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Polysaccharide-protein surface modification of titanium via a layer-by-layer technique: Characterization and cell behaviour aspects

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Abstract

To improve the surface biocompatibility of titanium films, a layer-by-layer (LBL) self-assembly technique, based on the polyelectrolyte-mediated electrostatic adsorption of chitosan (Chi) and gelatin (Gel), was used leading to the formation of multilayers on the titanium thin film surfaces. The film growth was initialized by deposition of one layer of positively charged poly(ethylene imine) (PEI). Then the thin film was formed by the alternate deposition of negatively charged Gel and positively charged Chi utilizing electrostatic interactions. The LBL film growth was monitored by several techniques. The chemical composition, surface topography as well as wettability were investigated by using X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), confocal laser scanning microscopy (CLSM) and water contact angle measurement, respectively. Quantitative XPS analysis showed the alternative change of C/N ratio after four sequential cycles coating of Ti/PEI/Gel/Chi/Gel, which indicated the discrete layer structure of coatings. Uncoated titanium (control sample) displayed a smooth surface morphology (root mean square (RMS) roughness was around 2.5 nm). A full coverage of coating with Gel/Chi layers was achieved on the titanium surface only after the deposition layers of PEI/$(Gel/Chi)_2$. The PEI/Gel/$(Chi/Gel)_3$ layer displayed a rough surface morphology with a tree-like structure (RMS roughness is around 82 nm). These results showed that titanium films could be modified with Chi/Gel which may affect the biocompatibility of the modified titanium films. To confirm this hypothesis, cell proliferation and cell viability of osteoblasts on LBL-modified titanium films as well as control samples were investigated in vitro. The proliferation of osteoblasts on modified titanium films was found to be greater than that on control ($p<0.05$) after 1 and 7 days culture, respectively. Cell viability measurement showed that the Chi/Gel-modified films have higher cell viability ($p<0.05$) than the control. These data suggest that Chi/Gel were successfully employed to surface engineer titanium via LBL technique, and enhanced its cell biocompatibility. The approach presented here may be exploited for fabrication of titanium-based implant surfaces.

Keywords: Titanium; Layer-by-layer composite; Chitosan; Gelatin; In vitro assay; Surface engineering; AFM; XPS; Contact angle; Laser scanning confocal microscopy; Interfaces; Surfaces

1. Introduction

Despite major developments in prosthetic technologies, implants often fail because of an inadequate direct bone-to-implant interface (osseointegration) [1]. Successful anchorage of the implants generally occurs via an intermediate layer (up to 10 μm in thickness) of fibrous connective tissue. Previous studies [2,3] revealed that such fibrous tissue plays an important role in implant fixation and the long-term stability of an implant. Therefore, maintaining mature bone/device interfaces is critical to the success of skeletal prostheses.

Commercial pure titanium (cpTi) and its alloys have been extensively used in dental [4] and orthopaedic [5] fields for manufacturing medical devices, such as dental implants or hip-joint replacement devices as well as

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for heart-valves. Titanium has excellent resistance to corrosion and superior biological performance because of the passivating titanium oxide layer [5]. However, even if a titanium implant osseo-integrates, it is only passively integrated with bone and, it cannot directly bind to bone [1].

The benefits of enhanced bone growth and improved initial stability of bone-contacting implants are clear. Diverse strategies have been implemented in pursuing this aim. One approach, biomimetic surface engineering, utilizes the specific molecular recognition between cell membrane and extracellular matrix to control implant–tissue interactions. The main strategy of biomimetic surface engineering is to immobilize biologically active peptides (normally well-known RGD containing peptides) on materials surfaces [6]. Another approach is surface modification by coating with hydroxyapatite, a common method to enhance the biocompatibility of orthopaedic and dental titanium-based materials [7].

We present here another approach, a layer-by-layer (LBL) self-assembly, to surface engineer titanium films. This technique is based on the consecutive adsorption of polyanions and polycations via electrostatic interactions. The electrostatic attraction between positively and negatively charged molecules seems to be a good choice as a driving force for multilayer build up [8]. Compared with the classic chemical immobilization method, the LBL technique has the least demand for chemical bonds. The multilayers built by the LBL method afford a more stable coating than that prepared by physical adsorption because of the electrostatic attractions between layer to layer and layer to substrate. One important feature of this method is the adsorption at every step of polyanion/polycation assembly, which results in recharging of the outermost layer during the film fabrication process. The overcompensating adsorption, more than equal charge, allows for charge reversal on the surface, which has two important consequences [8]: first, repulsion of equally charged molecules and thus self-regulation of the adsorption and restriction to a single layer; and second, the ability of an oppositely charged molecule to be adsorbed in a second step on top of the first one. The LBL self-assembly of polyanions and polycations into multilayered coatings has emerged as a versatile, inexpensive yet efficient technique to "build" biologically active surfaces [9,10] in recent years.

In this study, LBL self-assembly of chitosan (Chi) and gelatin (Gel) was employed to fabricate bioactive coatings on titanium films. Chi was extensively studied for its applications in tissue engineering [11,12] and drug delivery systems [13,14] in recent years. Chi has been recently used to surface modify titanium via organic silane linkage [15]. It was also used as composite coating with calcium phosphate/hydroxyapatite [16,17] on titanium substrates because of its good biocompatibility.

On the other hand, Zhu et al. [18] employed Gel to surface modify poly(D, L-lactic acid), which confirmed Gel to be having beneficial effects on improving biocompatible properties. Although the LBL technique has been applied on titanium oxide [19,20] previously, studies mainly focused on the production of composite with titanium oxide. To the best of our knowledge, this is the first study which employs LBL self-assembly to construct Chi/Gel layers on titanium films in order to improve their biocompatibility. Only very recently, Schultz et al. [21] have reported the LBL coating of titanium prosthesis with poly(L-lysine) (PLL)/poly(L-glutamic acid) (PGA) pair. A synthetic analogue of MSH peptide was chemically linked to PGA molecules on the coated multilayers to improve the anti-inflammatory property of the prosthesis.

In the present study, Chi and Gel were chosen as polycation and polyanion for a LBL self-assembly system, respectively. The rationale to select these two components is that Chi is the analogue of glycosaminoglycans (GAGs) and hyaluronic acid, and Gel is directly derived from collagen. It thus shares some characteristics with various GAGs and hyaluronic acid present in articular cartilage [22]. Since GAGs properties include many specific interactions with growth factors, receptors and adhesion proteins, it is likely that the analogous structure in Chi may also have related bioactivities. That is the interest in selecting Chi/Gel pair in the present study.

Either collagen coating [23] or collagen immobilization [24] on substrates displayed beneficial effects on osteoblast growth in previous studies. Gel is prepared by thermal denaturation of collagen, isolated from animal skin and bones, with very dilute acid. The biological origin of collagen-derived Gel makes this material an attractive candidate for tissue engineering [25,26] and drug delivery systems [27,28]. An alternative application for Gel, which we present here, would be to construct thin films with Chi for the coatings of titanium films in order to enhance their biocompatibility.

The objective of this study was to fabricate and characterize surface-modified titanium films with Chi/Gel via LBL technique. We hypothesized that this Chi/Gel coating would be helpful for improving osteoblast growth on titanium substrates. Therefore, the influence of such surface modification of titanium on osteoblast growth behaviour was investigated in vitro as well.

2. Materials and methods

2.1. Materials

Poly(ethylene imine) (PEI) ($M_w = 75,000$) was purchased from Aldrich (Munich, Germany). Gelatin (Gel, molecular weight 20,000 Da) and chitosan (Chi, medium