

Morphofunctional characteristics of the systemic impact of implants with a coating on the basis of superhard compounds

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Introduction

A comparative morphological study of implants made of copper (Cu), 12X18H9T medical steel, 12X18H9T steel coated with hafnium nitride and titanium nitride (HfN + TiN), 12X18H9T steel coated with zirconium nitride and titanium nitride (ZrN + TiN) having an effect on parenchymal organs and the heart is presented. Materials and methods

Results of implantations were reviewed in four groups of rats including two control (12X18H9T and Cu) and two experimental (HfN + TiN and ZrN + TiN) groups. The study is the latest research on the implants’ effect on vital organs of experimental animals.

Results

Analysis of the results showed that histological changes in the organs were the least in the 12X18H9T medical steel and the steel coated with HfN + TiN groups throughout the experiment and histological characteristics of the internal organs appeared to be normal in all animals at the end of the study. Marked pathological changes were observed in the copper group and the group of steel implants coated with TiN + ZrN despite the current widespread use of the latter in dentistry.

Conclusion

Implants made of copper and steel coated with ZrN + TiN were shown to have a negative impact on the animal body at both local and systemic levels. The findings indicated to the safe use of implants made of 12X18H9T medical steel and steel coated with HfN + TiN alloy.

Keywords: rat, implant, coating, titanium nitride, hafnium nitride, zirconium nitride

Materials used for manufacturing orthopaedic bone fixators and implants are characterized by several limitations [1]. Major drawbacks include biological instability and perseverance to corrosion that lead to toxic response to host and decrease in mechanical properties of an implant [2]. Life expectancy of the constructs is defined by response of surrounding tissues and main vital organs due to biological activity of the host material (metal) [3]. A metal implant coating is meant to minimize release of metal ions in biological fluids and facilitate resistance to a negative effect of the environment. Biocompatibility and interaction with aggressive biological environment must be explored with the use of implants with protective coatings [4]. It should be noted that there are no available implants with bioinert coating registered in the Russian Federation.

A frequently used anodic oxidation cannot be referred to such providing color differentiation. The authors reported earlier results of implant effect on bone condition at the site of implantation. The study is the latest research and meant to evaluate morphofunctional condition of vital organs of experimental animals with implantation of one of the constructs under review. [3]

The purpose of the study was to characterize a systemic effect of coated/uncoated implants made of different materials and placed transosseously in experimental rats using morphofunctional analysis of several vital organs.

MATERIAL AND METHODS

The research was performed at the department of veterinary surgery of Bauman Kazan State Academy of Veterinary Medicine. Experimental study, care, accommodation, housing, management, euthanasia were performed in compliance with requirements of “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (1986). Experiments were produced according to GOST ISO (R) 10993 (Cl.11,12) and approved by Local Ethic Committee of the Kazan SMAVM (Protocol № 5 of June 25, 2015).
Eighty nonlinear outbred white male rats with a body weight of 250-300 g selected according to analogy principles were used for the experiment. Pegs of 8–10 mm in length and 0.8 mm in the diameter were employed for the research. The animals were divided into four groups with regard to the implant material used. Two of the groups were controls with implants manufactured of 12X18H9T medical steel (n = 20, Group I) and copper implants (n = 20, Group II). Uncoated implants were used in control groups. There were two index groups with implants made of 12X18H9T medical steel coated with hafnium nitride and titanium nitride (HfN + TiN) (n = 20, Group III) and implants manufactured of 12X18H9T steel coated with zirconium nitride and titanium nitride (ZrN + TiN) (n = 20, Group IV).

Procedures were performed under general potentiated anesthesia under full aseptic conditions. With rats lying on a side a through hole was made in the middle third of the tibial shaft to place an implant. Experiment length was determined at 10, 30, 60 and 90 days. Decapitation was used for euthanasia at the end of each experimental stage.

Pieces of liver, kidneys, myocardium, lungs and spleen were obtained for morphological examination and fixed in 10 % neutral buffered formalin and embedded in paraffin. Histological sections 5–7 mcm thick were cut with a microtome and stained with hematoxylin and eosin and using Van Gieson technique. Morphological analysis was performed with Jenamed-2 light microscope [5].

Statistical analysis was performed with SPSS Version 13.0 statistic software package using Student’s t-test and Bonferroni correction. A value of P < 0.05 was considered statistically significant.

