HISTOLOGY AND MICRO-CT STUDY OF DIAMOND-COATED METAL BONE IMPLANTS IN RABBIT FEMURS

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Abstract

A conformal coating of a thin diamond layer on three-dimensional metal bone implants was shown directly on stainless steel and $TiAl_6V_4$ cortical screw implant using ultrasonic and composite polymer pretreatment method. The best conformation coverage was achieved in the case of the WO_3 interlayer for both stainless steel and $TiAl_6V_4$ screws. The process of osteointegration of the screw implants into rabbit femurs is evidenced by the formation of a bone edge via desmogenous ossification around the screws in less than six months after implantation. A detailed evaluation of the tissue reaction around the implanted screws shows good biocompatibility of diamond-coated metal bone implants.

Keywords: Screw implant, diamond coating, osteointegration, histology

1. INTRODUCTION

Diamond thin films represent a target material having a unique combination of intrinsic properties such as low coefficient of friction, high abrasion resistance and corrosion resistance, high thermal conductivity, high hardness, chemical stability, osteointegration, radiation resistance, optical transparency, semiconducting character (insulator vs. conductor), tunable photoluminescence, biocompatibility, zero cytotoxicity, or even antibacterial properties. [1-6]

Many medical applications require enhanced biocompatibility, low cytotoxicity, increased molecular bioactivity, osteointegration, bacterial biofilm resistance, reduced particle implantation, resistance to the environment, especially compared to commonly used medical steel and titanium alloys. The response of the body to commonly used metal implants cannot be avoided. [2,5,7] The long-term implant stability strongly depends on the surface properties (structural, mechanical, chemical) of the implant. Release of ions and wear particles from implants can be effectively prevented by robust, continuous, non-permeable and biocompatible coatings that adhere well to the bulk material. [8] Diamond proved as an excellent material supporting the adhesion, growth, viability, metabolic activity, and osteogenic differentiation of cells. [9]

The deposition of diamond on the non-diamond substrate requires pre-treatment of the surface to reduce the energy barrier to the formation and growth of diamond grains. Also, it is difficult to prepare diamond layers on non-diamond substrates which contain carbon diffusion-promoting elements. [10] After the diamond deposition, there is often a high internal stress created by the difference in lattice constants and the coefficients of thermal expansion of the diamond and non-diamond substrate.

The combination of layers consisting of either microcrystalline, nanocrystalline or boron-doped nanocrystalline diamond on a titanium alloy with a layer of hydroxyapatite was discussed by Strąkowska et al. [6] The authors

attribute the best adhesion of cells to hydroxyapatite on boron-doped nanocrystalline diamond. The suitability of use of diamond layers for endoprostheses was studied in the form of diamond-like layers on conventional metal implant materials, such as stainless steel AISI 316L, Ti_6Al_4V and CoCrMo. [1] The rate of corrosion of these implant materials in 10% HCl was 10,000 to 15,000 times lower compared to the uncoated material. No surface damage was observed for diamonds of passive implants for 6 months. Titanium coated with nanocrystalline diamond significantly increased surface hardness. The addition of O_2 or NH_3 to the working gas mixture during diamond growth affected its morphology and crystallographic quality, resulting in increased adhesion of osteoblasts to diamond. [11]

Dental implants made of diamond-coated $TiAl_6V_4$ titanium alloys exhibited a similar degree of osteointegration as sandblasted implants, i.e. uncoated. [12] An increased osteoinductive effect in irradiated bone, which would otherwise heal more slowly, was demonstrated on the implant surface with a bone morphogenetic protein-2 (BMP-2) functionalized nanocrystalline diamond layer. [13]

Nanocrystalline diamond surfaces have been shown to be non-cytotoxic in primary fibroblast cultures and induce cell proliferation and stimulation of differentiation markers in treatment with reference polystyrene surfaces. [2]

With a view to practical medical applications, we will show results of histology and micro-CT study after six and twelve months implantation of cortical screws in rabbit's femur. These results are correlated with material characterization (morphology, composition, surface chemistry, hydrophilicity) of uncoated and diamond coated screws before and after *in vivo* tests. For diamond deposition, we investigated two pre-treatment methods based on seeding by colloidal diamond nanoparticles in ultrasonic bath and immersion in a polymer composite with diamond nanoparticles. Application of a 150 nm a-Si, Cr, or WO_3 interlayer was also evaluated.

