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Structure and biocompatibility of titanium nitride coatings on polyurethane produced by laser ablation

Roman Major [1], *Elżbieta Czarnowska* [2], *Agnieszka Sowińska* [2], *Roman Kustosz* [3], *Jürgen M. Lackner* [4], *Wolfgang Waldhauser* [4], *Michał Woźniak* [5], *Tadeusz Wierzchoń* [5], *Bogusław Major* [1] *

[1] Polish Academy of Sciences, Institute of Metallurgy and Materials Sciences, ul. W. Reymonta 25, 30-059 Cracow, Poland; nmmajor@imim-pan.krakow.pl

[2] The Children Memorial Health Institute, Department of Pathology, Warsaw, Poland

[3] Foundation of Cardiac Surgery Development, Zabrze, Poland

[4] Joanneum Research Forschungsgesellschaft mbH, Laser Center Leoben, Austria

[5] Warsaw University of Technology, Faculty of Materials Science and Engineering, Warsaw, Poland

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Abstract: Titanium nitride (TiN) coatings on polyurethane (PU) were produced by pulsed laser deposition, by means of a system working with a Nd:YAG laser for ablation of titanium target. Nitrogen environment was applied in the reactive chamber. Structure examinations – X-ray diffraction (XRD), atomic force microscopy (AFM) and transmission electron microscopy (TEM) – were performed to study surface morphology (AFM) and microstructure of the cross-section (TEM) as well as crystallographic texture and residual stresses (XRD). AFM results revealed diminishing roughness of the surface when the coating thickness increased in the range from 0.25 via 0.5 and 1 up to 3 µm. The appearance of characteristic features on the surface leads to the conclusion that the contribution of kinetic processes in the formed morphology was dominant. XRD measurements showed an increase of the lattice parameter, accompanied by an increasing coating thickness, which could be explained by the change of the deposition mechanism. Crystallographic texture measured in TiN layers presented close to random crystallographic orientation, while pole figures of the residual stress distribution suggested isotropic character. Thin foils prepared from the cross-section by the microtome method showed a form of anchoring of the deposited TiN layer to the PU substrate with possible local remelting of substrate by the first deposited particles. Residual stress measured in the TiN layer was in the range of about 2 GPa and its value diminished together with the rise of coating thickness. Biological tight examinations of the coating were carried out with respect to cell proliferation, death and adhesion.

Introduction

Blood-contacting materials are routinely used in modern medicine [1- 4]. When blood comes into contact with a foreign matter, such as a biomaterial, the first clinically manifested process that occurs is the activation of haemostasis (clotting). The first step of haemostasis involves adsorption of blood proteins, followed by platelet adhesion and activation. A variety of agents, such as collagen, plasma proteins and the products of platelet metabolism, can cause this activation of platelets. The process is influenced by the fluid mechanical properties of the blood flow. A key issue in this context is the wall shear stress, meaning the force exerted by the flow per unit of wall surface area. The materials used in blood-contacting devices have often been chosen according to their physical characteristics, such as flexibility or rigidity, mechanical strength, transparency, degradability, etc. Thus, in the case of materials with the above-mentioned properties a reduction of thrombogenicity (the tendency to blood clotting) may not always be achieved. Furthermore, increasing evidence is now being reported that the thrombogenic properties of medical devices during clinical use are different from those observed during *in vitro* testing under static conditions. The response of the haemostatic system may differ for each application, depending on the flow conditions. Well-known titanium nitride (TiN) coatings have proven effective in making cutting tools more durable. TiN is now being considered as a perspective biomaterial. As a blood-contacting material, titanium nitride has to undergo more than the typical biocompatibility tests. It also has to meet the haemocompatibility criterion in both *in vivo* and *in vitro* tests.

