Surface Modification of Porous Titanium Matrix by Diamond-like (DLC) and Nanocomposite Nitrogen-Containing (CN_x) Carbon Films for Creation of Effective Bioimplants[1]

A.P. Rubshtein

Institute of Metal Physics UB RAS, 18, S. Kovalevskaya str., Ekaterinburg, 620041, Russia
Phone: +7(343) 378-58-66, Fax: +7(343) 374-52-44, E-mail: rubshtein@imp.uran.ru

Abstract – The study deals with biofunctional properties of diamond-like (DLC) and nanocomposite nitrogen-containing (CN_x) carbon films. The films were deposited by the method of pulsed arc sputtering of graphite. Nitrogen was leaked into the working chamber for deposition of CN_x coatings. The substrates were titanium matrices with a preset porosity, which were prepared by compaction. The research task was to select such a coating that will not only protect the metallic implant from corrosion, but also stimulate the osteosynthesis. The pilot *in-vitro* experiments led to the following results: 1. The colony-formation capacity (CFC) of the cohesive fraction of myelokaryocytes increased in the cultures incubated with samples of porous titanium with DLC as compared to CFC of the bone marrow cultivated in the presence of porous titanium and porous titanium with CN_x. 2. The CFC of the noncohesive fraction of myelokaryocytes increased in the series "porous titanium – porous titanium with DLC – porous titanium with the CN_x coating". The following facts were established in the *in-vivo* experiments: 1. The nanocomposite CN_x coatings facilitated the formation of a denser bone tissue in pores of the implant. 2. The nanocomposite CN_x coatings decreased the areas of the poor joint between the implant and the parent bed.

1. Introduction

Development of new materials for implants used as endoprotheses in traumatic and orthopedic surgery is a topical problem today. Orthoimplant materials should be biocompatible with tissues of living organisms, be mechanically strong, and possess bioactive properties, i.e., they should stimulate the osteosynthesis and integrate into the bone bed in patients.

These properties are inherent in porous permeable metal matrices (titanium and its alloys) with a bioactive surface [1–4]. One of the means for making the surface of a metal matrix bioactive is its modification by various coatings, specifically, diamond-like carbon (DLC) films including those doped with other elements [5]. Moreover, protective films prevent the metal corrosion and, thus, decrease the probability of "stress remodeling of the bone tissue" around the implant.

The present paper deals with an *in-vitro* study of the colony-formation capacity of different coatings and an *in-vivo* analysis of processes underlying the growth of the bone tissue into a porous titanium matrix with nitrogen-containing carbon films and hydroxyapatite particles in pores of the implant.

2. Experimental technique

Porous titanium with the porosity $\theta = 40\%$ was made from particles (2–3 mm) of the TG-OP-1 titanium sponge by compaction in special molds [2]. The system of pores included both microscale pores and macrochannels (200–400) μm in cross-section, which were necessary for accumulation, migration and adhesion of bone marrow cells [6]. The proportion of pores communicating to the surface accounted for ~75% of the total number of pores. Diamond-like (DLC) and nitrogen-containing carbon (CN_x) films ~20 nm thick were deposited by the method of pulsed arc sputtering of graphite in a vacuum [7].

Equal volumes of a hydroxyapatite powder and distilled water were mixed thoroughly to obtain a homogeneous suspension. The size of particles in the suspension was (0.5–5) μm. Internal pores of the implants were saturated with hydroxyapatite particles ($Ca_5(PO_4)_3OH$) by forcing this mixture along the axis of the cylinders, while surface pores were loaded by exposing the implants in the mixture to a 80 W ultrasound for 10 min. Then the implants were dried at 80 °C for 2 h and 150 °C for 4 h in air.

The effect of the porous titanium samples having different coatings (DLC and CN_x) on the colony-formation capacity of bone marrow precursors was studied *in vitro* by cloning of the nonadherent and adherent fractions of myelokaryocytes in the RPMI-1640 semi-viscous full culture medium in the presence of the samples. The cloning conditions were as follows: the temperature of 37 °C; the CO_2 concentration equal to 3%; 100-% humidity; and the culturing time of 7 days. The maturation rate of the precursor cells was estimated by the maturation index (the ratio of the

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number of clusters to the number of colonies grown in a single slot).