RESULTS

Morphological evaluation

The liver of animals examined at ten days showed the central venous plethora, sinusoidal extension (Fig. 1a) and granular dystrophy of hepatocytes in all groups. Animals of Group II having copper implants exhibited lymphohistiocytic infiltrates in portal tracts (Fig. 1b). The liver of rats with implants of medical steel coated with zirconium nitride and titanium nitride in Group IV featured thrombi inside vessels’ lumen, parenchymal microabscesses and small- and large-drop fatty degeneration. Histological structure of the liver was normal in Group I of medical steel and Group III of hafnium nitride and titanium nitride coating at 30 days of experiment.

Two other groups showed different findings. Plethoral effect was more intense at 30 days of experiment with erythrocytes being outside of sharply extended sinusoids. Hepatic tubules appeared to be squeezed with transudate and erythrocytes and lost its architectonics. Hepatocyte dystrophy was more intense with signs of ballooning degeneration as an extreme form of vacuolar dystrophy (Fig. 1c). Fatty degeneration was noted in the groups. Lymphohistiocytic infiltrates in portal tracts appeared to be more intense with some cells penetrating the liver parenchyma.

Pathological processes were shown to progressively grow in Groups II and IV at the next stage of observation. Areas of hepatocyte necrosis, rarefaction of the liver parenchyma and cavitation were seen in addition to degeneration, hemorrhages and cellular infiltration. The group of ZrN+TiN coating showed trombi in vessel lumen and microabscesses in the liver parenchyma (Fig. 1d). The changes persisted at 90 days of experiment.

Renal histology of animals showed interstitial edema, venous hyperemia (Fig. 2a) and granular dystrophy of convoluted tubules at ten days of experiment in each of the groups. Rats with uncoated implants of medical steel and steel coated with hafnium nitride and titanium nitride coating at 50 days of experiment.

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Fig. 1 Histograms of the liver showing:

- a the central venous plethora and sinusoidal extension at 10 days of experiment. Stained with hematoxylin and eosin, × 200;
- b perivascular lymphohistiocytic infiltrate. Stained with hematoxylin and eosin, × 400;
- c histogram of the liver showing ballooning degeneration of hepatocytes at 30 days of experiment. Stained with hematoxylin and eosin, × 400;
- d histogram of the liver showing microabscess at 6 days of experiment. Stained with hematoxylin and eosin, × 400.

Fig. 2 Histograms of a kidney showing:

- a venous hyperemia and interstitial edema at 10 days of experiment. Stained with hematoxylin and eosin, × 200;
- b thrombus in vessel lumen at 30 days of experiment. Stained with hematoxylin and eosin, × 400.
- c moderate interstitial edema at 10 days of experiment. Stained with hematoxylin and eosin, × 200;
- d marked perivascular and interstitial swelling and disarranged muscular fibers at 10 days of experiment. Stained with hematoxylin and eosin, × 200;
- e perivascular swelling and lymphohistiocytic infiltration at 60 days of experiment. Stained with hematoxylin and eosin, × 400.
Microscopic examination of myocardium showed minor interstitial swelling, plethora with no signs of disarranged muscles in Groups I and III at 10 days of experiment (Fig. 2c). Subsequently, myocardium was characterized by normal histological structure. Evident perivascular and interstitial swelling and disarranged muscular fibers (Fig. 2d) were seen in Group II at 10 days of experiment. Vessels appeared to be full of blood with perivascular lymphohistiocytic infiltration noted in some cases. Color variations were visualized in several groups of cardiomyocytes with absence of cross striation. Edema appeared to grow with foci of cardiomyocyte micronecrosis and lesion of cells. All the impairments persisted at 60 and 90 days of experiment with moderate hemorrhages under epicardium seen in some cases. The group of samples coated with zirconium nitride and titanium nitride showed identical histological findings. Thrombi, growing edema, plethora, disarrangement of muscular fibers, dystrophy and necrosis of some cardiomyocytes, lymphohistiocytic infiltration and microhemorrhages were observed in myocardium (Fig. 2e).

Microscopic examination of the lungs showed signs of venous hyperemia in Groups I and III at 10 days of experiment. Plethora decreased at 30 days and was nearly absent with normal pulmonary structure at 90 days. Group II exhibited evident signs of plethora and sharply extended vessel lumen at 10 days. Hyperemia appeared to grow with erythrocytes being outside of vessels and infiltration of inter-alveolar septa (Fig. 3a). Transudate could be visualized in lumen of several alveoli. Lymphohistiocytic components were seen in pulmonary tissue in presence of enhancing hyperemia at 60 days of experiment. The bronchial epithelium exfoliated in the lumen. Cases of bronchitis and focal serohemorrhagic pneumonia were noted. These manifestations persisted with intermittent patchy emphysema and atelectasis observed in some cases at the end of experimental observation. Pulmonary changes were more serious in Group IV with application of steel implants coated with zirconium nitride and titanium nitride as compared to those in Group II. Foci of serohemorrhagic pneumonia were common in addition to plethora and edema (Fig. 3b) [6, 7].

