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Full Length Article

Structure and hemocompatibility of nanocrystalline titanium nitride produced under glow-discharge conditions

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ABSTRACT

Significant efforts are being made towards developing novel antithrombotic materials. The purpose of the presented study was to characterize two variants of nitrided surface layers produced on alloy Ti-6Al-4V in different areas of low-temperature plasma – at the plasma potential (TiNp) or at the cathode potential (TiNc). The layers were characterized in terms of their microstructure, surface topography and wettability, and platelet response to the environment of different pH.

The produced layers were of the $TiN + Ti_2N + \alpha TiN$-type, but the layer produced at the plasma potential was thinner, smoother and had lower surface free energy compared with that produced at the cathode potential. Biological evaluation demonstrated more fibrinogen buildup, less platelet adhesion and aggregation, and fewer strongly activated platelets on the TiNp surface compared with those parameters on the TiNc surface and on the titanium alloy in its initial state. Interestingly, both surface types were significantly resistant to fibrinogen adsorption and platelet adhesion in the environment of lower pH.

In conclusion, the nitrided surface layer produced at the plasma potential is a promising material and this basic information is critical for further development of hemocompatible materials.

1. Introduction

Although titanium and it alloys are materials widely used for blood-contacting implants and medical devices, they may trigger various unfavorable reactions including thrombosis, inflammation, and fibrosis [1]. In order to eliminate these effects, different methods of surface modification are used [2]. Among them, the glow-discharge nitriding produces diffusion layers of the $TiN + Ti_2N + \alpha Ti(N)$-type with a nanostructured titanium nitride (TiN) surface zone. The layers have good resistance to frictional wear and corrosion, high hardness, and a better biocompatibility than titanium/titanium alloys in their initial state [3–5]. Nitrided-layer properties can be widely modified by selecting specific technological parameters and also by shifting between the methods of glow-discharge nitriding, such as the process with the use of the 'active screen' (also known as nitriding at the plasma potential) and the process conducted at the cathode potential. In conventional glow-discharge nitriding the treated specimen is the cathode. Therefore, the cathode sputtering phenomenon plays a key role in nitrided layer formation [6–9]. The process of nitriding at the plasma potential involves the use of an active screen which is composed of the same material as the specimen and serves as the cathode during the nitriding process. This screen is a perforated cage allowing the reactive gases to freely flow through. The treated specimen is placed inside the active screen and isolated. During the process at the cathode potential, it is possible to control the surface energy of the treated specimen by introducing (through bombardment with ions) defects into the crystalline surface structure of treated specimen thereby increasing surface roughness, so as to control the chemisorption and diffusion processes, which are decisive in nitrided-layer formation [10,11]. Nitriding at the plasma potential involves a limited effect of cathode sputtering on the layer being formed, which ensures that the treated specimen have smooth surfaces [9]. Moreover, recent studies suggest that implants or devices with a nanocrystalline surface structure exhibit better functionality in contact with blood [12,13]. We have already shown that diffusion layers with an outer zone of nanocrystalline TiN or titanium oxide (TiO_2) decrease platelet adhesion and activation [14–16].

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It is known that surface chemical composition, topography and surface energy of titanium and it alloys exert complex physicochemical influences on protein adsorption and cell integrin expression subsequent the cell biological response [17,18]. Studies have shown that plasma proteins accumulate to a greater extent on more hydrophobic surfaces [19], with greater amounts of accumulated fibrinogen inducing platelet and leukocyte adhesion [20–22]. Low pH in the area surrounding the implant can also play an important role in this process [23].

Plasma surface treatment however leads to increased hydrophilicity of the surface but different surface roughness may additionally change cells adhesion and response. Generally is believed that smooth surfaces with high-surface energy better protect against thrombus formation [reviewed in Ref. [24]. But complex the influence of surface roughness, surface energy and TiN nanocrystalline character on its hemocompatibility have not yet been studied.

The purpose of our study was to analyze the structure of nitrided surface layers produced on the Ti-6Al-4V titanium alloy in different areas of the plasma, called the plasma and cathode potentials, and assess these layers with respect to their hemocompatibility, taking into account different ambient pH values. We showed that $TiN + Ti_2N + \alpha Ti(N)$ layers produced at the cathode potential were characterized by increased nanoroughness, surface energy, and platelet adhesion and aggregation compared with the layers produced at the plasma potential. Thrombosis decreased when the ambient pH value was lowered.

2. Material and methods

2.1. Specimen preparation

Specimens with nitrided surface layers produced on titanium alloy Ti-6Al-4V were prepared as previously described [15]. Ti-6Al-4V titanium alloy meets ASTM B348 (Standard Specification for Titanium and Titanium Alloy Bars and Billets). The samples were cut from a bar of 8 mm diameter. In brief, Ti-6Al-4V specimens underwent nitriding under glow-discharge conditions, in the atmosphere of nitrogen (95%), in a gas mixture containing 5% volume of hydrogen, for 4 h, at a temperature of 680 °C, and pressure of 2 mbar. Two variants of nitrided surface layers were produced: TiNc, produced at the cathode potential, and TiNp, produced at the plasma region (Fig. 1). Both the titanium alloy specimens used in layer formation and those used as control specimen**s** were ground with fine-grain sandpaper (i.e. grit size 1200), and polished in an aqueous suspension of aluminum oxide (Al_2O_3), with particle size of 1 μm. All specimens were sterilized in plasma (Sterrad 100, atmospheric H_2O_2, temperature 54 °C, pressure 7 mbar) before tests.