Three types of implants were used in the *in-vivo* experiments (Table 1). The implants were inserted into channels formed in the clivus of condyles of shin and femoral bones of rabbits. A porous titanium implant was inserted into the femur (sample 1, Table 1) of each rabbit as the check sample. In 4 months, all the animals were withdrawn from the experiment.

Table 1. Types of implants for the in-vivo experiments

Ord. No.	Implant material	Porosity θ, %	Implant
1	Titanium	40	Ti*
2	Titanium	40	(Ti + GA)**
4	Titanium	40	(Ti+$CN_{0.5}$+GA)***

Note. * Control sample without additional treatment. **Hydroxyapatite particles are introduced into the implant pores from a water suspension in an ultrasonic bath. *** Hydroxyapatite particles are introduced into the implant pores. Nitrogen-doped carbon $CN_{0.5}$ films of 20 nm in thick are deposited on the surface.

The bones with the implants, which were removed from the rabbits, were potted in paraffin and sliced so as to divide the implant into two halves. X-ray images were used for this purpose. One half of each implant was intended for examination of the structure and the composition of the neogenic bone tissue by the method of the scanning electron microscopy (SEM), while the other half served for the morphological analysis of thin sections of tissues in a light microscope. The composition of the bone tissue formed in pores of the implants was determined by the X-ray spectroscopic analysis of metallographic sections of bone blocks. The titanium implant was etched from the bone block for the SEM study of the structure and the morphology of the tissues [8]. The bone blocks were decalcified before making of thin sections.

3. Results and discussion

The *in-vitro* study demonstrated that the colony-formation capacity (CFC) of the adherent fraction of myelokaryocytes increased in the cultures incubated with samples of DLC porous titanium as compared to CFC of the bone marrow cultivated in the presence of porous titanium and CN_x porous titanium (Fig. 1).

The colony-formation capacity of the nonadherent fraction of myelokaryocytes (hemopoietic precursors) increased in the series "Ti – (Ti + DLC) – (Ti + CN_x)". Nitrogen-containing CN_x films were chosen for the *in-vivo* experiments.

The SEM study of sections of bone blocks after etching of the titanium matrix showed that through pores in all the implant samples were filled with a neogenic bone tissue (NBT) (Fig. 2).

However, areas of a loose union were present at the boundaries of the contact between the outer surface of the implants and the parent bed. These areas were small in the Ti implant (Fig. 2, *a*).

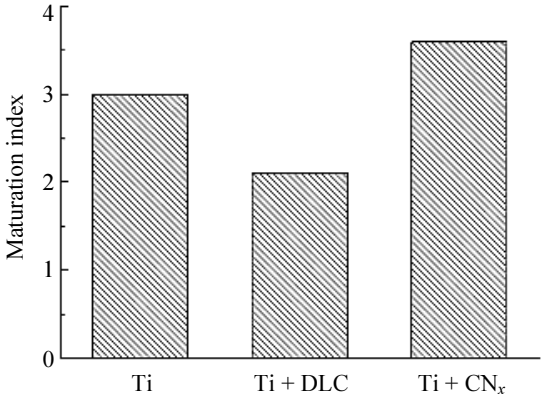

Fig. 1. Maturation intensity (maturation index) of cells, which are precursors of the adherent fraction, during incubation of different samples

