Acta Biomaterialia 9 (2013) 4926–4934

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

The effect of polyelectrolyte multilayer coated titanium alloy surfaces on implant anchorage in rats

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ARTICLE INFO

Article history:
Received 18 April 2012
Received in revised form 11 July 2012
Accepted 8 August 2012
Available online 16 August 2012

Keywords:
Titanium alloy
Polyelectrolyte multilayer
Layer-by-layer
Chitosan
Gelatine

ABSTRACT

Advances have been achieved in the design and biomechanical performance of orthopedic implants in the last decades. These include anatomically shaped and angle-stable implants for fracture fixation or improved biomaterials (e.g. ultra-high-molecular-weight polyethylene) in total joint arthroplasty. Future modifications need to address the biological function of implant surfaces. Functionalized surfaces can promote or reduce osseointegration, avoid implant-related infections or reduce osteoporotic bone loss. To this end, polyelectrolyte multilayer structures have been developed as functional coatings and intensively tested in vitro previously. Nevertheless, only a few studies address the effect of polyelectrolyte multilayer coatings of biomaterials in vivo. The aim of the present work is to evaluate the effect of polyelectrolyte coatings of titanium alloy implants on implant anchorage in an animal model. We test the hypotheses that (1) polyelectrolyte multilayers have an effect on osseointegration in vivo; (2) multilayers of chitosan/hyaluronic acid decrease osteoblast proliferation compared to native titanium alloy, and hence reduce osseointegration; (3) multilayers of chitosan/gelatine increase osteoblast proliferation compared to native titanium alloy, hence enhance osseointegration. Polyelectrolyte multilayers on titanium alloy implants were fabricated by a layer-by-layer self-assembly process. Titanium alloy (Ti) implants were alternately dipped into gelatine (Gel), hyaluronic acid (HA) and chitosan (Chi) solutions, thus assembling a Chi/Gel and a Chi/HA coating with a terminating layer of Gel or HA, respectively. A rat tibial model with bilateral placement of titanium alloy implants was employed to analyze the bones' response to polyelectrolyte surfaces in vivo. 48 rats were randomly assigned to three groups of implants: (1) native titanium alloy (control), (2) Chi/Gel and (3) Chi/HA coating. Mechanical fixation, peri-implant bone area and bone contact were evaluated by pull-out tests and histology at 3 and 8 weeks. Shear strength at 8 weeks was statistically significantly increased ($p < 0.05$) in both Chi/Gel and Chi/HA groups compared to the titanium alloy control. No statistically significant difference ($p > 0.05$) in bone contact or bone area was found between all groups. No decrease of osseointegration of Chi/HA-coated implants compared to non-coated implants was found. The results of polyelectrolyte coatings in a rat model showed that the Chi/Gel and Chi/HA coatings have a positive effect on mechanical implant anchorage in normal bone.

1. Introduction

Advances have been achieved in the design and biomechanical performance of orthopedic implants for fracture fixation and joint arthroplasty in the last decades. Concerning implant design, conventional straight plates or nails were adjusted to the shape of bones; anatomically shaped implants have been introduced, which now can be more easily attached to the bone, providing more comfort for the patient (and the surgeon) [1]. The biomechanical stability of osteosynthesis was significantly improved by introducing angle-stable locking, the fixed connection between screws and plates or nails [2–4].

Future modifications of implants will address the biological function of their surfaces. By surface modifications, the anchorage of implants in bone (osseointegration) can be promoted or reduced. There are many examples in orthopedic implant surgery, where the regulation of osseointegration is desirable. One is the early loosening of hip and knee prostheses, a serious complication

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http://dx.doi.org/10.1016/j.actbio.2012.08.013

of total joint arthroplasty [5]. If osseointegration of implants is incomplete, micromotions can occur at the bone–implant interface and instability together with activation of osteoclasts leads to further implant loosening and finally implant failure [6]. Another example is the use of functionalized implants in osteoporotic fractures [7].

On the contrary, increased osseointegration or overgrowth of bone/heterotopic bone formation is not desired in implants which are intended to be removed after fracture healing. This situation exists in young patients with fracture fixation devices on prominent anatomical sites, e.g. after fracture fixation of clavicula, olecranon, distal radius (dorsal), supracondylar femur, proximal tibia or ankle fractures.

Therefore, the question arises if osseointegration of titanium alloy implants can be regulated.

Osseointegration properties depend strongly on the initial cell–implant interaction at the implant interface; in particular, osteoblast adhesion, spreading and proliferation. The most important factors influencing cell–biomaterial interaction are surface topography [8–11] and surface energy and chemistry [12,13]. Thus, cell–biomaterial interaction in titanium alloy implants can be, for example, controlled by the adjustment of surface roughness and topography, e.g. accomplished by polishing or sandblasting.

Recently, the functionalization of titanium and titanium alloy with bioactive molecules has been applied for controlling the cell adhesion and proliferation at the cell–implant interface [14,15]. Titanium alloy surfaces can be coated by alternate immersion in anionic or cationic polyelectrolyte solution by a layer-by-layer (LBL) process [14,16–20]. In the study of Chua et al., it was shown that the adhesion and proliferation of osteoblasts can be improved by using polyelectrolyte multilayers of chitosan and hyaluronic acid coupled with surface-immobilized cell-adhesive arginine–glycine–aspartic acid (RGD) peptide [18]. As an alternative to polysccharides, glycosamino glycans and sulfated glycosamino glycans were used to construct polyelectrolyte multilayers for applications in bone contact in a different study [21], where the adhesion and proliferation of osteoblasts depend on the stiffness of such multilayers.

