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Plasma functionalization of titanium surface for repulsion of blood platelets

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ABSTRACT

Thrombosis and restenosis are the most common problems during insertion of biocompatible implants like titanium stents into human blood, due to aggregation of platelets on their surfaces. Because of this reason, we studied the response of blood platelets to a plasma treated titanium surface. The aim was to design a functionalized surface which would repel blood platelets or prevent their adhesion. Therefore, we functionalized surfaces with low-temperature inductively coupled oxygen plasma treatment, which in the first stage cleaned the surface of titanium, and in the second promoted incorporation of oxygen functional groups as well as the growth of a titanium dioxide film. In this paper we show that oxygen atoms or oxygen containing groups play an important role in the repulsion of platelets and their deactivation. At the same time, increased surface temperature of samples either through sequential thermal deactivation in oven at 150 °C or heating the surface with ion bombardment during the treatment, lowers the oxygen content and the surface repulsion for platelets.

1. Introduction

Titanium is an important material for biocompatible artificial implants e.g. coronary stents in human blood, which is comparable in characteristics to polymer materials that have been recently increasing due to low cost and flexibility [1–3]. The advantage in using titanium for stents instead of poly(ethylene terephthalate) (PET) or poly(tetrafluoroethylene) (PTFE) polymer materials is increased durability and wear resistance to circulating and eroding human blood [4]. However, when vein diameters are smaller than 6 mm, thrombosis and restenosis can occur after stent implantation [1,5,6]. The predominant reason for this is the adhesion of blood platelets to the stent which become activated. Attached and activated platelets attract other platelets from the circulating blood, leads to platelet aggregation on the stent. This can eventually lead to agglomeration, which leads to health problems, e.g. clogged veins. The aim, in this paper, is to prepare artificial implant surfaces, (including stents) in such a way that they will mimic the original biological components (e.g. veins) [1,4]. In this case, the best stent would be one that is coated with endothelial cells (the building units of veins) which naturally prevent adhesion of blood platelets. The platelets, however, can adhere to the surface during the growth process of endothelial cells from human blood. Due to this limitation, we are investigating new ways to prevent adhesion and activation of platelets on the implant surface, which in our case is a titanium stent.

One of the best ways how to prepare titanium stent surfaces is with an oxygen plasma. A typical low-temperature oxygen plasma can be used in many ways during processing of the stent surface, for example; it can clean the surface from organic impurities, sterilize the surface and possibly prevent adhesion of platelets. It has already been proven that plasma surface cleaning [7–9] and sterilization processes [10–12] are effective in oxygen plasmas, generated either with microwave, radiofrequency, DC discharges or similar sources [13–16]. However despite this, the link between plasma functionalized titanium surfaces and the behaviour of blood platelets has been explored only to a very limited extent. It has been found that ion implantation of oxygen can improve the repulsion of platelets [17,18]. Moreover it was reported that the layer of titanium dioxide prepared by wet chemical treatment in H_2O_2 increased the probability for the adhesion of platelets. Extensive heating of the same samples was shown to reduce the probability for adhesion [19], however, when the TiO_2 was prepared by thermal heating only, the probability for adhesion of platelets reduced with oxide layer thickness. The adhesion of platelets was reported to be influenced also by composition of oxide layer [20].

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Motivated by this observation, we explored the repulsion and adhesion of platelets to a modelled titanium surface after different plasma treatment procedures. Moreover, we tried to link the surface properties of plasma treated samples with platelet activity near their surfaces.

2. Experimental

The experiments were performed using an industrial grade titanium (96 at.% Ti and 4 at.% impurities) sheet with a thickness of 0.25 mm. The sheet was cut into 0.5×2 cm^2 sized pieces, which were washed ultrasonically for 2 min in demineralized water and air dried. The samples were then exposed to an oxygen plasma in the discharge chamber shown in Fig. 1. The system was pumped down using a two-stage oil rotary pump with a pumping speed of $16.7 \cdot 10^{-3}$ m^3/s. The discharge chamber was a Pyrex cylinder with a length of 270 mm and an inner diameter of 320 mm. The plasma was created using an inductively coupled RF generator, operating at a frequency of 27.12 MHz and a maximum output power of approximately 1.5 kW. The plasma parameters were measured with a double Langmuir probe and a catalytic probe. Commercially available oxygen was introduced into the discharge chamber. The pressure was measured by an absolute vacuum gauge and adjusted during continuous pumping by a precise leak valve. In our experiments, the pressure was fixed at 100 Pa, with plasma discharge parameters, the ion and neutral atom densities (close to the holder) of the plasma discharge were $7 \cdot 10^{15}$ m^{-3} and $1 \cdot 10^{22}$ m^{-3}, respectively. The samples were placed into the discharge chamber on a stainless steel holder with aluminum hook which was also connected to a RF coil for biasing as shown in Fig. 1. The samples were treated in the oxygen plasma for varied treatment times. When a bias was applied to a holder, the titanium samples could be heated up to 450–550 °C. Thermal treatment of samples was performed after the plasma treatment in the dry heat oven at 150 °C for 1 h, in order to study influence of temperature on deactivation of the surface. These samples were marked as plasma pre-treated samples.