Polyurethane

Segmented polyurethane (SPU) is the critical biomaterial used in the majority of clinical applications worldwide [5]. SPU is similar in chemistry to DuPont's Lycra Spandex. This series of solvent-based elastomers is based on an aromatic polyetherurethaneurea with a soft segment of poly(tetramethylene oxide) (PTMO) and a hard segment of diphenylmethane diisocyanate and mixed diamines. It contains an additive package consisting of an antioxidant and a copolymer of decyl methacrylate and diisopropylaminoethyl methacrylate. Surface properties can be tailored to your specifications with surface-modifying end groups. Numerous medical devices and technologies have benefited from the combination of softness, excellent mechanical properties, and good biocompatibility of segmented polyurethane. However, the stability of polyurethane in prolonged contact with blood is not sufficient. Thus, it is desirable to develop a method suitable for coating the surface of polyurethane objects with other compatible materials protecting polyurethane from contact with blood.

Titanium nitride

The use of TiN coating for orthopaedic prostheses [6,7], cardiac valves, and dental prostheses has been well documented, along with the haemocompatibility of TiN. The conclusion is that TiN is well tolerated by the human body, in interactions with the blood system. In the specific use of dental prostheses coated with TiN, Berhandt and Lunk [8] have found that TiN does not exhibit any cytotoxic effects. Further documentation available in Russian and German journals indicates that these prostheses gain widespread acceptance of patients [9]. However, it has also been reported that leaching of the metal ions from metal prostheses can accumulate in the body to toxic levels. The corrosion resistance of TiN-coated prostheses – although reducing the number of base metal ions in the body – depends strongly on the porosity of the coating, and thereby limits the coating's protective qualities. TiN coatings were chosen as a blood contacting material. Thin coatings of TiN can be deposited by a number of physical and chemical vapour deposition methods, including evaporation, ion plating and sputtering. In many cases, however, the substrate cannot withstand the elevated temperature entailed by these methods. Thus, there is a great demand for developing low-temperature deposition processes, such as pulsed laser deposition (PLD).

Pulsed laser deposition (PLD)

Lasers can be used to fabricate thin extended films by condensing the material ablated from a target (with the laser light) on a substrate surface [10,11]. Depending on the specific laser and material parameters, ablation takes place under quasi-equilibrium conditions, as in the laser-induced thermal vaporization, or far from equilibrium, as in many cases of pulsed laser ablation (PLA). Thin film formation based on PLA is termed pulsed laser deposition (PLD). Instead of PLD, terms such as laser sputter deposition (LSD), pulsed laser evaporation (PLE), and others, are also used in the literature. PLD is of particular interest because it enables one to fabricate multicomponent stoichiometric films from a single target. From the point of view of film formation, detailed ablation mechanisms are of minor relevance. It is only important that ablation takes place on a time scale that is short enough to suppress the dissipation of the excitation energy beyond the volume ablated during the pulse. Only under this condition, damage of the remaining target and its segregation into different components can largely be avoided. In this regime of interactions, the relative concentrations of species within the plasma plume remain almost unchanged for successive laser plumes and almost equal to those within the target material. This is the main reason why PLD has been found to be useful.

Results and discussion

Microstructure

The thickness and deposition parameters are given in Tab. 1. At the initial stage of modification pure titanium was deposited forming on the polyurethane film a layer of a few nanometers thickness. This was done with the purpose to improve the adhesion of the main TiN layer.

Tab. 1. Deposition parameters of the TiN layers coated onto the polyurethane substrate (sccm: standard cubic centimeters per minute)

Amount of layers	Target	Deposition time in min	Atmosphere	Total thickness
1	Ti	30	5 sccm Ar 30 sccm N_2	0.5 µm
1	Ti	15	5 sccm Ar 15 sccm N_2	0.25 µm

