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Surface functionalized titanium thin films: Zeta-potential, protein adsorption and cell proliferation

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Abstract

The relationship between electric charge at a material surface and protein adsorption is essential to understand the mechanism of biological integration of materials with tissues. This study investigated the influence of titanium thin films' surface chemistry and surface electric charge (zeta-potential) properties on protein adsorption and cell proliferation. Titanium thin films were surface functionalized with different functional end groups, such as –CH=CH_2, –NH_2 and –COOH groups in order to produce surfaces with a variety of electric charge properties. The chemical compositions, electric charges and wettability were investigated by using X-ray photoelectron spectroscopy (XPS), zeta-potential measurements and water contact angle measurements, respectively. XPS revealed the surface functionalization of titanium films with –CH=CH_2, –NH_2, and –COOH groups, which were converted from –CH=CH_2 groups. Ti–COOH samples showed the lowest water contact angles and zeta-potential compared to all other samples investigated in this study. NH_2-terminated titanium films displayed intermediate contact angles of $70.3 \pm 2.5^\circ$. Fibrinogen adsorption on titanium films and surface functionalized titanium films were investigated in this study. Ti–COOH samples displayed a lower protein adsorption than all other groups, such as NH_2–, –CH=CH_2-terminated titanium thin films. A tendency that the lower zeta-potential of the samples, the lower the protein adsorption at their surfaces was observed. In vitro cell proliferation tests were also performed on the different surface functionalized titanium films. NH_2-terminated titanium films displayed good cell proliferation and cell viability tendency. However, a lower cell proliferation on COOH-terminated titanium films was observed compared with NH_2-terminated titanium films. This effect was attributed to the difference in protein adsorption of these samples.

Keywords: Titanium film; Surface functionalization; Zeta-potential; XPS; Fibrinogen; Protein adsorption; In vitro study; Biointerface; Nano structure

1. Introduction

Interactions between medical implants and their surrounding tissues are affected by the physicochemical surface properties of the implants. Surface properties of implants, such as the surface chemistry, charge and the topography, influence such interactions and biological performance and response of materials [1]. The surface chemical compositions of substrates have a strong effect on the protein adsorption process and are well documented [2–4]. For example proteins tend to adsorb preferentially on hydrophobic surfaces compared to hydrophilic surfaces [2].

Titanium has superior bulk and surface properties to other metal biomedical implants, such as excellent resistance to corrosion and good biocompatibility. It has been extensively used in dental, orthopaedic and cardiovascular fields in biomedical devices, such as dental implants or hip-joint replacement devices [5] and heart-valves [6]. It is one of the most successful biomaterials.

In our previous study [7], reproducible flat thin titanium films were produced and well characterized. The main advantage of such titanium films is that surface roughness (nanometre scale) and grain size are well tuneable without affecting the surface chemical compositions significantly. Subsequently, we investigated the influence of the different nanometre scale topographies of these titanium films on protein adsorption and

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cell proliferation [8]. It was shown that differences of surface roughness on a nanometre scale do not have a detectable effect on protein adsorption and cell growth. Titanium thin films can be surface modified to improve their surface biological performance [9,10]. In another study [11], we reported a layer-by-layer technique using alternatively chitosan and gelatin layers to functionalize titanium films. It was shown that such surface modification greatly improved the cell proliferation on titanium films.

Self-assembly offers a popular way of materials surface functionalization. Therefore, self-assembly monolayer (SAM) techniques have been employed to titanium and related material developments in recent years. A previous study [12] reported octadecyltrichlorosilane (OTS) self-assembled monolayer on ultra-thin TiO_2 layer as gate insulator used in microelectronics. In these biomaterials, calcium phosphate nucleation and hydroxyapatite growth on titanium that were functionalized with self-assembled silane monolayer were investigated previously [13,14]. These studies showed that various functional end groups had different effects on calcium phosphate nucleation and hydroxyapatite growth behaviours. Huang et al. [15] investigated poly (L-lysine)-g-poly (ethylene glycol) layers (self-assembly like structure) on metal oxide surfaces including titanium and their effects on protein adsorption. So far, however, no self-assembled monolayers with various functional end groups on titanium thin films and their effects on biological reactions have been studied to the knowledge of the authors. Despite the thorough chemical and physical characterization of the thin titanium films, the electric charge properties of those films have not been characterized so far.