**Fig. 3** Histogram of the lung showing a inter-alveolar septum infiltrated with erythrocytes at 30 days of experiment. Stained with hematoxylin and eosin, × 400; b focal organizing pneumonia at 60 days of experiment. Stained with hematoxylin and eosin, × 200. Histogram of the spleen showing c venous hyperemia and lymphoid follicular hyperplasia with reactive center at 10 days of experiment. Stained with hematoxylin and eosin, × 200; d follicular hyperplasia with dumb-bell shaped structures at 30 days of experiment. Stained with hematoxylin and eosin, × 200
Venous hyperemia and follicular hyperplasia with reactive centers were characteristic of the spleen in groups of medical steel and steel coated with NHf+TiN at 10 days (Fig. 3c). Then plethora appeared to decrease and follicular reaction took place. Lymphoid follicles were not hyperplastic and contained no reactive centers in presence of moderate plethora at 60 days. The splenic structure was normal at 90 days.

Copper implants caused hypertrophy of lymphoid follicles with emerging reactive centers in addition to venous plethora in the spleen at 10 days of experiment. Hyperemia persisted and follicular reaction intensified with large merging follicles and dumb-bell shaped structures visualized at 30 days (Fig. 3d).

Follicular hyperplasia decreased at 60 days with small lymphoid follicles persisting without reactive centers with signs of splenic hyperemia. Identical manifestations were revealed in animals with implants coated with zirconium nitride and titanium nitride. Lymphoid follicular hyperplasia with the subsequent reduction was observed in addition to marked venous hyperemia.

**DISCUSSION**

The study of cytotoxicity of plasma condensates of metals with regard to passaging cultures of live cells showed no evident toxic effect on live cells with hafnium nitride and titanium nitride alloy that was defined as biologically compatible [8]. Our findings indicated to biological compatibility and safety of implants coated with NHf + TiN with less changes in the liver, kidneys, lungs and spleen observed in groups of steel coated with hafnium nitride and titanium nitride and 12X18H9T steel at all stages of experiment and normal histological manifestations in all vital organs at the end of observation. The present data on morphofunctional changes in experimental animals were close to those obtained with earlier experiment and showed normal or nearly normal histological structure of vital parenchymal organs of rabbits with implants made of 12X18H9T steel and coated with NHf + TiN at 180 days. Minor changes observed in the group were characteristic of a certain functional stress on the organ [9].

Copper was reported to be most toxic for vital organs of experimental animals (rats) that received intragastrically chronic doses of zinc, copper, ferrous iron and cobalt during 3 months. Copper salts were primarily detected in the liver, small intestine, lungs and kidneys with electrothermal atomization [10]. Microelement analysis of tissues evaluating effect of multiple exposure to copper nanoparticles showed that copper nanoparticles were responsible for changes in micro-element status of the liver [11]. Evident copper-induced cytotoxicity on live cell cultures was also reported [8].

Our series showed expressed pathological changes in rats with copper implants that resulted in dystrophy, edema, extended vessels and hemorrhages, micronecrosis of cardiomyocytes, focal serohemorrhagic pneumonia, patchy emphysema and atelectasis in the lungs and dumb-bell shaped structures in the spleen. The processes being identical to those in copper implantation group were noted in animals that had steel implants coated with zirconium nitride and titanium nitride. Histological picture was somewhat worse in several cases demonstrating thrombi in vessels of the liver and kidneys, microabscesses of hepatic parenchyma, necrosis of some cardiomyocytes and microhemorrhages. Complicated postoperative period previously reported in rats with implants made of 12X18H9T steel and coated with zirconium nitride and titanium nitride was characterized by hemorrhagic discharge from nasal passage and conjunctival cavity, mucosal ulceration of nasal and oral cavities and orchitis. Pathological restructuring of osseous tissues being adjacent to implantation site was revealed with the same constructs [12]. Systemic respiratory exposure to zirconium and zirconium compounds as fine dust was reported to cause elevated immunoglobulin E, alanine aminotransferase and alkaline phosphatase in addition to respiratory dysfunction [6]. Exposure of experimental animals to zirconium compounds showed that zirconium lactate and barium zirconate both produced severe, persistent, chronic interstitial pneumonitis [7].

**CONCLUSION**

Overall morphological changes in parenchymal organs observed in the present study and earlier series primarily indicated to the safe use of implants made of 12X18H9T steel and steel coated with hafnium...
nitride and titanium nitride and a negative effect of implants made of copper and steel coated with zirconium nitride and titanium nitride on animals’ organism at local and systemic levels.

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