Materials Science and Engineering C 63 (2016) 359–366

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Effect of cathodic polarization on coating doxycycline on titanium surfaces

Sebastian Geißler, Hanna Tiainen, Håvard J. Haugen *

Department of Biomaterials, Institute of Clinical Dentistry, University of Oslo, PO box 1109 Blindern, 0317 Oslo, Norway

ARTICLE INFO

Article history:
Received 4 December 2015
Received in revised form 29 February 2016
Accepted 1 March 2016
Available online 3 March 2016

Keywords:
Titanium
Doxycycline
Cathodic polarisation
Surface modification
Antibacterial

ABSTRACT

Cathodic polarization has been reported to enhance the ability of titanium based implant materials to interact with biomolecules by forming titanium hydride at the outermost surface layer.
Although this hydride layer has recently been suggested to allow the immobilization of the broad spectrum antibiotic doxycycline on titanium surfaces, the involvement of hydride in binding the biomolecule onto titanium remains poorly understood. To gain better understanding of the influence this immobilization process has on titanium surfaces, mirror-polished commercially pure titanium surfaces were cathodically polarized in the presence of doxycycline and the modified surfaces were thoroughly characterized using atomic force microscopy, electron microscopy, secondary ion mass spectrometry, and angle-resolved X-ray spectroscopy. We demonstrated that no hydride was created during the polarization process. Doxycycline was found to be attached to an oxide layer that was modified during the electrochemical process. A bacterial assay using bioluminescent *Staphylococcus epidermidis* Xen43 showed the ability of the coating to reduce bacterial colonization and planktonic bacterial growth.

1. Introduction

Titanium based materials are the prevalent choice for bone-anchored implants in orthopedic and dental applications. The reason for this is the well-known biocompatibility of titanium which originates from the combination of good mechanical properties, high corrosion resistance and biostability [1]. The latter two factors can be attributed to the presence of a stable native oxide layer that covers the surface making the material passive and therefore minimizing the host response [2]. However, such passive implant surfaces are susceptible to bacterial colonization [3]. The often resulting biomaterial-associated infections represent a significant risk to the establishment of host tissue integration which is crucial for the intended function of the implant [4].

A variety of surface modifications has therefore been explored in order to make the passive implant surfaces more interactive with the host tissue, and by this, influencing the bone-to-implant interface. Beside the established techniques to enhance osseointegration (e.g. grit-blasting and acid-etching, anodic oxidation, calcium phosphate coatings), biochemical methods have become a popular tool to actively influence biological processes [5,6]. The main principle of these methods is the immobilization of specific bioactive molecules such as extracellular matrix proteins, RGD-containing peptides, growth factors or antimicrobial agents onto the implant surface [5,7–12]. A covalent chemical binding of such biomolecules is mainly obtained by an activation of the surface oxide followed by the actual attachment of the molecules [8].

One method for activating the surfaces of titanium implants was proposed by Videm et al. who reported the formation of a titanium hydride (TiH_2) layer by applying cathodic polarization in organic acids [13]. This hydride layer has been shown to increase bone attachment to implants in vivo [14]. The same process has also been applied on dental abutments revealing positive effects on human gingival fibroblast proliferation [15]. Furthermore, the higher reactivity of the hydride layer has been suggested to be beneficial for attaching biomolecules onto the surface of an implant [13]. In a study by Frank et al., cathodic polarization was successfully used to bind enamel matrix derivate (EMD) onto titanium based materials in order to improve bone regeneration around implants [16]. A further approach explored the suitability of this polarization process to coat titanium-zirconium surfaces with the broad spectrum antibiotic doxycycline [17]. However, the surfaces used in this study were grit-blasted and acid-etched, resulting in very rough surfaces which already contain titanium hydride in their subsurface layer as shown by Szmukler-Moncler et al [18]. This complexity of the initial surface restricts the characterization of the modified surface properties as the effect of the modification process may be overshadowed by the original surface features. Furthermore, the complex surface morphology and high roughness limits the use of many surface specific characterization techniques that can only be applied on surfaces with low to moderate roughness.

The aim of the present study was to investigate the effect of the polarization process previously reported by Walter et al. [17] on the

* Corresponding author.
E-mail address: h.j.haugen@odont.uio.no (H.J. Haugen).

http://dx.doi.org/10.1016/j.msec.2016.03.012
0928-4931/

chemical surface properties of titanium. The main objective was to examine the potential role of hydride formation in binding doxycycline onto titanium surfaces during this polarization process. Using a variety of characterization techniques on mirror-polished surfaces, we assessed the events occurring during the polarization process and their effect on the biomolecule coating.

2. Materials and methods

2.1. Sample preparation

Grade IV commercially pure titanium (Ti) was used in this study. Disc-shaped samples with a diameter of 6.2 mm and a height of 2 mm were mirror-polished and washed as described before [19]. For the doxycycline coating, the same cathodic polarization process as described by Walter et al. was used [17]. Briefly, the samples were cathodically polarized in a 2 M acetic acid/sodium acetate buffer (Sigma Aldrich, Oslo, Norway) with a pH of 3 containing 1 mg/ml doxycycline hyclate (AK Scientific Inc., Union City, CA, USA). A current density of 1 mA/cm^2 was applied for 3 h. After the process, the samples were rinsed with deionized (DI) water and dried with nitrogen gas. Unmodified samples were used as a first control group ("Control"). Samples polarized without doxycycline served as a second control group ("Polarized"). A release study was performed by immersing doxycycline coated samples in DI water for 10 h ("Doxycycline imm.").

