experimental groups: no 3-with implants made of steel coated with a combination of titanium and hafnium nitrides (TiN+HfN) (n=5), №4–with implants made of steel coated with titanium and zirconium nitrides (TiN + ZrN) (n=5).

To assess the impact of implants on the rats, morphological changes of bone tibia at the end of each experimental period: on the 10th, 30th, 60th and 90th days were examined that is, the remodeling processes of bone tissue under transosseous osteosynthesis with the mentioned above implants after experimental trauma: quality of the bone and bone marrow remodeling, initial and final time of the remodeling process. At the end of each experimental period, rats were withdrawn from the experiment humanely by decapitation, and the tibia bones in the area of direct contact with the implants were removed from rats. The resulting material was fixed in 10% neutral buffered formalin (Alexandrova et al., 1987). Decalcification was carried out using the drug “BIODEC” (Italy) (Sokolov and Chumakov, 2004). After dehydration in increasing concentrations of alcohols and xylene, the material has been embedded in paraffin (Almazov and Sutulov, 2002). On the microtome, histologic sections 5-7 microns in thickness were produced and then stained with hematoxylin and eosin and by the use of the Van Gieson’s staining method.

Results

On the 10th day at the interface with the implants made of steel 12X18H9T, connective tissue structures with the growth of collagen fibers and the phenomena of bone formation already was identified. Bone beams with transverse partitions were formed; the space between them was filled by a fibrous connective tissue that is, a non lamellar (trabecular) bone was formed. In all cases, at this stage, granulation tissue and cartilage, as well as foci of necrosis were not visible. Inflammatory cell response was absent or consisted in formation of non significant lymphohistiocytic infiltrates.

On the 30th day of the experiment trabecular bone was totally formed (Figure 1a). Along the edges of the perforative hole with the remodeling of the trabecular bone into lamellar bone, trabeculae begun to resorb (Figure 1b). In the bone marrow new trabeculae developed as the adipose tissue was being filled by hematogenically originated cells (Figure 2).

On the 60th day, at the interface with the implants, lamellar bone with a developed system of Haversian canals and recovered marrow was formed.

At the 90th day, the picture remained similar as on the 60th days.

In the groups with implants made of copper (Cu) on day 10, along the edge of the bone defect, the proliferation of connective tissue and the formation of bone trabeculae took place. However, there were also cases of formation of cartilaginous tissue at the interface of bone with copper-based implants (Figure 3a). At the same time, areas of granulation tissue could be registered, which was usually accompanied by an inflammatory response. Accumulations of lymphocytes and macrophages, sometimes with an admixture of neutrophils were observed. At the bone interface with the implants, necrotic processes were observed.

On the 30th day, in the majority of cases, trabecular bone was formed and its remodeling into the lamellar bone was initiated. But often, the edge of the bone defect was lined with cartilage without evidence of ossification and deeper; the defect was presented by immature trabecular bone (Figure 3b). In the bone marrow, at the same time, adipose tissue sections not yet filled with hematopoietic elements were preserved. There were also signs of inflammation that sometimes bore purulent nature, with the dissolution of the bone surrounding the implants.

