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ScienceDirect

Ciência & Tecnologia dos Materiais 29 (2017) e130–e134

http://ees.elsevier.com/ctmat

Special Issue "Materiais 2015"

In vitro study of platelet behaviour on titanium surface modified by plasma

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Abstract

Biomedical devices introduced in the human body interact initially with blood cells, their success being dependent on the result of this interaction. This work aimed to obtain optimum biocompatibility and hemocompatibility of the titanium by plasma treatment. For this, discs of commercially pure titanium (CP) were subjected to plasma nitriding using different mixture of nitrogen and hydrogen; the effect of addition of hydrogen to the nitriding plasma was investigated. Before and after treatment, samples were evaluated in terms of topography and wettability using atomic force microscopy and sessile drop tests, respectively. The titanium biological response was evaluated *in vitro* through the application of platelet-rich plasma (PRP) on the surfaces modified and analysis of their behaviour from the point of view of surface tension and cell adhesion. Surface properties, such as roughness and wettability, were sensitive to the hydrogen/nitrogen ratio in the nitriding plasma, suggesting a strategy for producing different surfaces of biomedical devices. Results showed to be possible to obtain surfaces with different response to the adhesion of platelets, covering different applications.

Keywords: Biocompatibility; hemocompatibility; titanium; plasma treatment; biological response.

1. Introduction

Biocompatibility and hemocompatibility are important properties to ensure success of an artificial implant biomedical since initially it will interact with blood cells. Blood is a mixture of plasma, various types of cells and platelets. Adsorption of plasma proteins is one of the first events that occur when surfaces of implants are exposed to the contact with blood [1], followed by others process such as adherence, activation and proliferation of platelets [2]. Activated platelets attract others platelets forming aggregates; thus, activations are characterized by morphological changes and by the presence of aggregates [3].

Commercially pure titanium (CP-Ti) is an important material used as artificial implant due to its characteristics of biocompatibility, corrosion resistance and high strength-to-weight ratio [4,5]. However, the surface characteristics desired to medical devices depend on the application. For example, to ensure bone-biomaterial interface integrity it is desired a good surface wettability. Unlike, a low wettability of the blood is required to avoid adhesion and activation of platelets on surfaces of stents and valves. Such opposite conditions indicate the need for surface modification after manufacture of biomaterials in order to direct them for specific applications [6]. Modification of the surface without adversely changing the characteristics of biocompatibility of the biomaterial is a challenge which motivated the present work. The purpose of this investigation was to study CP-Ti with the surface modified by nitriding plasma

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http://dx.doi.org/10.1016/j.ctmat.2016.09.003

and the influence of the addition of hydrogen in the nitriding atmosphere on that modification, with respect to the behaviour of platelets *in vitro*.

2. Experimental Methods

Discs of commercially pure titanium (CP-Ti) were subjected to a nitriding atmosphere of plasma and the effect of addition of hydrogen to the plasma was investigated. Seven different experimental groups, including polished samples used as the control, were studied.

Discharge plasma was produced in a vacuum at a fixed pressure of 1.0 mbar while the temperature and time of tests were maintained at 450°C and 1 hour, respectively. A flow of 5 sccm of Argon, and varying proportions of nitrogen and hydrogen, as shown in Table 1, was maintained during the plasma treatment.

Table 1. Gaseous flow used in the plasma treatment.

$_{H2}A_{N2}$	Flow (sccm)		
	Ar	H_2	N_2
control	-	-	-
$_{15}A_0$	5	15	0
$_{12}A_3$	5	12	3
$_9A_6$	5	9	6
$_6A_9$	5	6	9
$_3A_{12}$	5	3	12
$_0A_{15}$	5	0	15

Before and after the plasma treatment, the samples were evaluated in terms of topography and wettability. Atomic force microscopy (AFM) was used to characterize the surface topography utilizing the contact mode and images with areas of 10x10 μm^2 were obtained. These results enable to calculate the roughness parameters (Ra, Rp, Rz). Wettability was evaluated by the sessile drop tests, depositing 10 µL of distilled water, glycerol or components of the blood on the surfaces of the samples, control and treated. Surface energy and interfacial tension were determined through a contact angle using methods of calculating based on the work of Huang *et al.* [7].

The biological response was evaluated by *in vitro* test through the application of platelet-rich plasma (PRP) on the titanium modified surfaces. Dilution was made to guarantee that $8x10^7$ platelets were present in 1 mL of plasma. Analysis of their behaviour from the point of view of adherence of platelets was investigated trough images by scanning electronic microscopy (SEM).

3. Results

Figs. 1 and 2 present the roughness and contact angle of the titanium surface before (control) and after the plasma treatment with different flows of nitrogen (N_2) and hydrogen (H_2). Plasma treatment resulted in rougher surfaces and sharp peaks, confirmed by the Ra value and by the Rp/Rz higher than 0.5, respectively, as can be seen in Fig. 1. Rp/Rz ratio indicates the surface shape; values higher than 0.5 refer to sharp peaks and values lower than 0.5 indicate round peaks and, according Zhu *et al.* [8], round peaks promote the scattering of liquids.

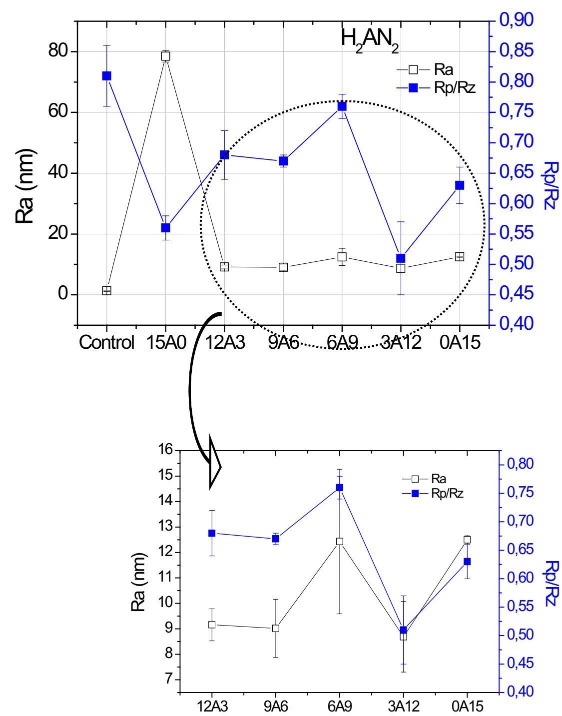

Fig. 1. Roughness parameters before and after plasma treatment obtained by AFM.

$_3A_{12}$ sample presented Rp/Rz = 0.51, thus on the threshold between round and sharp peaks. Roughness Ra of the sample treated with only H_2 ($_{15}A_0$) presented the higher values (Ra≅78 nm), probably in this case prevailed the etching caused by hydrogen, corroborated by reduction in the Rp/Rz. However, roughness increased smoothly when nitrogen was added in the plasma atmosphere, indicating the possible deposition of TiN, overlapped to etching.

All the treated surfaces showed a reduction in wettability for liquids, water and glycerol, as observed by contact angle values presented in Fig. 2, highlighting the sample treated only with hydrogen which showed the highest hydrophobic character.