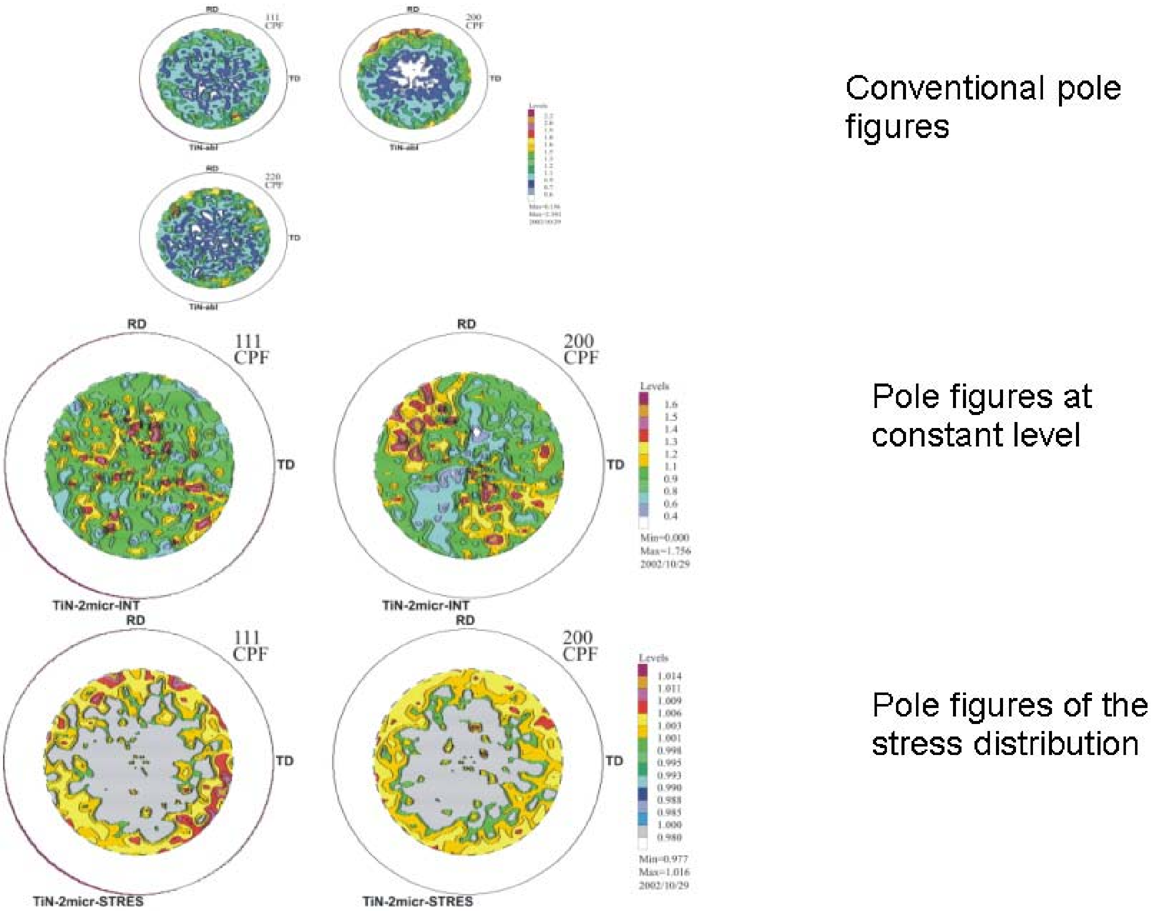

Fig. 1. Crystallographic texture and the stress distribution examination of the TiN deposited on PU

