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Experimental and Morphological Study of the Osseointegration of Dental Implants Using the Method for Deep Etching of Titanium.

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ABSTRACT

This article describes an original method for the preparation of morphological bone tissue medications with integrated titanium implants, which consists of deep etching of titanium. The objective of the research is to study the osseointegration of a dental implant in the jaw of an animal using a special preparation of morphological medications for removing a metal implant without destroying the adjacent bone. It describes the dynamics of bone maturation in contact with a titanium implant for 1, 3 and 6 months under experimental conditions of introducing implants into the wells of extracted teeth in dogs. The methods of macroscopy, scanning electron microscopy and histological study of bone tissue are used. The efficiency of the titanium bone tissue implant removing is shown through its etching to further prepare medications of bone tissue. It has been shown that mature bone tissue repeats threaded implant configuration after 6 months of implant's presence in the bone tissue, and the implant osseointegration process begins with its apical portion and terminates at the implant collar. A new interpretation of osseointegration is suggested, including such criterion as an exact repetition of the titanium implant thread by the surface of a newly formed jaw tissue.

Keywords: implantation, osseointegration, morphology, preparation methodo

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INTRODUCTION

The clinical use of dental implants as supports for dental prostheses that replace defective dentitions is widely spread in the surgical and prosthetic dentistry (Gileva *et al.*, 2012; Dolgalev *et al.*, 2014; Zhuruli *et al.*, 2010; Zhusev, 2012; Zagorskiy *et al.*, 2013; Kairbekov, 2013; Kolesov, 2008; Nikholskiy *et al.*, 2010; Olesova, 2015; Rudakov, 2013; Block, 2010; Greenberg, 2015).

However, experimental studies in the field of dental implantology are constrained by the complexity of the preparation of morphological medications for interaction between the bone tissue and titanium implant. There are publications about the boundary zone microscopy "implant-bone" in sections or fractures, as well as about the bone tissue microscopy separated from the implant by mechanical means (Gunko, 2009; Kulakov *et al.*, 2006; Paraskevich, 2011; Robustova, 2010; Shashmurina, 2008; Babbush *et al.*, 2010; Misch, 2014). Wherein, the bone tissue is often broken on the border with an implant, which prevents from studying the nature of titanium osseointegration. The objective of this research was to study the original method of preparation for microscopic examination of bone tissue medications with integrated implants of the "chemical deep etching method according to Mirgazizov" (MCDE).

METHODOLOGY

The essence of the MCDE method according to Mirgazizov is as follows: a bone implant block cut out of the implantation zone is placed for 30-45 days into a solution (40%-solution of hydrofluoric acid – 220 g; metallic zinc – 100 g; ethylene glycol – 800 g). During this period, the metal disappears completely; the bone tissue is exposed to demineralization and becomes suitable for obtaining histological sections.

The composition of the etching solution is chosen based on the following propositions: titanium readily reacts with weak acids even in the presence of complexing agents, for example, with hydrofluoric acid HF it interacts through the formation of a complex anion $[TiF_6]^{2-}$. Zn is a complexing agent in the oxidation state +II (Zn^{II}). In the oxidation of ethylene glycol, depending on the oxidizing agent conditions, a mixture of glycol aldehyde $HOCH_2CHO$, glycolic acid $HOCH_2COOH$, glyoxal $OHCCHO$, glyoxylic acid $OHCCOOH$ and oxalic acid is obtained. The oxidation with molecular oxygen leads to occurrence of peroxides, formaldehyde, and formic acid.

The described method is applied in the study of osseointegration of 18 screw implants made of nanostructured titanium, installed in the wells of the lower jaw premolars in 6 dogs. Removal of bone blocks with implants was performed after 1, 3 and 6 months, followed by the deep etching of titanium and conducting a macroscopic, electron-microscopy and histological examination.

RESULTS

Under macroscopic examination of bone tissue medications after Titanium etching, the bone surface adjacent to the implant was consistent with the helical surface of the implant, which is a characteristic feature of its osseointegration.

Scanning electron microscopy of bone blocks has shown that already in the first month of the implant interaction with bone tissue, the manifestation of a structure of the threaded implant surface began. However, in these terms a complete picture of "bone carving" is missing. In terms of 3 and 6 months, there is full compliance with the bone picture of carving with the parameters of threaded implant surface (Figures 1, 2, 3).