2.2. Layer characteristics

The microstructure of $TiN + T_2N + \alpha Ti(N)$ surface layers was examined under a scanning-transmission electron microscope (Hitachi HD2700) and scanning electron microscope (Hitachi S3500N). The thin foil sheets intended for microscopic examination were cut with a focused gallium ion beam (FIB) using a Hitachi NB500 FIB/SEM device. Identification of the phase composition based on electron diffraction of the selected area as previously described by Morgiel and Wierzchon [25].

Cross-section chemical composition of the produced layers was analyzed by means of secondary ion mass spectrometry (SIMS), with a Cameca IMS 6F device. Cesium ions (Cs^+) were used as the etching beam (5.6 keV energy and 150 nA current). The etching area was a square with a 200 μm side. The area of secondary ion analysis

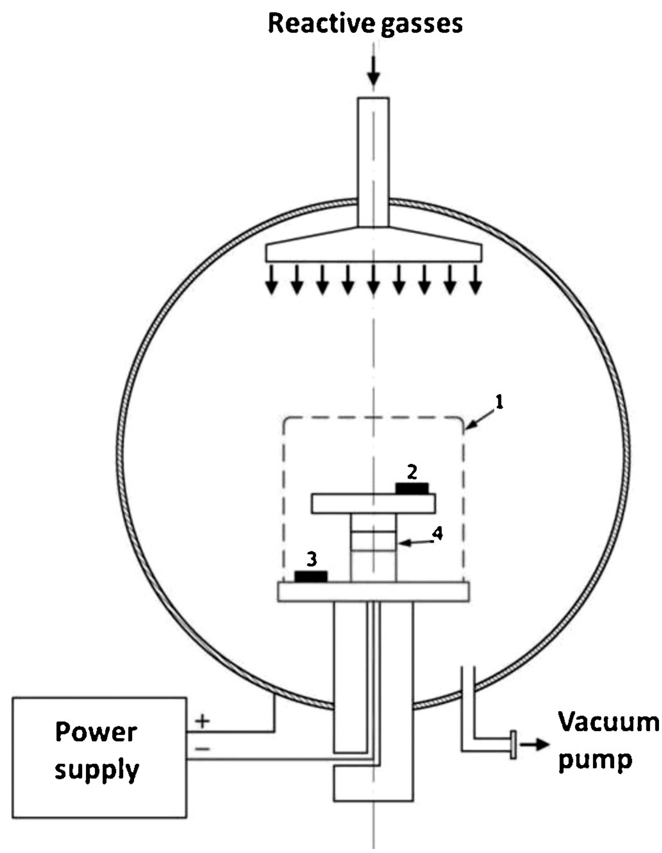

Fig. 1. A schematic representation of the reaction chamber for glow-discharge treatments, where the nitriding process can be carried out at the cathode or plasma potential. 1 – active screen, 2 – specimen at the plasma potential, 3 – specimen at the cathode potential, 4 – insulator.

was a circle with a 60 μm diameter. The following elements were analyzed: Ti, Al, V, N.

The resulting nitrided layers were compared in terms of their nanoscale surface topography, with a nanoscope VIII Multimode atomic force microscope (Tapping mode; ACSTA AppNano tip) (Veeco), and in terms of their microscale surface topography, with a Wyko NT9300 optical profilometer.

Surface wettability was evaluated with a Contact Angle system OCA 20 goniometer (DataPhysics) at room temperature. Two different liquids were used for this experiment: deionised water and diiodomethane. A droplet of liquid (0.4 μL) was used and the measurement was repeated 10 times on specimens with both surface types. Mean values and standard deviations were calculated. Droplet profiles were analyzed using SCAZO software. Based on the obtained contact angle values for diiodomethane and deionised water, the surface free energy was calculated for each specimen using the Owens-Wendt method [26], with free energy assumed to be the sum of dispersive and polar components.

2.3. Protein adsorption

Blood plasma was obtained from the blood of healthy donors. Protein adsorption was investigated after applying 150 μL aliquots of blood plasma onto specimens at pH values of approximately 7.6 and 6.0 and incubating the specimens for 1 h (at 37 °C in an atmosphere of 5% CO_2). The lower pH was obtained by the use 0.1 mol of a citric acid solution (Chemipur). Then, the specimens were rinsed with phosphate-buffered saline (PBS; Sigma – Aldrich) (to remove any loosely bound proteins) and the proteins strongly bound to the material (constituting its biofilm) were fixed with 4% paraformalde-