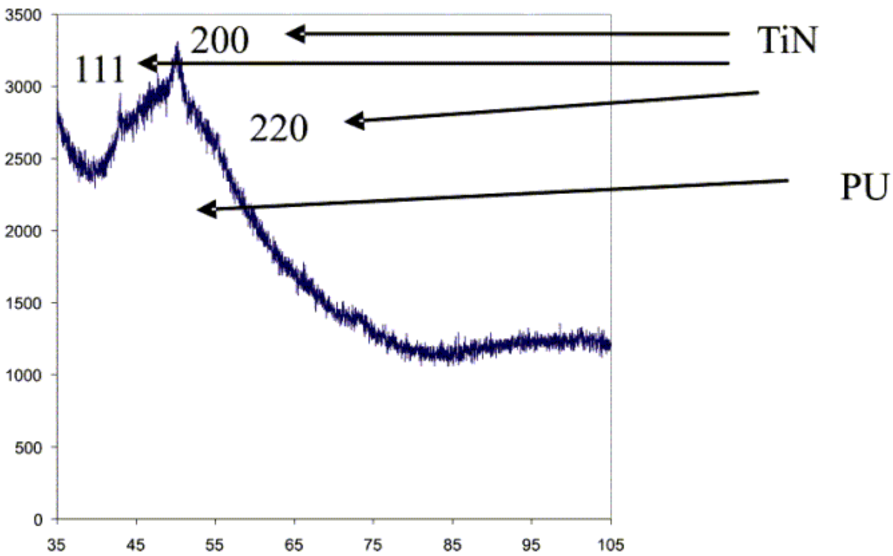

Fig. 2. X-ray diffraction pattern of the TiN layer on segmented PU

Investigations of the texture of thin films are very difficult since pole figures of TiN on the segmented polyurethane present a weak orientation (Fig. 1). No observations indicated any influence of the texture of the substrate on the texture created in the deposited layer.

Application of the pseudo-positive sensitive detector allowed drawing pole figures of the residual stress distribution. The macro residual stress character is close to the isotropic one. The weak character of the texture of the deposited layer could be explained by a high contribution from the amorphous substrate (Fig. 2).

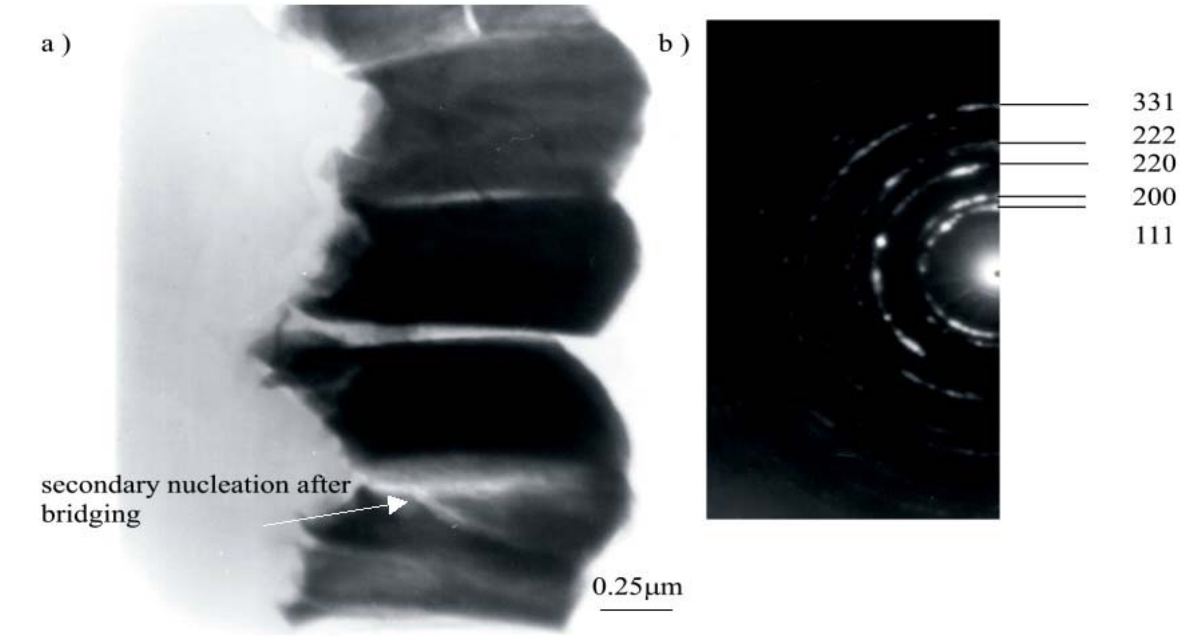

Fig. 3. TEM micrograph of a) the cross-section and b) the electron diffraction pattern achieved by selected area diffraction of TiN on PU

Results of the texture examination show non-uniform distribution of crystallites. This means that there is no influence of the texture developed in the substrate on the texture of the layer. Transmission electron microscopy revealed columnar character of the deposited layer produced at kinetic conditions (Fig. 3).