2. EXPERIMENTAL

Self-tapping cortical screw HA 3.5×8 mm made of stainless steel (SS) and Ti_6Al_4V alloy (Ti) were employed. Cortical screws were covered by approximately 150 nm a-Si, Cr or WO_3 thin layers prepared by radio frequency chemical vapour deposition (CVD), thermal evaporation or magnetron sputtering, respectively. Prior the diamond deposition screws were pretreated either by suspension of ultra-dispersed detonation diamond (UDD, ø5-10 nm) or by immersion into polyvinyl alcohol and UDD colloid. The diamond coating was then deposited by CVD in a low-pressure linear antenna microwave plasma system [14] on bare SS and Ti cortical screws and screws with interlayers. The growth of diamond layer in the LAMWP system was performed for 60 hours at a total gas pressure of 10 Pa, microwave power of 2×1.7 kW and the gas mixture of 3.3 % CH_4 and 13.3 % CO_2 to H_2. The substrate temperature was set to 450 °C using heatable substrate holder. The deposition was repeated two times overcoating top and bottom part of the screws. Surfaces of the diamond-coated screw implants were further modified by radio frequency oxygen plasma, a process expected to improve their hydrophilicity and adhesion of bone cells. Standard material characterization was done by SEM and Raman spectroscopy.

In an in vivo experiment, a total of 20 rabbits were implanted with 20 different screws. While the control screws were uncoated, some of the nanodiamonds were coated by the above procedure. Already after 3 months, healing of the operating field and the onset of osteointegration were observed. Sampling was performed at 6 and 12 months after implantation. Extracted distal femurs were subjected to micro-CT examination, followed by decalcification and the integrity check of the coating on the removed screws and subsequent histological processing of the bone.

3. RESULTS AND DISCUSSION

Figure 1 shows SEM images of the cortical screw and the morphology of deposited nanocrystalline diamond thin film with typical Raman spectra. Low magnification SEM image (**Figure 1a**) shows conformal coating of

the screw thread without any cracks and visible delamination of the NCD layer. We have found out that for conformal coating better results were obtained by ultrasonic pretreatment of SS screws while for Ti screws pretreatment by polymer composite gave better results. This behaviour is explained by the original material properties of SS and Ti cortical screw. By comparison of coated screws with and without interlayers, it was found that the best coating conformation and coverage was achieved in the case of the WO_3 interlayer for both SS and Ti screws. Stainless steel screws preserve a smooth, glossy surface which, however, exhibits additional, optically unnoticeable nano-roughness due to diamond coverage. The noticeably rougher morphology of the coating on Ti screw is given by its primary rough surface (apparent from the matte appearance). The coating preserves well the micro-roughness of the implant and adds nano-roughness of NCD film. The typical nanocrystalline character of the diamond thin film (grain size < 100 nm) is shown in **Figure 1b**. **Figure 1c** shows typical Raman spectra of diamond films. The spectrum is dominated by one sharp peak centred at ~1330 cm^{-1} (sp^3 diamond phase) and three bands centred at the frequencies of ~1150 cm^{-1}, ~1370 cm^{-1} and ~1600 cm^{-1}. The D-band at 1 370 cm^{-1} is observed when small graphite crystallites are present. A broad band from approximately 1450 to 1630 cm^{-1} is a superposition of several bands, including trans-polyacetylene band (1450 cm^{-1}) and G-band (related to sp^2 graphite phase) at 1550-1620 cm^{-1} [15,16]. The trans-polyacetylene band (at 1150 cm^{-1}) is attributed to grain boundaries when small grains (see **Figure 1b**) are present [18].