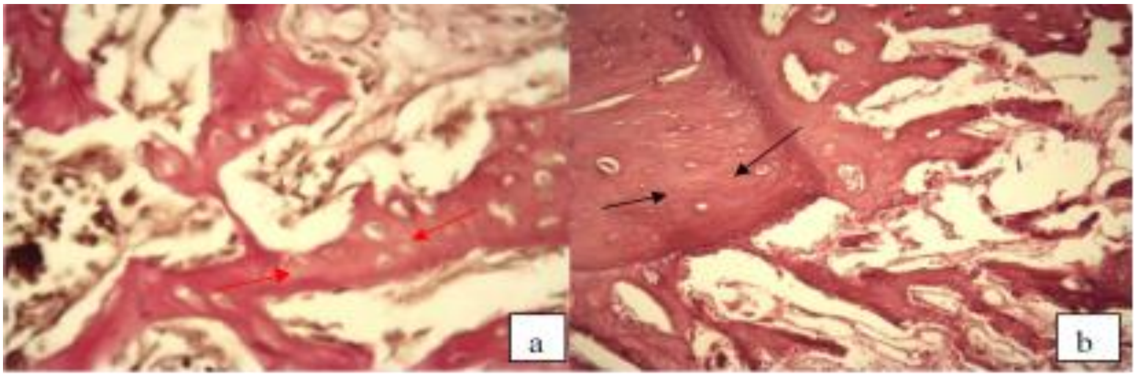

Figure 1. a) Trabecular bone initiation with beam structure (red arrows). Van Gieson staining. x 400. Group with implants made of steel 12X18H9T. 10th days of experiment.
b) Restructuring non lamellar bone into lamellar (black arrows). H/E staining. x 200. Group with implants made of steel 12X18H9T. 30th days of experiment.

Şekil 1. a) Işın yapısı ile trabeküler kemik başlaması (kırmızı oklar) Van Gieson boyaması. x 400. 12X18H9T çelik ile yapılmış implant grubu. Deneyin 10. günü.
b) Non-lamelar kemiğin lamelar hale yeniden yapılanması (siyah ok). H/E boyaması. x 200. 12X18H9T çelik ile yapılmış implant grubu. Deneyin 30. günü.

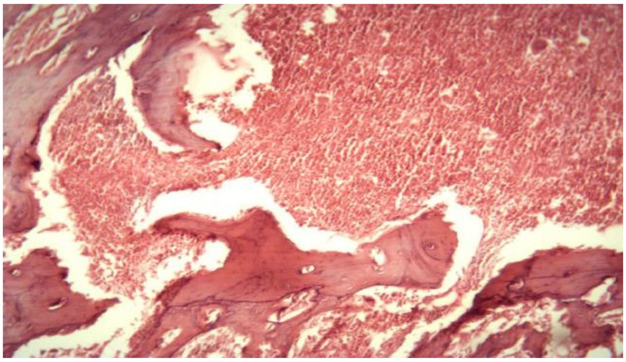

Figure 2. Bone marrow: trabeculae formation and filling of adipose tissue with cells of hematogenic origin. H/E staining. x 200. Group with implants made of steel 12X18H9T. 30th days of experiment.

Şekil 2. Kemik iliği; Yağ dokusunun trabeküler oluşumu ve hematojenik kökenli hücreler ile doldurulması. H/E boyaması. x 200. 12X18H9T çelik ile yapılmış implant grubu. Deneyin 30. günü.

On the 60th day, along the bone interface with implants, in some cases lamellar bone was formed, while in others (in cases when the healing process went through cartilage) at this stage the cartilage resorption, calcification and its replacement by bone tissue was registered (Figure 4a). In case of purulent inflammation at this specified period, there was an increased volume of leukocyte-necrotic masses that occupied the bone – implant interface (Figure 4b).

On day 90 in case if there was an already formed lamellar bone, the cartilage was not detected, as well as trabecular bone. In cases if, on the previous term, purulent inflammation occurred, it grew progressively into osteomyelitis with the dissolution of bone structures around the implants (Figure 5).

The process of healing of the defect at the interface with implants made of steel coated with titanium and zirconium nitrides (TiN + ZrN) was similar to the bone repair process in the group with copper-based implants (Cu): on day 10, along the bone defect, the proliferation of connective tissue and the cartilaginous tissue, and then necrotic processes of osteoid tissue at the bone interface with the implants were observed. The inflammatory cell response was characterized by an accumulation of lymphocytes and macrophages, with an admixture of neutrophils. However, the formation of bone trabeculae was initiated.