The thickness of the deposited layer is firmly associated with the mechanism of film growth. The initial stage concerns initial modes, controlled by the surface diffusion, which can be: two-dimensional (2D), two-dimensional associated with three-dimensional (2D+3D), and three-dimensional (3D). Then the mechanism of late growth starts to operate, controlled mainly by the kinetic processes, like columnar or polycrystalline growth. The most appropriate mechanism observed would be 2D or 2D associated with 3D. Microstructure investigations were performed on the 0.5 μm thick layer. It was proven that the thickness was too high. The TEM micrograph showed the influence of the plasma temperature on the elastic blocks participation. Polyurethane is a copolymer, consisting of two types of phases: elastic and rigid blocks. The higher the temperature, the stronger is the participation of elastic blocks and the possibilities of substrate deformation. Moreover, the re-sputtering effect should also be considered. When there is some degree of roughness of the initial substrate material, one can observe so-called hills and valleys. The re-sputtering effect occurs less frequently in the valleys then on the hills, which is due to a lower plasma temperature, which could also have an influence on the material behaviour. Bridging of coating as well as secondary nucleation could be observed during growth. The applied method of deposition on the polymer substrate should consider the low melting temperature of the substrate. Pulsed laser deposition would allow to deposit

TiN layers on polyurethane substrate without substrate degradation, however, the temperature of the plasma is too high to eliminate the waving effect of the substrate.

Observations of the coating – at higher magnifications – revealed texturing of the structure (Fig. 4a). This could be explained with the gradual film growth, which is illustrated in the corresponding scheme (Fig. 4b).

Application of the scratch technique allowed observing the upper part of the columns. Thus it was possible to calculate the average value of the area covered by a single column of the layer (Fig. 5a,b). It was found that, in most cases, a single column covered the area of 275 nm^2 of the substrate. This means that the crystallites were very fine, thus uniformly distributed.

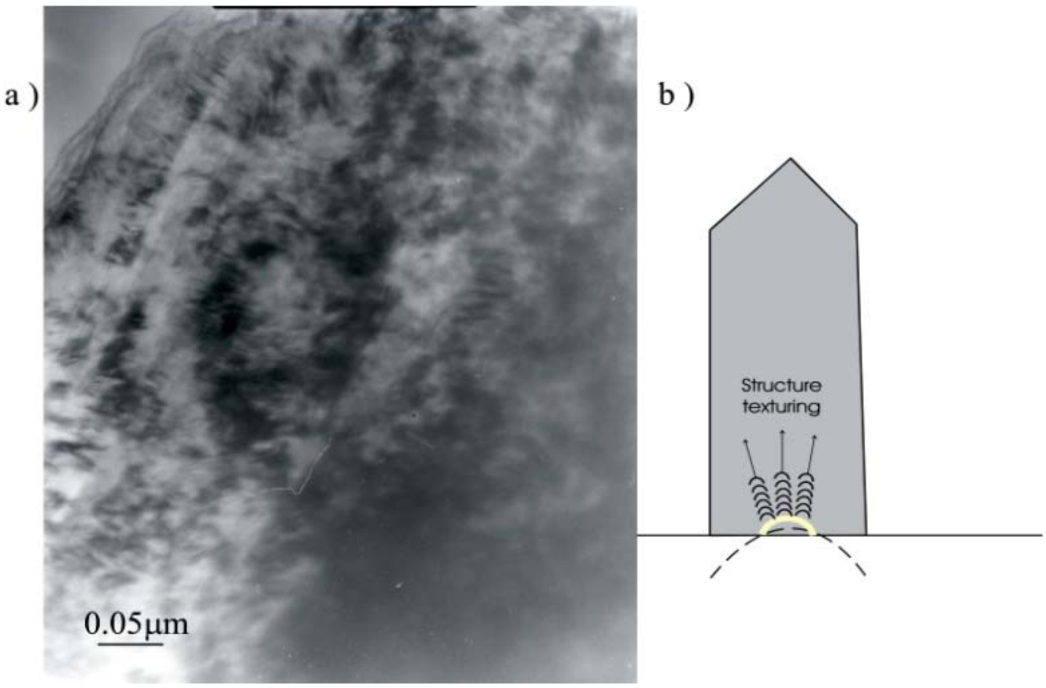

Fig. 4. a) TEM micrograph showing the structure texturing effect, and b) proposed physical explanation