The electric charge of a material surface is considered to be one of the main physical factors involved in the biological evolution of the tissue around an implant. This charge depends on several factors, such as the chemical composition of the material surface in contact with the tissues, the inflammatory situation, the composition of the surrounding body fluid and the environmental pH value [16]. Investigation of the relationship between surface chemistry, electric charge on a surface and protein adsorption is essential for understanding the mechanism of biological integrations with tissues. Consequently, it is beneficial to the improvement of the quality of prostheses and biomedical devices.

A biomaterial's zeta-potential indicates its electric surface properties. The zeta-potential is measured by electrophoresis or alternatively by streaming potential methods [17]. The result of a zeta-potential measurement is the potential of a material in an ionic solution at the boundary between the so-called Stern layer (Fig. 1) and the diffuse layer. It is based on the charge displacement in the electric double layer caused by an external force shifting the liquid phase tangentially against the solid [18]. Charge carriers temporarily bound in double layer will be removed by the external flow with pressure, and the potential can be measured between two electrodes. The Stern layer is the layer of anions that attach to a charged surface. These ions are temporarily bound and screen the surface charge. Larger positive values of the zeta-potential at a fixed pH indicate a positive charge of the surface which would attract negatively charged entities such as anions or charged proteins while lower values

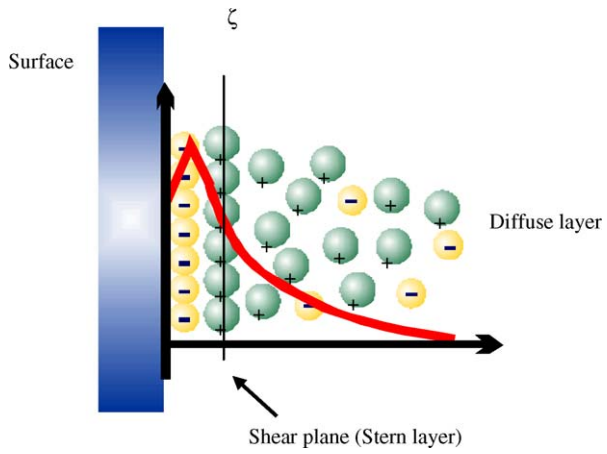

Fig. 1. Schematic diagram of the electric double layer in zeta-potential measurement. The Stern layer is the layer of counter ions (layer of bright grey (yellow) ions) that attach to a charged surface. Ion concentrations near the surface decrease further from the surface, thus forming the diffuse layer. The zeta-potential is generated when a liquid is forced to flow directly through a small gap formed by two sample surfaces, thus charge carrier bound in double layer will be removed. This potential (ζ) is defined directly at the interface between the Stern layer and diffusive layer (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article).

of the zeta-potential at a fixed pH reflect a negative charge of a material surface that tends to attract positively charged particles.

In a previous study [17], Roessler et al. studied the zeta-potentials of passive and anodic titanium oxide layers on Ti6Al4V but without protein adsorption. An isoelectric point (IEP) of about 4.4 was observed in this previous study. Krajewski et al. [16,18] confirmed that surface charge properties have an influence on protein adsorption. They investigated the correlation of ceramic and glasses' zeta-potentials with albumin adhesion behaviour. It was found that the lower the absolute value of zeta-potential of the surface, the lower the driving force that brings albumin molecules to adhere to the surface.

Fibrinogen adsorption and cell growth on titanium is well documented. Fibrinogen is one of the most important proteins in the human body. It takes part in blood coagulation, facilitates adhesion and aggregation of platelets, and it is important in the process of thrombosis [19]. Nevertheless, the relationship between titanium surface functionalities and zeta-potential has not been reported to the knowledge of the authors. Therefore, it is interesting and important to investigate their relationship in the present study. Thus, fibrinogen was introduced in this study because it is one of the most relevant proteins that are adsorbed on many biomaterials surfaces.

The aim of the present study was to investigate the relationship between surface zeta-potential (surface charge property), protein adsorption and cell proliferation on native and SAM functionalized titanium thin films. Titanium films were surface functionalized with different functional end groups such as $-CH{=}CH_2$, $-NH_2$ and $-COOH$ group. Then, the relationship between their wetting properties, zeta-potentials and fibrinogen adsorption were investigated in this study. Finally, an in vitro cell proliferation study was performed on native titanium films and surface functionalized titanium films.