SURFACE MODIFICATIONS OF CLINICALLY USED METALLIC IMPLANTS FOR ENHANCING THEIR BIOCOMPATIBILITY AND BIOACTIVITY – A REVIEW

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In hard tissue surgery, i.e. orthopaedics, traumatology, cranial, maxillofacial and dental surgery, metallic material for construction of bone implants cannot be avoided, because these implants are often exposed to a high load and mechanical stress. Ceramic materials are usually too brittle for these applications, and polymers are too elastic, unless they are reinforced with metallic, ceramic or carbon components [1-3]. Thus, the number of operations requiring metallic implants is continuously increasing. It is often desirable if the bone-contacting surface of the implant enables tight conjunction of the implant with the surrounding bone. In other words, the implant should have the primary and secondary stability. The primary stability is determined mainly by the shape of the implant and the quality of the bone preparation, but its duration is limited to several weeks or months. For example, in hip endoprostheses, the primary stability is limited to 3 months. The secondary stability comes after the primary stability, and is due by the bone tissue ingrowth into the surface structure of the implant. Therefore, the secondary stability strongly depends on the surface properties of the endoprosthesis. If a high secondary stability is achieved, a good and painless function and durability of an implant can be expected, completely without or with considerably delayed revision surgery and need of reimplantation.

In order to obtain the secondary stability of bone implants, i.e. its integration with the surrounding bone tissue, many surface treatments have been experimentally developed (with participating of our group), such as grinding, polishing [4], electric discharge machining, shot peening, acid etching [5-7], oxidation procedures [8,9], or ion implantation [10]. Some of these modifications have been also introduced into clinical practice (mostly for dental implants), e.g. electric discharge machining [11], acid etching alone [12] or in combination with sandblasting [13], microarc oxidation [14] and CO ion implantation [15].

Although some of the mentioned modifications, e.g. polishing, electric discharge machining or sandblasting, strengthened the material surface, these modifications, which are mostly referred as subtractive, i.e. degrading or reducing the material surface layer, are not able to fully prevent the release of cytotoxic ions and wear particles from the bulk material of the bone implant. Clinically used metallic bone implants are usually made of Ti (mainly stomatologic implants [11,13]), Ti-6Al-4V alloy (total hip arthroplasty [16], craniomaxillofacial surgery [17]), Co-Cr-Mo alloys [18,19] and stainless steel [20]. When exposed to load and corrosive biological environments, these materials can release cytotoxic and immunogenic ions, which can damage the adjacent and remote tissues and thus cause the implant failure or systemic diseases (e.g. neurodegenerative diseases caused by Al, carcinogenicity of Cr). These ions have been detected in the blood of patients with bone implants [18,19]. Even titanium, which is generally considered as highly biocompatible metal, caused allergy in stomatologic and other patients [21].

The release of ions and wear particles from the bone implants could be effectively prevented by a strong, continuous, non-permeable and biocompatible coating, well-adhering to the bulk material. Clinically used coatings include e.g. zirconium [18], hydroxyapatite [22], bioactive glass [23], apatite-wollastonite-containing ceramics [24], titanium dioxide [25], or biphosphonate coating [26]. Other promising coatings are nanocrystalline diamond (NCD) films developed in our earlier studies [27-30].

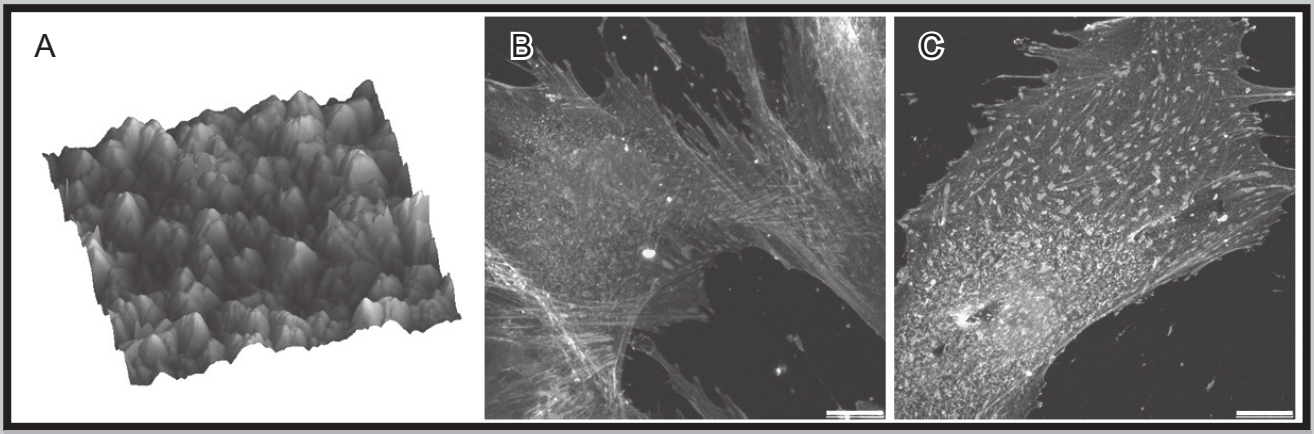

FIG. 1. Morphology of a nanocrystalline diamond film (NCD) evaluated by atomic force microscopy (AFM), picture 1 x 1 μm (A), and the adhesion of primary human osteoblasts on H-terminated (B) and O-terminated (C) NCD films. The cells were stained for talin (green, immunofluorescence), actin (red, phalloidin/TRITC), and DNA (blue, DAPI). Scale bar = 25 μm.