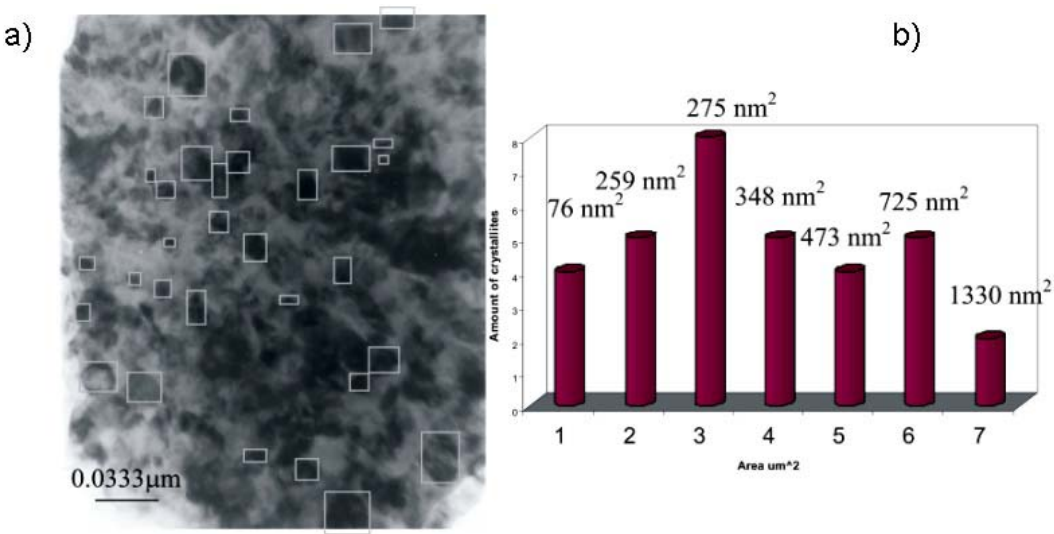

Fig. 5. a) Calculation of the average area covered by a single column basing on the TEM micrographs, and b) histogram of the average area

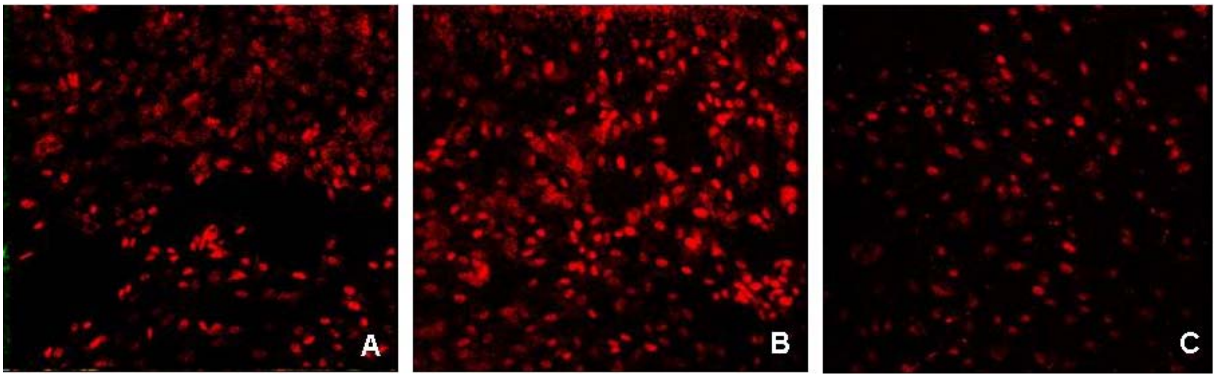

Fig. 6. Fibroblast proliferation on control culture dish substrate (A), on the polyurethane with Ti layer (B), and on the polyurethane with TiN layer (C)