Prior to and after the plasma treatment, the samples were analysed though wettability contact angle (WCA) measurements and X-ray photoelectron spectroscopy (XPS). Wettability was investigated using See System Advex Instruments immediately after plasma treatment by measuring the water contact angle with a demineralised water droplet of volume 1 μl. Each determination was obtained by averaging results of 7 measurements. The relative humidity (45%) and the room temperature (25 °C) were monitored continuously and were found not to vary significantly during the contact angle measurements. The measurement error of wettability angle was 1°.

The surface of the sample was analyzed using an XPS instrument TFA XPS Physical Electronics. The base pressure in the XPS analysis chamber was approximately $6 \cdot 10^{-8}$ Pa. The samples were excited with X-rays over a 400 μm spot area with monochromatic Al $K_{\alpha\ 1,\ 2}$ radiation at 1486.6 eV. The photoelectrons were detected with a hemispherical analyzer positioned at an angle of 45° with respect to the normal to the sample surface. The energy resolution was about 0.5 eV. Survey-scan spectra were made at a pass energy of 187.85 eV and a 0.4 eV energy step, while for C 1s, O 1s and Ti 2p individual high-resolution spectra were taken at a pass energy of 29.35 eV and a 0.125 eV energy step. The XPS spectra were measured on both the pristine and oxygen plasma treated samples. The spectra were fitted using MultiPak software from Physical Electronics. A Shirley-type background subtraction was used.

The plasma treated surfaces at room temperature were then tested for repulsion of blood platelets. The blood from a healthy human volunteer was collected into a vacutainer containing sodium citrate as an anticoagulant. Six samples were prepared for single plasma treatment and an untreated control, which was used as a control, were incubated in 1 ml of human blood in a 24-well cell culture plate at ambient conditions at a shearing rate of 300 rpm. After 1 h of incubation, the samples were taken out and dip-rinsed several times with 1 ml PBS (phosphate buffer saline) in order to remove platelets which were not attached to the surface, and fixed with 2,5 (v/v) glutaraldehyde for 30 min. Subsequently, the samples were washed with distilled water and dried. Samples were then examined using a confocal light microscope (Axio CSM 700). The platelets were counted on 5 samples with at least 5 counted areas of size 234 μm × 188 μm per treated sample. Parallel to counting, we also performed control with in vitro Sulforhodamine B based toxicology assay. This is a colorimetric assay originally developed for the cytotoxicity screening of the cancer drugs based on the incorporation of sulforhodamine B on intracellular proteins. The quantity of incorporated colour is proportional to a total cell mass, and is measured with absorbance at 564 nm with UV/VIS spectrophotometer.

3. Results and discussion

Surface functionalization or modification in a plasma treatment can normally be directly observed by simple wettability contact angle measurements. Due to this, we tested titanium samples for surface wettability after different treatment times in an oxygen plasma. The samples were either treated only with an inductively coupled plasma or they were additionally biased in order to increase the effective ion flux to the surface, which could rapidly heat the surface to temperatures around 450 °C. Since the increased temperature after the plasma treatment is expected to reduce the surface functionalization and deactivate the surface, the samples were treated in a dry heat oven at 150 °C for 1 h. From Fig. 2, we can clearly see the activation

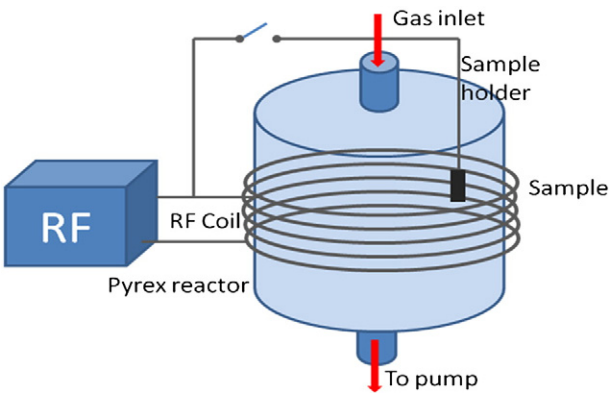

Fig. 1. Schematic of plasma system with different configurations.

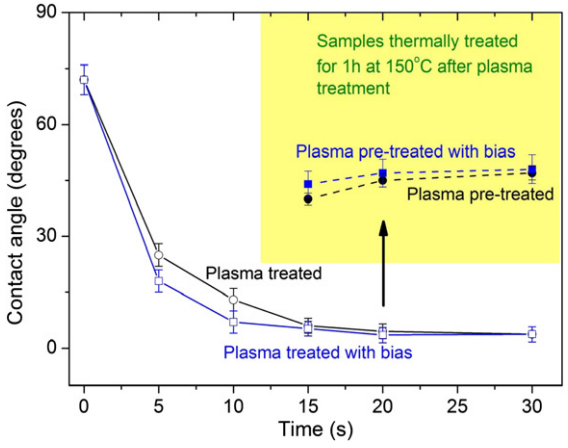

Fig. 2. Wettability contact angle (WCA) measurements of demineralized 1 μm water drop for different plasma treatment times, where samples were subjected to O_2 plasma with floating (-○-) or biasing (-□-) sample holder. Both types of plasma treated samples were additionally subjected to heating in the dry heat oven for 1 h at 150 °C (-●- floating, --■-- biased samples).