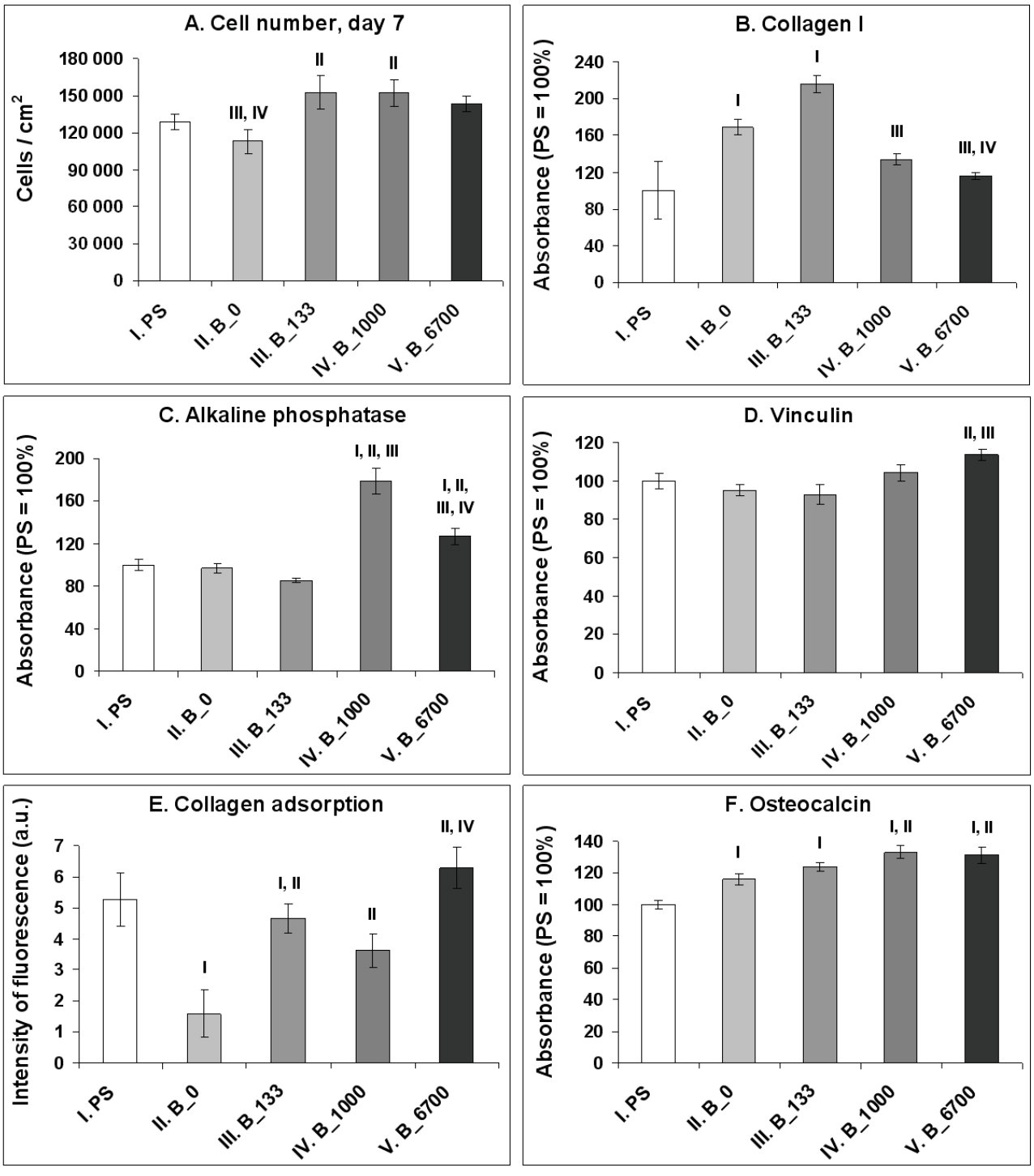

FIG. 2. Number of human osteoblast-like MG-63 cells (A), concentration of early markers of osteogenic cell differentiation collagen I (B) and alkaline phosphatase (C), concentration of integrin-associated focal adhesion protein vinculin (D), adsorption of collagen I, conjugated with Texas Red C_2-Maleimide, on the tested materials after 2 h at room temperature (E) and concentration of osteocalcin, a marker of late osteogenic differentiation (F) in MG-63 cells on day 7 after seeding on polystyrene culture dishes (PS), pure NCD films (NCD_0) and NCD films with B:C ratio of 133, 1000 or 6700 ppm (B_133, B_1000 and B_6700, respectively). Mean ± S.E.M. from 3 experiments. ANOVA, Student-Newman-Keuls Method. Statistical significance: $^{I, II, III, IV}$: $p \leq 0.05$ compared to the group labeled with the same Roman number.