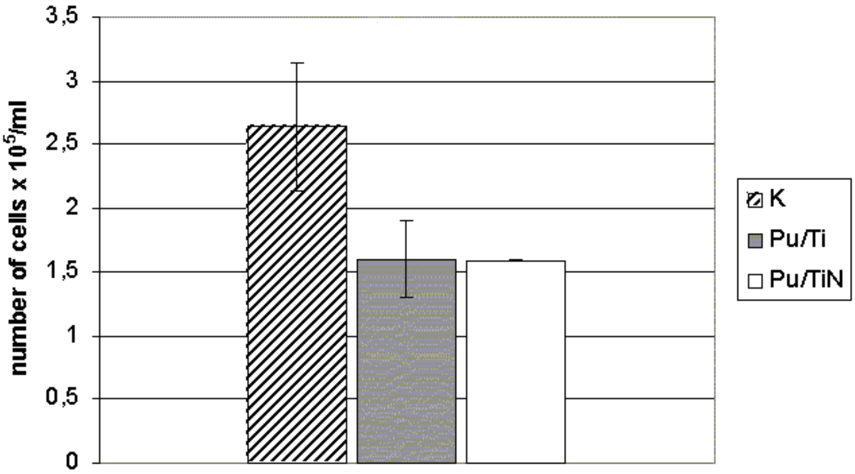

Fig. 7. Number of living cells detached from substrate and counted in a Burcker cell

Biocompatibility

The data show that the number of fibroblasts (Fig. 6) adhered on polyurethane with TiN layer was lower than on polyurethane with Ti layer and on control substrate. Cell populations were characterised by high viability (Fig. 7) and only single cells were dead (not shown).

Concluding it can be stated that the obtained results of the TiN layer deposited on polyurethane substrate confirm that the deposited layers were uniformly distributed. There was not observed degradation of the substrate, but the plasma temperature influenced the rigid blocks participation. Transmission electron microscopy investigations of the top view revealed uniform and fine microstructure, although a cross section showed the unwanted kinetic mechanism of film growth typical of brittle coating.

Biological observations revealed that fibroblasts (cells known for their easy adhesion to various materials) adhered in significantly lower number to polyurethane modified by coating with a TiN surface layer than on other tested materials. It has to be noted that contact with TiN-coated polyurethane did not induce cell death. Thus, we could conclude that the TiN surface layer produced on polyurethane provides good biocompatibility and decreases the surface affinity for cell adhesion. These properties are especially valuable for materials for elements of cardiovascular implants.

Experimental part

High-purity titanium targets were used for the ablation experiments using a pulsed Nd:YAG laser system, which provides four beams of 1064 nm wavelength, 0.6 J pulse energy and 10 ns pulse duration at a repetition rate of 50 Hz. In this multi-spot evaporation system the targets were rotated during laser irradiation in order to avoid the formation of deep craters. The emitted species were deposited at room temperature (approx. 25°C).

The crystalline phases present in the coatings were studied by means of X-ray diffraction (XRD). Crystallographic texture examination was performed with the same method. The application of a pseudo-position sensitive detector allowed measurement of the macro residual stress distribution simultaneously with the crystallographic texture. The microstructure of the TiN layers was investigated with a transmission electron microscope. Application of the scratch technique allowed observing the top view of the columns of the layer.

Biological examinations were performed on human fibroblast cells in 48 h culture. Samples of each type of biomaterial were investigated with the same population of cells and analysed under light and confocal microscope. For light microscopical analysis the cells were harvested from the samples by non-enzymatic chemical 'cell dissociation solution' (Sigma), then analysed with trypan blue staining and counted using a Burcker camera. For confocal microscopical investigations the cells adhered to the substrates were fixed in 4% parafomaldehyde followed by ice-cold 70% methanol, then cell nuclei were visualised by incubation with 7-ammonioactinomycin D (Merck) and finally were imaged by confocal microscopy (Olympus, FV-500 system).

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