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# Blood platelets in contact with nanocrystalline diamond surfaces

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**Abstract**

The stainless steel (AISI 316L) and nanocrystalline diamond (NCD) deposited on stainless steel substrates by Radio Frequency Plasma Chemical Vapour Deposition (RF PCVD), predicted for use as coatings for medical applications, were studied by a meaning of blood platelet adhesion. After 12 h contact with platelet poor plasma (PPP) and additional 1 h with citrated whole human blood, platelets present at the surface were found to spread on the stainless steel but not on nanocrystalliane diamond surfaces. The only exception made damaged fragments of NCD surfaces, where we have found adhered blood platelets and other blood cells. The results indicate favorable properties of NCD surface in contact with human blood.


*Keywords:* Platelet adhesion; Surface modifications; Stainless steel; Nanocrystalline diamond—NCD

## 1. Introduction

The surface of an implant in contact with blood (heart valve, stent, blood vessel) may be a place of unfavorable changes caused by activation of the haemostatic system. This contact may lead to thrombus formation and, as a consequence, thromboembolic complications [1]. When artificial surface gets in contact with blood flow, quick adsorption of plasma proteins occurs [1–3]. Platelets adhere to the implant via a layer of adsorbed proteins. The rate and extensiveness of this process depends on the type of proteins adsorbed on the surface of biomaterial [1–3]. Fibrinogen activates the adsorption of platelets, while albumin prevents from it. Thus, the ratio of albumins to fibrinogen is a possible marker of thrombocompatibility [4]. The smaller the proportion, the higher the number of adhering platelets. High thrombogenity may be favorable when implants are used for the process of osteosynthesis. Recent study by Hong et al. [5] demonstrated that titanium implants routinely used in orthopedic surgery are highly osteointegrative due to activation of a large number of platelets, and thus to increasing a level of growth factors produced by platelets and released from α-granules.

An intentional surface modification of biomedical materials applied for contact with blood results in significant improvement in their clinical features. Mitamura [6] and Dion et al. [7] reported high biocompatibility of implants coated with titanium nitride (TiN), while Chen et al. [8] observed an increase in haemocompatibility with $Ti(Ta^{+5})O_2$ layer (titanium oxide films doped with $Ta^{+5}$).

Recently, thin layers of crystalline carbon—diamond like carbon (DLC) and nanocrystalline diamond (NCD) have received considerable attention [9,10]. Studies on the carbon layers application on surface of implants made of medical steel, titanium and cobalt alloys were started in the early 70th years of the last century [11,12]. The structure and physicochemical properties of the so obtained layer depend on the method and conditions of its deposition. The early methods for DLC coating of metal surface appeared to show low adhesion to the substrate [13], which excludes them from medical applications. Thus, some researchers have used intermediary layers to increase adhesion. Jones et al. [4,14] deposited adherent DLC onto titanium using interlayers of titanium nitride (TiN) and titanium carbide (TiC), and he found [14], that in the case of DLC layers

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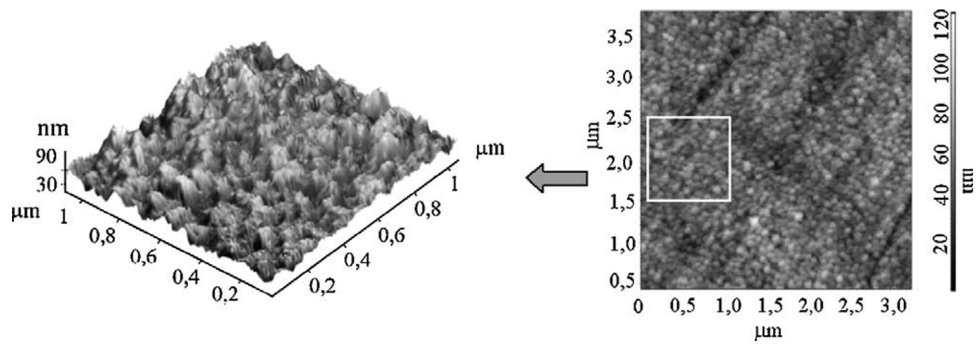


Fig. 1. Topography of small surface element of a sample of AISI 316L steel coated with NCD.