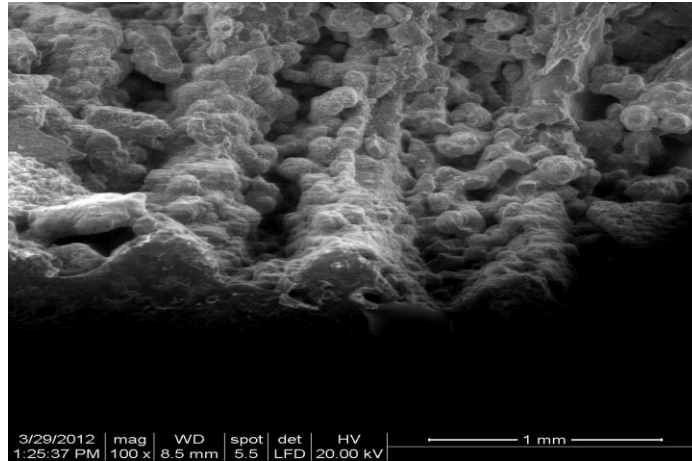


Figure 1. Scanning electron microscopy of the bone tissue contact area with the titanium implant (1 month)

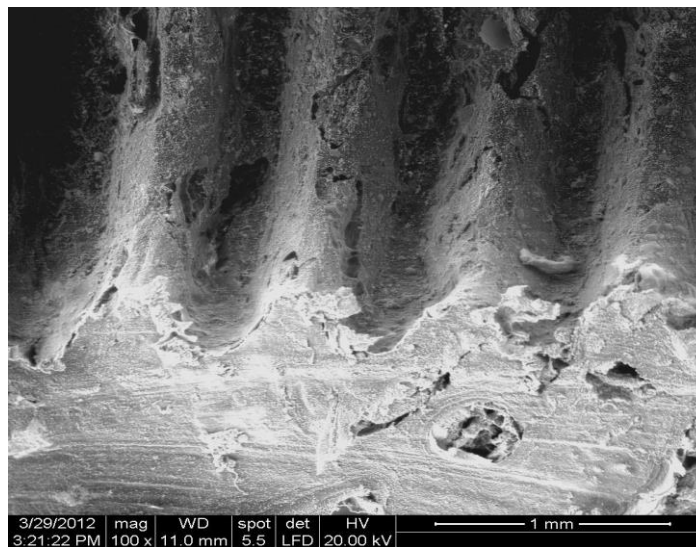


Figure 2. Scanning electron microscopy of the contact zone of bone tissue with a titanium implant (3 months)

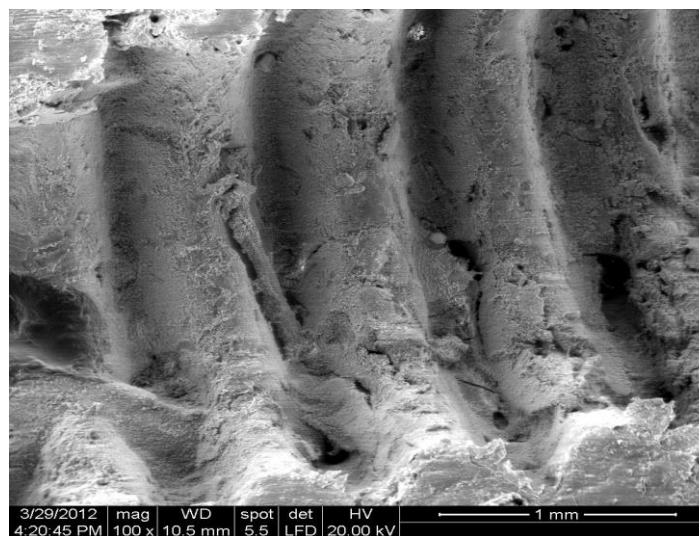


Figure 3. Scanning electron microscopy of the bone tissue contact area with the titanium implant (6 months)

As a result of the histology study of the standard paraffin sections of bone issue, it was found out that one month after the operation, the upper part of the implant retained tissue with microvascular proliferation

and migration of fibroblasts. Proliferation of collagen fibers and formation of fibrous tissue was insignificant. Moreover, in some cases, there were edema, congestion of vessels and lymphohistiocytic infiltration with admixture of neutrophils. In the middle of this period, the granulation tissue was almost completely replaced with fibrous tissue that had a marked proliferation of collagen fibers. Some observations were early signs of bone formation. At the bottom of the implant, the fibrous tissue in most cases started transforming into the coarse-fibered bone that was formed from the newly formed bone trabeculae (Figures 4, 5, 6).