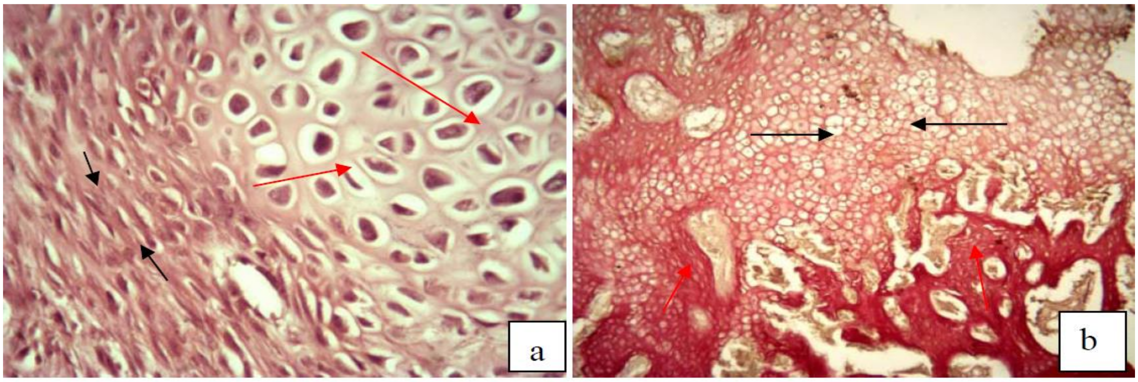

Figure 3. a) The proliferation of connective tissue (black arrows) and cartilage (red arrows) formation along the bone-implant interface. H/E staining. x 400. Group with copper-based implants (Cu). 10^{th} of experiment.

b) Bone defect: Cartilage (black arrows) and immature trabecular bone (red arrows). Van Gieson staining. x 200. Group with copper-based implants (Cu). 30^{th} days of experiment.

Şekil 3. a) Bağ dokusunun çoğalması (siyah oklar) ve kemik implantı interfazı boyunca kıkırdak (kırmızı oklar) oluşumu. H/E boyaması. x 400. Bakır bazlı yapılmış implantlar (Cu) grubu. Deneyin 10. günü.

b) Kemik hasarı. Kıkırdak (siyah oklar) ve olgunlaşmamış trabeküler kemik (kırmızı oklar). Van Gieson boyaması. x 200. Bakır bazlı yapılmış implantlar (Cu) grubu. Deneyin 30. günü.

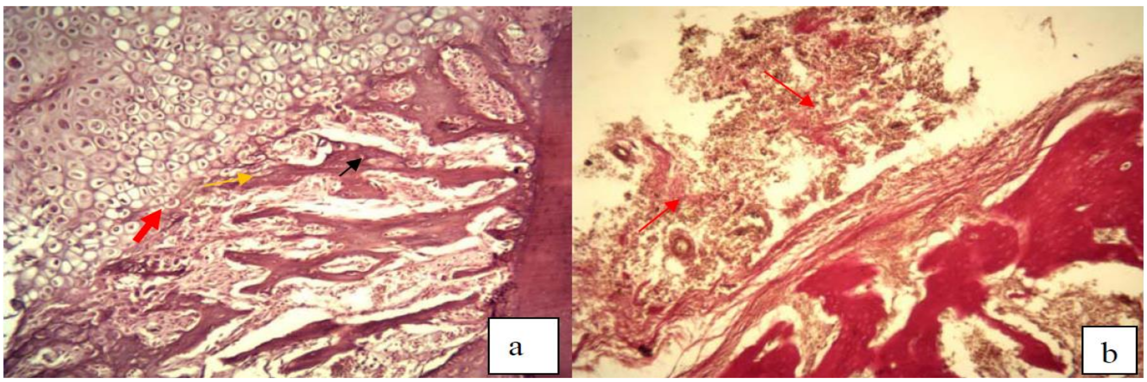

Figure 4. a) Histologic section showing cartilage resorption (red arrow), ossification initiation (yellow arrow) and replacement of cartilage tissue by bone tissue (black arrow) in group with copper-based implants (Cu). H/E staining. x 200. 60^{th} days of experiment.

b) Histologic section showing an accumulation of leucocyte-necrotic masses (red arrows) in the bone-implant interface in group with copper-based implants (Cu). Van Gieson staining. x 200. 60^{th} of experiment.