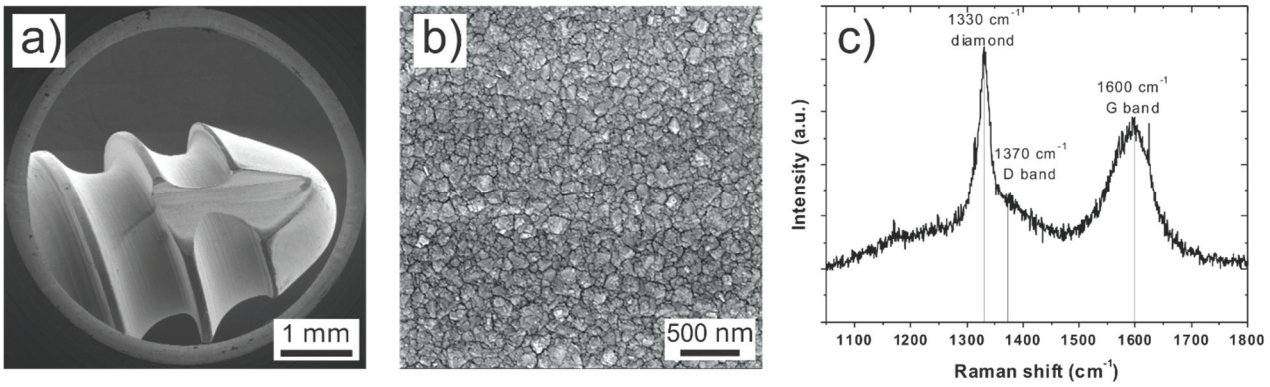

Figure 1 SEM macroscopic and microscopic view of Ti screw together with typical Raman spectra

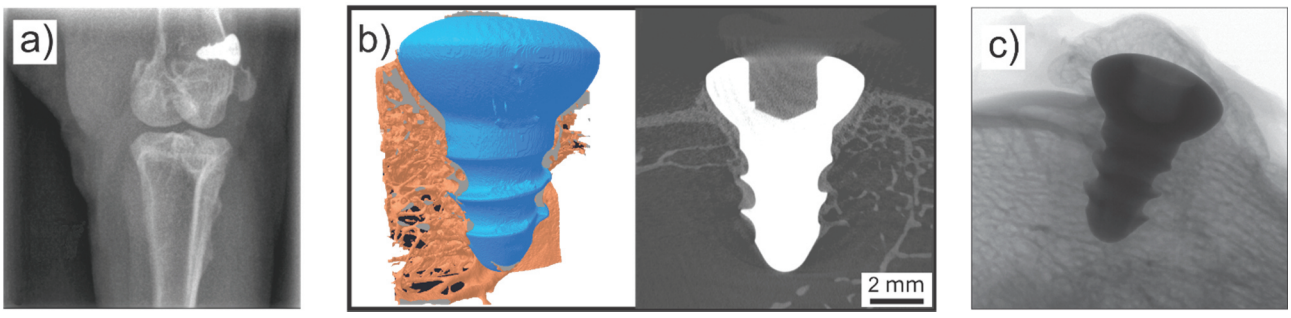

Figure 2 X-ray image of implanted Ti screw (a), micro-CT image of reaction in the vicinity of Ti screw (b) and Ti screw with diamond over-coating layer six months after implantation

Figure 2a shows X-ray image of the Ti implanted screw into rabbit femur bone. **Figure 2b** shows the reconstructed 3D image and a selected plane of micro-CT. **Figure 2c** shows how the bone overgrows screw head. It could be seen that both normal and uncoated screw was well integrated into the host tissue with no separation between the bone and metal. Coated screw after 6 months appeared to be at least as well embedded within the trabecular bone as was the uncoated one after 12 months, including active covering of its head.