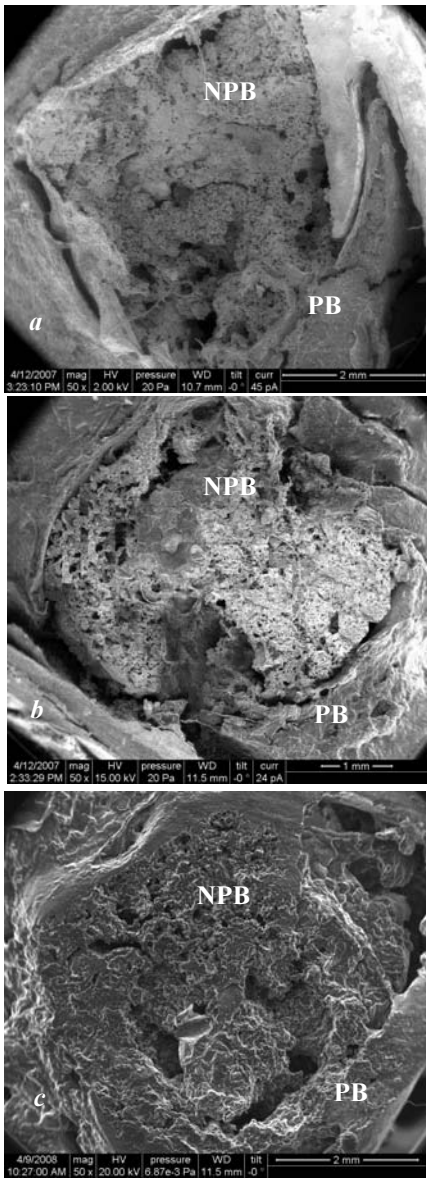

Fig. 2. SEM image of sections of bone blocks after etching of the titanium matrix: *a* – with Ti implant; *b* – with (Ti + HA) implant; *c* – with (Ti + $CN_{0.5}$ + HA) implant. (NBT – neogenic bone tissue; PB – parent bed)

The implant modification by addition of hydroxyapatite particles (Ti + HA) and deposition of a nitrogen-containing carbon film (Ti + $CN_{0.5}$ + HA) increased the extent of the solid union between the implant and the parent bed (Fig. 2, *b* and *c*). The technology used for fabrication of the implants by compaction of porous titanium granules leads to partial "smoothing" of their surface through friction on the mold walls. This causes closing of through pores in places, which most probably present the areas of a loose union between the implant and the parent bone.

A detailed examination of the sections showed that the union is due to well-formed bone trabecules (Fig. 3, *a*).

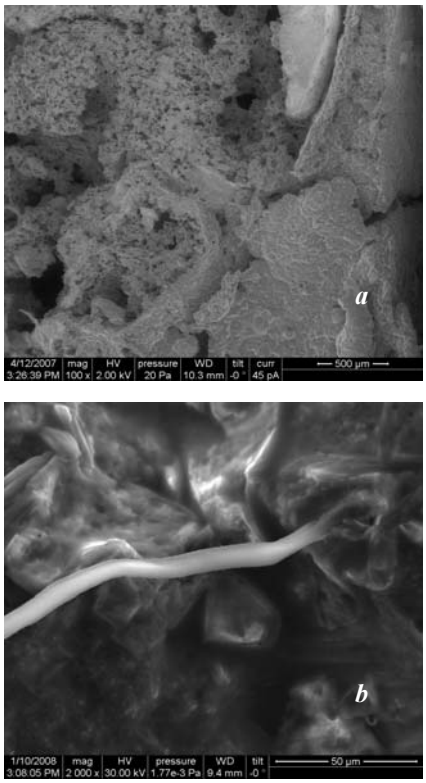

Fig. 3. SEM image of sections of bone blocks after etching of the titanium matrix

Furthermore, vessels formed from the parent bone tissue were seen in sections of all the samples (Fig. 3, *b*). Elements of connective tissue capsules were detected in areas of a loose union only in sections with the Ti implant. Such formations were absent for the (Ti + HA) and (Ti + $CN_{0.5}$ + HA) implants. These observations were confirmed by the morphological analysis of thin sections.

Results of the study of the bone tissue composition (the Ca/P ratio) are given in Table 2.

It is seen from this table that an especially solid bone tissue (approaching the cortical bone in composition) was formed in the (Ti + HA) and (Ti + $CN_{0.5}$ + HA) implants. An additional treatment of the implant surface with hydroxyapatite particles and deposition of nanocomposite nitrogen-containing coatings facilitated the formation of a mature bone tissue.

Table 2. Ca/P ratio in different bone tissues after the in-vivo experiment

Bone tissue	Ca/P, weight %
Cortical bone (parent bone)	2.5
Spongy bone (parent bone)	1.8
Bone tissue in pores of the Ti implant	2.2
Bone tissue in pores of the Ti implant with GA	2.5
Bone tissue in pores of the Ti implant with GA and $CN_{0.5}$	2.6

4. Summary

The *in-vivo* experiments demonstrated that pores in titanium implants having the porosity of 40%, which were made by compaction, were homogeneously filled with a neogenic bone tissue in 4 months. It was found that implants with hydroxyapatite particles in the porous matrix (Ti + HA) and implants coated with a nanocomposite nitrogen-containing carbon film (Ti + $CN_{0.5}$ + HA) offer some distinctions over Ti implants. These distinctions are as follows:

1. A more solid bone tissue, which is similar in composition to the cortical bone, is formed in the pores.
2. The area of a solid union with the parent bed increases.
3. The connective tissue capsule is not formed in the implants.

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