In general, nanostructured substrates are considered as extraordinary suitable cell carriers for implantology and tissue engineering. The nanoscale irregularities on their surface mimic the nanoarchitecture of the native extracellular matrix (ECM) molecules, e.g. nano-sized irregularities in these molecules, e.g. folded (wavy) or helical structure, branching etc. Nanoscale irregularities enlarge the surface area of a material and also increase its wettability. On nanorough surfaces, the cell adhesion-mediating ECM molecules, such as vitronectin, fibronectin, collagen or laminin, are spontaneously adsorbed in a bioactive geometrical conformation, which allow binding between specific sites in these molecules, e.g. RGD-containing amino acid sequences, and cell adhesion receptors. In addition, it is believed that among the cell adhesion-mediating molecules, nanorough surfaces adsorb preferentially vitronectin (due to its relatively small and linear molecules), which is recognized preferentially by osteoblasts through the amino acid sequence KRSR, specific for osteoblast binding [31-34]. Thus, the nanostructured surfaces are much more suitable for bone implants and bone tissue engineering than conventional flat or microstructured substrates.

In accordance with these ideas, in our earlier studies conducted *in vitro*, as well as in studies by other authors, the NCD films proved as excellent substrates supporting the adhesion, growth, viability and metabolic activity of human osteoblast-like cell MG-63 and Saos-2 cells [27-30], and also their osteogenic differentiation, as indicated by an increased activity of alkaline phosphatase [35,36], production of osteocalcin, i.e. an ECM glycoprotein binding calcium [30], and ECM mineralization [36]. The biocompatibility and bioactivity of NCD can be further enhanced by its termination with specific atoms and chemical functional groups, e.g. oxygen, which increases the material surface wettabillity, and thus the adsorption of cell adhesion-mediating molecules and cell colonization [37,38]. In our experiments, the O-terminated NCD coatings promoted the proliferation, synthesis of collagen I, and calcium deposition in human osteoblast-like Saos-2 cells. Primary human osteoblasts showed better developed talin-containing focal adhesion plaques and actin cytoskeleton on O-terminated than on H-terminated NCD films (FIG. 1).

The oxygen termination can be further utilized for immobilization of biologically active molecules promoting bone tissue formation, such as bone morphogenetic protein-2 (BMP-2). Primary human bone marrow mesenchymal stromal cells cultured on these substrates strongly activated the expression of osteogenic markers, and when inserted into sheep calvaria, the implant showed enhanced osseointegration [39]. Osteogenic cell differentiation can be also supported by BMP-7, which is approved for clinical use in the Czech Republic in the form of commercially available preparation Osigraft [40,41].

Another possibility how to increase the NCD bioactivity is doping the NCD with boron. Boron induces electrical conductivity of NCD films, and thus enables electrical stimulation of osteoblasts, which accelerates osteogenic cell differentiation, and finally integration of the implant into the bone [42,43]. In our experiments, boron-doped NCD films were prepared with the B:C ratio of 133, 1000 and 6700 ppm. The NCD films doped with the low and medium B:C ratio (i.e., 133 and 1000 ppm of B) showed the most pronounced increase in the number of human osteoblast-like MG-63 cells in 7-day-old cultures. As measured by an enzyme-linked immunosorbent assay (ELISA) per mg of total protein, the cells on NCD with the B:C ratio of 133 and 1000 ppm also contained the highest concentrations of collagen I and alkaline phosphatase, respectively. On the NCD films with the B:C ratio of 6700 ppm, the cells contained the highest concentration of focal adhesion protein vinculin, and the highest amount of collagen I was adsorbed. The concentration of osteocalcin also increased with increasing level of boron doping (FIG. 2).

In addition, due to their strength, continuity, mechanical resistance and chemical stability, the NCD films could prevent the release of harmful ions from metallic implants. In our studies, the NCD films coated hermetically silicon substrates and fully prevented their cytotoxicity *in vitro*, which was clearly apparent in bare Si substrates [28].

Thus, it is of worth to test the NCD films in preclinical studies on laboratory animals and to introduce in future these coating into clinical practice, at least in the case of stomatological and other small implants (due to a still relatively high cost of these coatings).

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