deposited on titanium the albumin to fibrinogen ratio is much higher than in the case of titanium implants and modified titanium surfaces (TiN and TiC). Greater adsorption of albumins is attributed to smooth surface and hydrophobicity of material. This observation was confirmed [15] by analyzing of interactions between titanium surfaces of various microstructure and blood. It was demonstrated that the greater the surface roughness, the higher the number of adhering platelets accompanied by a higher number of microparticles formed and a higher the level of P-Selectin (the marker of blood platelet activation) at flowing platelets. Also Tang et al. [12] studied haemocompatibility of various biomaterials in vivo and in vitro, and they found that carbon coating deposited by CVD method causes smaller adhesion of polymorphonuclear lymphocytes than titanium and medical steel. However, even the most sophisticated implants, made of metal, may lead to biocorrosion and deposition of metal ions in the human body [16]. In this case passivation with albumins may not be sufficient [17] and coating of metal surface with well adhered carbon layer could prevent an implant from biocorrosion. Our herein presented results indicate very promising feature of nanocrystalline layer in contact with blood, with the assumption that the diamond layer must be free from any defects.

## 2. Materials and methods

### 2.1. Sample preparation

Commercially available stainless steel (AISI 316L) round bars, diameter 8 mm, were cut into 3 mm thick discs. The discs were manufactured using machining procedures identical to those used for commercial implants. Final mechanical polishing of surfaces was performed using 1200 grit metalographic polishing paper, and after that electropolishing was employed.

The discs predicted for coating with NCD were passivated and used for the Radio Frequency Plasma Chemical Vapour Deposition (RF PCVD) technology.

### 2.2. Nanocrystalline diamond synthesis

The NCD layer is formed by decomposition of methane in a vacuum reactor chamber by RF PCVD. The apparatus used for RF PCVD consists of a plasma reactor, high frequency electrode—fixed on a base plate and connected via a condenser to a high frequency generator, vacuum system and measuring unit. NCD layers were synthesized out in Department of Biomedical Engineering, Technical University of Lodz, headed by Prof. Stanislaw Mitura. More detailed description of the method and conditions used for synthesis of NCD coating were already reported by us [11]. Briefly, nanocrystalline diamond coating was synthesized by decomposition of hydrocarbon in a radio frequency plasma (13.65 MHz) with the accompanying negative self-bias potential of several hundred volts, at a relatively high gas pressure of 8 to 50 Pa. After that, each sample was cleaned out with water, for 5 min, and sonicated in ethanol.

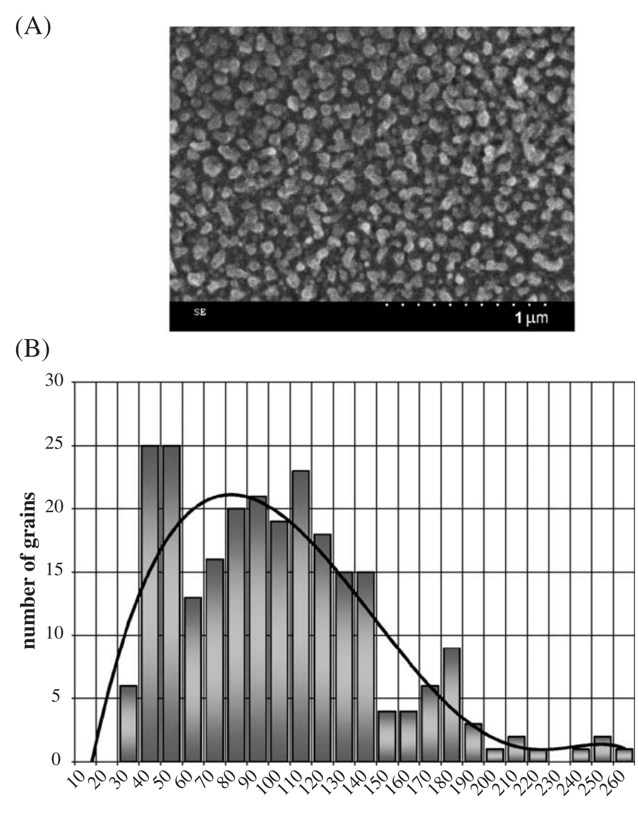


Fig. 2. The SEM image of NCD surface synthesized on the medical steel sample (A) and the range of the maximal linear size of the crystals (grains) (B).