Şekil 4. a) Bakır-bazlı implantlar (Cu) grubunda kıkırdak resorpsiyonunu (kırmızı oklar), kemikleşme başlamasını (sarı oklar) ve kıkırdak dokusunun kemik dokusu yerine geçmesini gösteren histolojik kesit . H/E boyaması. x 200. Deneyin 60. günü.

b) Bakır-bazlı implantlar (Cu) grubunda kemik-implantı interfazında lökosit-nekrotik kütleler (kırmızı oklar) birikimini gösteren histolojik seksiyon. Van Gieson boyaması . x 200. Deneyin 60. günü.

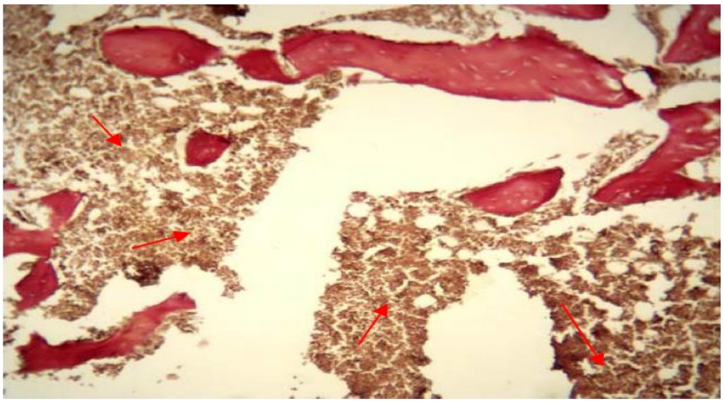

Figure5. Histologic section showing a bone marrow purulent inflammation-osteomyelitis (red arrows) in group with copper-based implants (Cu). Van Gieson staining. x 200. 90th of experiment.

Şekil 5. Bakır-bazlı implantlar (Cu) grubunda bir kemik iliği cerahatli inflamasyon-osteomiyelitini gösteren histolojik kesit. Van Gieson boyaması. x 200. Deneyin 90. günü.

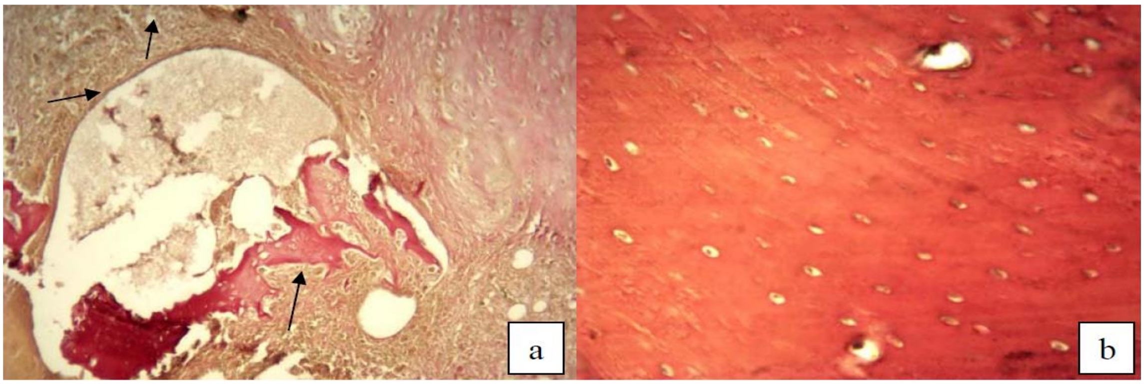

Figure 6. a) Histologic section showing destruction, necrosis and lysis (arrows) of bone in the group with implants made of steel coated with titanium and zirconium nitrides (TiN+ZrN). Van Gieson staining. x 200. 90th days of experiment.
b) Histologic section showing a mature lamellar bone with Haversian canals in group with implants made of steel coated with titanium and hafnium nitrides (TiN+ HfN). Van Gieson staining. x 400. 90th days of experiment.