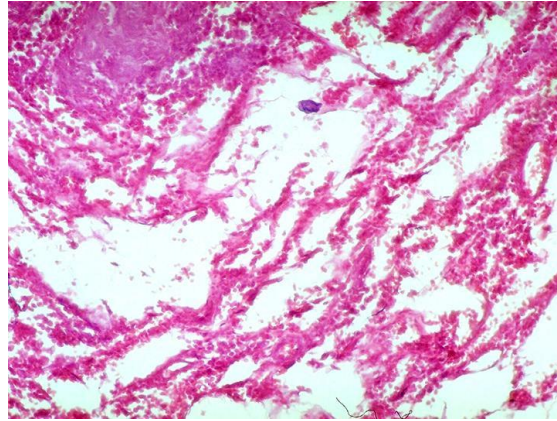


Figure 4. The upper segment, staining with hematoxylin and eosin, x400

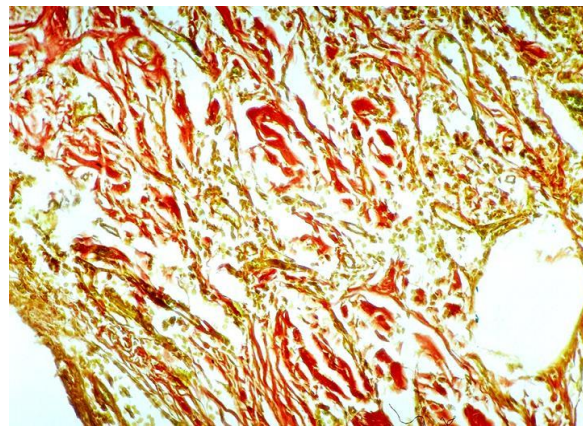


Figure 5. The middle segment, the hematoxylin-eosin staining technique, x400

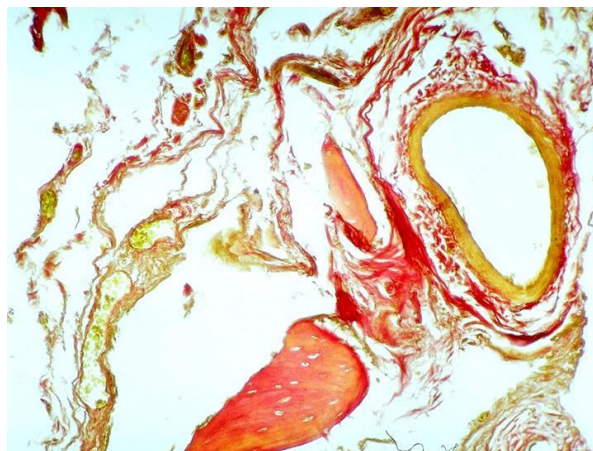


Figure 6. The lower segment, Van Gieson's stain, x 400

After 3 months after surgery, the upper zone demonstrated almost a universal transformation of the connective tissue with the presence of bone trabeculae of varying maturity degrees. Edema, hyperemia, and

inflammatory cell infiltration were not observed. In the median section in all cases, a spongy bone with trabeculae structure was formed, which completely replaced the fibrous tissue. In the lower part around the implant, together with a mature spongy bone, there was a lamellar bone tissue with ingrowth of blood vessels into the Haversian canals. At this period of the experiment, in the middle and especially lower sections, there was a clear dental line from the implant cuts

After six months, all over the implant border, there was formed a mature lamellar bone with individual sections of mature trabecular bone with distinct teeth on the inner surface of the implant lines, respectively.

It should be noted that at all stages of the experiment, the surrounding soft tissues retained their normal structure, and after 1 month, the stratified plain non-squamous gum epithelium did not differ from the norm.

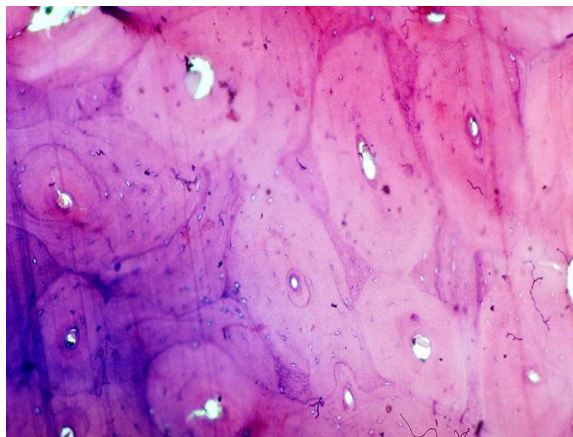


Figure 7. Histological examination of the bone tissue contact area with a titanium implant after 6 months of the experiment, staining with hematoxylin and eosin, x400

DISCUSSION

As can be seen, the proposed method of deep titanium etching of the intraosseous implant provides safety of the implant-contacting jaw tissue, which allowed us to demonstrate a 6-months transformation dynamics of the connective tissue along the implant boundary into a mature bone. The osteogenesis activity increases from the collar to the top of the implant. At all stages of control, normal structure of the oral mucosa around the implant collar is retained. The high degree of osseointegration of titanium implants is illustrated by the formation of bone adjacent to the implant surface, by the repetition of the formed bone tissue surface of the threaded implant configuration.

CONCLUSION

Based on the conducted study, we have formulated a new concept about the osseointegration of screw implants. In our opinion, an osseointegrated screw implant is a condition where the surface of each cavity and top of the implant thread is completely filled with newly formed mature cortical bone, the picture of which very accurately mirrors the geometry of the implant's threaded surface. The results of the morphological study obtained by using titanium etching method made it possible to trace the dynamics of bone tissue maturation during the osseointegration of a titanium intraosseous implant and confirmed the possibility of using implants of artificial nano-titanium as supports for replacement of missing teeth.

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