Figure 3 shows the vicinity of the implanted screw after four (a) and six (b) months of implantation. Almost a continuous edge of lamellar bone tissue was seen 4 months after implantation. The width of this border was greatest at the head of the screw, where it reached 200-250 μm. towards the tip of the screw, the bone tissue border narrowed to about 20 μm. At the tip of the screw, it was discontinuous, interrupted by a thin layer of collagen tissue. The new bone formation around the screw had the character of a desmogenous ossification. A thin layer of collagen ligament was present on the bone border under which osteoblasts adhered to the bone. After six months of implantation, both the micro CT (**Figure 2b**) and the microscopic image (**Figure 3b**) showed a contiguous edge of the bone tissue, at the screw head reaching 300 μm, at the tip of the screw about 50 μm.

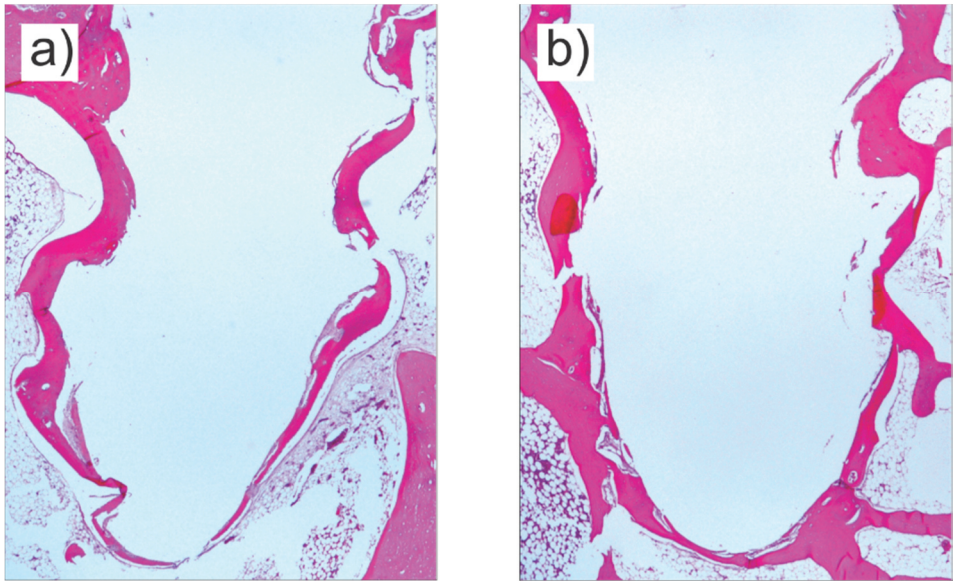

Figure 3 Bone tissue lesion at the edges of the screw after 4 and 6 ray image of implanted Ti screw (a) and micro-CT image of reaction in the vicinity of Ti screw (b) three months after implantation. Hematoxylin and Eosin histological staining, transmitted light, 20x objective

4. CONCLUSION

The bone tissue reaction around the diamond-coated cortical self-tapping screw introduced into the rabbit femur was evaluated using micro CT and optical microscopy. The best coating conformation and coverage was achieved in the case of the WO_3 interlayer for both SS and Ti screws. The coating preserves the micro-roughness of the implant and adds the nano-roughness of NCD film. Histology of control implants (standard Ti alloy) after both 6 months and 1 year in rabbit femurs showed good osteointegration without any inflammatory reaction. Micro-CT examination after 1 year showed lamellar bone around the entire implant without any empty pockets. Diamond-coated screws examined after 6 months exhibited equally good integration into the bone and results from the 6 months interval indicate faster osteointegration. The diamond-based coating thus provides a promising outlook for core material replacement (for avoiding Ti allergy) and for taking advantage of versatile and stable surface functionalization of diamond by diverse molecules (for faster osteointegration).

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