Şekil 6. a) Titanium ve zirkonium nitrit (TiN + ZrN) ile kaplı çelikten yapılmış imlantlı grupta kemiğin bozulmasını, nekrozunu ve dağılmasını (oklar) gösteren histolojik kesit. Van Gieson boyaması. x 200. Deneyin 90. günü.
b) Titanium ve hafnium nitrit (TiN + HfN) ile kaplı çelikten yapılmış imlantlı grupta Haversian kanallar ile bir olgun lamellar kemiği gösteren histolojik kesit. Van Gieson boyaması. x 400. Deneyin 90. günü.

On the 30th day, in the majority of cases, trabecular bone was formed and its remodeling into the lamellar bone was initiated. But often, the edge of the bone defect was lined with cartilage without an evident ossification and deeper; the defect was presented by immature trabecular bone (Figure 6a). In the bone marrow, adipose tissue sections were not filled with hematopoietic elements. There were also signs of inflammation that sometimes bore purulent nature, with the dissolution of the bone surrounding the implants.

On the 60th day, along the bone interface with implants, in some cases lamellar bone was formed, while in others (in cases when the healing process went through cartilage) at this stage the cartilage resorption, calcification and its replacement by bone tissue was registered. In case of purulent inflammation at this specified period, there was an increased volume of leukocyte-necrotic masses that occupied the bone – implant interface.

On the 90th day in some cases, a definitive formation of lamellar bone and an absence of the cartilage were registered. In other cases, the purulent inflammation that occurred previously grew progressively into osteomyelitis with the dissolution of bone structures around.

In brief, bone repair process in this group was characterized by delayed remodeling of bone as well as of bone marrow and healing process went through the formation of cartilage, and by the presence of cases of suppurative inflammation with destruction of the bone.

In the group with implants made of steel coated with titanium and hafnium nitrides (TiN + HfN) in general, the dynamics of transformation of bone was the same as in group № 1 (12X18H9T): On day 10 trabecular bone with beam structure was formed, on the 30^{th}-60^{th} days: transformation of trabecular into lamellar bone (Figure 6b); the bone marrow repair and the absence of any changes on day 90. Granulation tissue and cartilage, foci of necrosis and inflammation process were also absent.

Discussion

Osseointegration or osteointegration refers to a direct bone-to-metal interface without interposition of non-bone tissue. This concept has been described by Branemark, as consisting of a highly differentiated tissue making "a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant" (Branemark, 1959; Branemark, 1983).

The first biological component to come into contact with an implant is blood. Blood cells including red cells, platelets, and inflammatory cells such as polymorphonuclear granulocytes and monocytes emigrate from post-capillary venues, and migrate into the tissue surrounding the implant. The blood cells entrapped at the implant interface are activated and release cytokines and other soluble, growth and differentiation factors (Davies, 1998). The clinical efficacy of implants depends on many different factors related to the surface properties of the implant materials and their components.

Histologically, already by the 10^{th} day of observation, in the group of rats with implants made of steel 12X18H9T the formation of trabecular bone could be detected and in all cases, at this stage, granulation tissue and cartilage, as well as foci of necrosis were not visible. Inflammatory cell response was absent or was less pronounced.

Recently, the application of nitride coatings on Ti (Titanium) and its alloys have been proposed as a surface treatment aiming to increase the mechanical, physical, and esthetic properties of dental implants. Generally, nitride coatings increase the surface hardness (Tamura et al., 2002), wear resistance (Alsabeeha et al., 2011), and corrosion resistance (Endo et al., 1994) of implant materials. Additionally beneficial, coated Ti surfaces have also been connected with antimicrobial properties (Yoshinari et al., 2001).