2.2. Field emission scanning electron microscopy

Images of the surface micro- and nanostructure were attained using a field emission scanning electron microscope (FE-SEM; *S*-4800, Hitachi, Tokyo, Japan). The samples were sputter-coated with platinum (Cressington 308R, Watford, UK) prior to imaging. Acceleration voltage was set to 5 kV and the samples were imaged at a working distance between 8.6 mm and 8.8 mm.

2.3. Atomic force microscopy

Further surface characterization and visualization was conducted using an atomic force microscope (AFM; MFP-3D-SA, Asylum Research, Santa Barbara, CA, USA) in combination with OMCL-AC240TS cantilevers (Olympus Corporation, Tokyo, Japan). Scanning mode (contact or AC), scanning rate, set point and integral gain were adjusted accordingly in order to obtain a good following of the trace and retrace signal. Surface parameters were measured on 5 spots per sample (10 μm × 10 μm) and 3 samples per group ($n = 15$). Obtained datasets were analyzed using the open source software Gwyddion. Height and amplitude signal graphs were overlaid for an optimal visualization of the surfaces. Furthermore, an area measuring 2.5 μm × 2.5 μm was scanned for obtaining three-dimensional (3D) images of the surfaces.

2.4. Transmission electron microscopy

Transmission electron microscopy (TEM) was used to visualize the surfaces in cross section. The samples were prepared by two different routes. The doxycycline coated sample was prepared by a Helios NanoLab DualBeam focused ion beam scanning electron microscope (FIB-SEM, FEI, Hillsboro, OR, USA). First, a platinum protection layer was deposited with electron beam assisted deposition to avoid Ga^+ ion beam damage of the sample surface. Ga^+ ion beam assisted deposition was then used to deposit further platinum and carbon protection layers. After this, the sample was thinned at 30 kV ion beam acceleration voltage, followed by a final thinning step at 5 kV to minimize surface damage of the TEM sample. The control sample and the polarized sample were prepared by mechanical tripod wedge polishing as described by Eberg et al. down to a thickness of 5–10 μm [20]. The final thinning was performed by FIB-SEM as described above. TEM was performed on a double C_s corrected (probe- and image-corrected) cold-FEG JEOL ARM200F (JEOL, Tokyo, Japan) operated at 200 kV.

2.5. Fluorescence microscopy

A confocal laser scanning microscope (Leica TCS SP8, Leica Microsystems, Wetzlar, Germany) equipped with a 10× Leica HC PL APO objective was used for imaging the sample surfaces. Fluorescence images were obtained using an excitation wavelength of 405 nm while the signal was detected in the range between 440 nm and 560 nm.

2.6. X-ray photoelectron spectroscopy

The elemental composition on the sample surfaces was analyzed by X-ray photoelectron spectroscopy (XPS; Axis Ultra^{DLD} XP spectrometer, Kratos Analytical, Manchester, UK) using monochromatic Al Kα X-rays ($h\nu = 1486.69$ eV). The measurement area was 300 μm × 700 μm. Survey spectra were recorded between 1100 eV and 0 eV binding energy. Detail spectra were recorded in the energy regions of Ti2p, O1s and C1s.

Angle-resolved XPS was performed on a Thetaprobe XP spectrometer (Thermo Scientific, Waltham, MA, USA) using monochromatic Al Kα radiation ($h\nu = 1486.69$ eV). The spectra were acquired in parallel angle-resolved mode over an analysis area of approximately 400 μm in diameter.

Spectra analysis was conducted using CasaXPS (Casa Software Ltd, Teignmouth, UK).

2.7. Secondary ion mass spectrometry

The depth profiles of the isotopes ^1H and ^{12}C were measured by secondary ion mass spectrometry (SIMS; IMS 7f, CAMECA, Gennevilliers, France). The samples were bombarded by a 15 keV primary ion beam (Cs^+) and emitted secondary ions were collected from the central part (67 μm × 67 μm) of a crater measuring 150 μm × 150 μm. The isotope concentration distribution was indicated by count intensity (c/s) of secondary ions as a function of sputter time.

2.8. Fourier transform infrared spectroscopy

A PerkinElmer Spectrum 400 FT-IR/FT-NIR spectrometer (PerkinElmer, Waltham, MA, USA) was used to investigate the presence of doxycycline on the sample surface. Samples were measured with a diffuse reflectance accessory in the mid infrared range between 4000 cm^{-1} and 450 cm^{-1}. Per sample, 16 scans with a resolution of 4 cm^{-1} were conducted. The background spectrum was collected from an unmodified sample.

2.9. Bacteria assay

The antibacterial properties of the modified Ti surfaces were assessed by culturing bioluminescent *Staphylococcus epidermidis* Xen43 on non-polished polarized and doxycycline coated discs. Doxycycline samples were tested both immediately after coating and after immersion in DI water for 24 h. *S. epidermidis* Xen43 were cultured in tryptic soy broth (TSB) at 37 °C in aerobic atmosphere. When optical density (OD_{600}) reached 0.4–0.5, the bacteria were diluted 1:100 in TBS and incubated overnight. Sample discs ($n = 16$) were placed in 96-well opaque microplates (OptiPlate-96, PerkinElmer, Waltham, USA) and inoculated with 150 μl of the overnight bacteria suspension diluted 1:100 in TSB ($OD_{600} = \sim 0.05$). The well plates were sealed with transparent adhesive seals (TopSeal™ A-Plus, PerkinElmer, Waltham, MA, USA) and incubated at 37 °C in a multi-detection plate reader (Synergy HT, BioTek, Winooski, VT, USA). Luminescence was measured every 15 min for 16 h, after which the sample discs were removed from the wells and prepared for SEM imaging. Bacteria on the