In a previous study, we used the natural polyelectrolytes chitosan (Chi), hyaluronic acid (HA) and gelatine (Gel) for the fabrication of polyelectrolyte multilayers on the titanium surface [22]. In this previous in vitro study on human osteoblasts, we have shown that cell proliferation on titanium functionalized with a Chi/Gel multilayer was significantly increased compared to pure titanium. In contrast, the Chi/HA multilayer reduced osteoblast proliferation significantly; after 4 days osteoblasts were almost completely detached from the substrate surface.

To our best knowledge, the effect of polyelectrolyte multilayers on osseointegration in vivo has not been reported before. The aim of the present study was to determine if the in vitro results of polyelectrolyte multilayer coated titanium can be confirmed in an in vivo model. Three hypotheses were tested based the results of in vitro osteoblast proliferation tests [22]: (1) polyelectrolyte multilayers have an effect on osseointegration in vivo; (2) Chi/HA multilayers decrease osteoblast proliferation compared to native titanium alloy, hence reduce osseointegration; and (3) Chi/Gel multilayers increase osteoblast proliferation compared to native titanium alloy, hence enhance osseointegration.

A modified rat tibial model, described by Gao et al. [23], with bilateral placement of titanium alloy implants, was used to evaluate enhancement or reduction of osseointegration in vivo. Implants with three different surface modifications were analyzed in mechanical pull-out tests and histomorphometry: (1) titanium alloy (control) (Ti), (2) Chi/Gel coating and (3) Chi/HA coating. We demonstrate for the first time that LBL coatings consisting of polysaccharides and gelatine improve implant fixation in vivo compared to native titanium alloy. We found no decrease of osseointegration of Chi/HA-coated implants compared to non-coated implants.

2. Materials and methods

2.1. Preparation of implants

2.1.1. Implants

Implants made of ground and ceramic-blasted TiAl6V4 provided by Königsee Implantate GmbH (Aschau, Germany) were used as a control. This titanium alloy, ground and ceramic-blasted, was the basic material for all other implants. A representative scanning electron microscopy (SEM) image of the implant surface with its overall topography is shown in Fig. 1a. The implants had a cone point on one end and measured 0.8 mm in diameter and 10 mm in length.

2.1.2. Materials for multilayers

Poly(ethylene imine) (PEI) 50 wt.% water solution, hyaluronic acid potassium salt from human umbilical cord (HA, high molecular weight polymer), gelatine (Gel) and chitosan (Chi, medium molecular weight) with a degree of deacetylation of 75-85% were purchased from Sigma–Aldrich GmbH, Munich, Germany. All materials were used without further purification. Solutions of 5 mg ml^{-1} PEI, 20 mg ml^{-1} Gel, 1 mg ml^{-1} and 5 mg ml^{-1} Chi and 1 mg ml^{-1} HA were used for the preparation of multilayer films. These were prepared by direct dissolution of the polyelectrolytes in 0.14 M NaCl (for PEI and HA), 0.1 M HCl/0.14 M NaCl (for Chi) or 0.1 M NaOH/0.14 M NaCl (for Gel). All solutions were filtered using Whatman® quantitative filter papers grade 40 prior to use. The pH of solutions was adjusted to 6.0 (for Chi/Gel multilayers) and 4.5 (for Chi/HA multilayers) using diluted HCl and NaOH.

2.1.3. Fabrication of multilayers

Polyelectrolyte-multilayer coatings on TiAl6V4 implants were fabricated using a layer-by-layer (LBL) technique. Details of the fabrication procedure can be found in Ref. [22]. Briefly, the implant surface was pre-treated with poly(ethylene imine) by immersion in PEI solution for 20 min and subsequent washing with deionized water for 2 min. Thus, a precursor layer with a stable positive charge was generated in order to initiate the LBL self-assembly process. Polyelectrolyte multilayers were deposited by alternate dipping of implants into Gel (or HA) and Chi solutions for 10 min, starting with Gel (or HA), and subsequent washing in deionized water for 2 min. Finally, the samples were dried with compressed air. Two multilayer coatings, namely $(Gel/Chi)_4/Gel$ terminated with Gel and $(HA/Chi)_5/HA$ terminated with HA, were obtained. These are noted hereinafter as Chi/Gel and Chi/HA, respectively. The implants were divided into three groups as summarized in Table 1.

2.1.4. Characterization of multilayers: ellipsometry

The thickness of the polyelectrolyte layers deposited onto the implant surface was measured using an EP3SE ellipsometer (Accurion, Göttingen, Germany) operated at a wavelength of 532 nm and an angle of incidence of 60°. For each sample, ten different points were measured. The thickness was calculated using EP3 software provided by the manufacturer.

2.1.5. Characterization of multilayers: X-ray photoelectron spectroscopy (XPS)

Chemical composition analysis was performed using an XPS (Quantum 2000, PHI Co., Chanhassen, MN, USA) with a focused monochromatic Al K_α source (1486.7 eV) for excitation. The elec-