Hafnium nitride is characterized by a high melting point, chemical inertness and relatively good oxidation resistance in extreme environments (Gasch et al., 2004; Srivatsan et al., 2007).

A combination of titanium and hafnium nitrides (TiN+HfN) as coatings made the implants biologically inert, the reason why in this group, bone remodeling occurred at an early date and without any complication.

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 (Nelson, 2002).

The cytotoxicity of copper-based shape memory alloys, and particularly single-crystal copper-based shape memory alloys is well documented. The prior art generally teaches away from using exposed single-crystal Cu-based alloys because of this presumed cytotoxicity. For example, Yahia et al. (2006) discusses the presumed cytotoxicity of copper-based single-crystal alloys. Even as recently as 2008, copper-based single-crystal alloys are presumed to be cytotoxic (Creuziger and Crone, 2008). Various factors may inhibit osseointegration. They include implant-related factors such as implant design and chemical composition (Marco et al., 2005).

In our study on the 10^{th} day of experiment, in the group with copper-based implants (Cu), cases of formation of cartilage at the interface with implants were reported. At the same time, areas of granulation tissue could be registered, which was usually accompanied by an inflammatory response. Accumulations of lymphocytes and macrophages, sometimes with an admixture of neutrophils were observed. In the bone bordering the implants, necrotic processes were noted which, according to us, attests the toxicity of the copper-based implants.

Although we didn't find any report in the literature concerning the 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 leads us to conclude that zirconium is as cytotoxic as copper, because, as in groups with copper-based implants, identical phenomena in the bone tissue after transosseous introduction of implants coated with nitrides of zirconium and titanium such as delayed appearance of the trabecular bone, delayed transformation of the trabecular into lamellar bone, inflammation, lysis and necrosis of bone tissue were observed.

Initial interactions of blood cells with the implant influence clot formation. Platelets undergo morphological and biochemical changes as a response to the foreign surface including adhesion, spreading, aggregation, and intracellular biochemical changes such as induction of phosphotyrosine, intracellular calcium increase, and hydrolysis of phospholipids. The formed fibrin matrix acts as a scaffold (osteoconduction) for the migration of osteogenic cells and eventual differentiation (osteoinduction) of these cells in the healing compartment. Osteogenic cells form osteoid tissue and new trabecular bone that eventually remodels into lamellar bone in direct contact with most of the implant surface (osseointegration) (Murai et al, 1996).

On the 30th day of the experiment in the groups 12X18H9T and TiN+HfN trabecular bone was formed, the transformation of non lamellar bone into lamellar, adipose tissue of the bone marrow is already filled with cells from hematogenic origin. On the 60th day of the experiment in groups 12X18H9T and TiN+HfN at the interface with the implants lamellar bone with a developed system of Haversian canals and marrow recovery was formed. This trend continued as on the 90th days.

Whereas in groups of Cu and TiN+ZrN based implants, on this term (30th day), the formation of trabecular bone just begins, and only some of its parts transformed into lamellar bone. In bone marrow there were still adipose tissue sections not yet filled with hematopoietic elements. In general, the processes of bone remodeling in these groups were characterized by the presence of purulent inflammation that grew progressively into osteomyelitis with the dissolution of bone structures around the implants and this tendency went on the following days.

Conclusion

The Conducted morphological analysis of bone showed that the bone healing and bone marrow recovery after experimental trauma occurred at an earlier date and without complications in the group with implants made of steel coated with titanium and hafnium nitride (TiN+HfN) and this confirms that these coatings (TiN+HfN) can be recommended for use in clinical practice.

But in groups with copper-based implants and with implants coated with a combination of titanium and zirconium nitrides such phenomena as necrosis, lysis and destruction of the bone around these implants were registered and the bone remodeling after experimental trauma went through formation of the cartilaginous tissue.

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