Acta Biomaterialia 10 (2014) 968–974

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Morphological zeta-potential variation of nanoporous anodic alumina layers and cell adherence

Birgit Joana Pedimonte [a,*], Tobias Moest [b], Thomas Luxbacher [c], Cornelius von Wilmowsky [b], Tobias Fey [a], Karl Andreas Schlegel [b], Peter Greil [a]

[a] Department of Materials Science (Glass and Ceramics), University of Erlangen-Nuernberg, Martens-str. 5, 91054 Erlangen, Germany
[b] Department of Oral and Maxillofacial Surgery, Medical Faculty, University of Erlangen-Nuernberg, Glückstr. 11, 91054 Erlangen, Germany
[c] Anton Paar GmbH, Anton Paar Str. 20, 8054 Graz, Austria

ARTICLE INFO

Article history:
Received 24 April 2013
Received in revised form 1 August 2013
Accepted 19 September 2013
Available online 29 September 2013

Keywords:
Nanoporous alumina
Zeta potential
Isoelectric point
Cell activity

ABSTRACT

Nanoscale surface modification of biomedical implant materials offers enhanced biological activity concerning protein adsorption and cell adherence. Nanoporous anodic alumina oxide (AAO) layers were prepared by electrochemical oxidation of thin Al-seed layers in 0.22 M $C_2H_2O_4$, applying anodization voltages of 20–60 V. The AAO layers are characterized by a mean pore diameter varying from 15 to 40 nm, a mean pore distance of 40–130 nm, a total porosity of ~10% and a thickness of 560 ± 40 nm. Zeta potential and isoelectric point (iep) were derived from streaming potential measurements and correlated to the topology variation of the nanoporous AAO layers. With decreasing pore diameter a shift of iep from ~7.9 (pore diameter 40 nm) to ~6.7 (pore diameter 15 nm) was observed. Plain alumina layers, however, possess an iep of ~9. Compared to the plain alumina surface an enhanced adherence and activity of hFOB cells was observed on the nanoporous AAO after 24 h culture with a maximum at a pore size of 40 nm. The topology-induced change of the electrochemical surface state may have a strong impact on protein adsorption as well as on cell adhesion, which offers a high potential for the development of bioactive AAO coatings on various biomaterial substrates.

1. Introduction

Bioceramics applied to bone replacement and regeneration should trigger minimal inflammatory responses while stimulating osteoblast adhesion, proliferation and differentiation [1]. It has long been known that substrate topography, including grooves, ridges, islands, nodes and pores, can affect cell response and osseointegration behavior of a bioceramic implant in contact with bone [2]. Interaction with nanotopographies can alter cell morphology, adhesion, motility, proliferation, endocytotic activity, protein abundance and gene regulation [3,4]. Among diverse cell types (fibroblasts, osteoclasts, endothelial, smooth muscle, epithelial) osteoblasts were observed to interact with nanotopographical features of substrate materials [5].

Nanoporous Al_2O_3 surfaces offer a high potential for a load-bearing implant in contact with bone due to excellent chemical and mechanical stability as well as biocompatibility [6,7]. The topology of nanoporous alumina can be easily controlled by changing the pore diameter, shape, periodicity and density by applying anodic oxidation of an Al-seed layer in a variety of polyprotic acids at voltages ranging from 10 to 200 V [8,9]. Anodic alumina oxide (AAO) is subtly different from conventional alumina in that it has a regular self-organized porous nanostructure with pores orthogonal to the surface and pore diameters ranging from 5 nm to 10 μm [10]. Previous work on the biomedical application of AAO mainly referred to AAO membrane structures serving as a tissue engineering scaffold for cultivating different types of human cells (hepatoma, osteoblasts, epithelium), as an immune-isolation for cell carrier devices, as a sensor array for cellular diagnostics as well as for immobilizing enzymes [11]. AAO surface coatings, however, may offer further potential for improving the interface bonding of load-bearing implants to bone [12] and for enhancing osseointegration [13]. Favorable osteoblast adhesion was observed on AAO membranes fabricated using a two-step anodization process in phosphoric acid with a pore size ranging from 30 to 80 nm [6,14]. Nanotopography with pores ranging from 20 to 200 nm was reported to affect monocyte/macrophage behavior with the 20 nm pores, resulting in a reduced pro-inflammatory response compared to the large pore surface [15]. Furthermore, nanoporous alumina surfaces were modified by physically absorbing vibronectin protein and covalently immobilizing the arginine–glycine–aspartic amino-acid sequence (RGD) peptide to enhance adhesion of bone-forming osteoblast cells [14].

* Corresponding author. Tel.: +49 9131 85 27565; fax: +49 9131 85 28311.
E-mail address: Joana.pedimonte@ww.uni-erlangen.de (B.J. Pedimonte).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.09.023

Because of the nanoporous structure and the hydrophilic nature of the alumina, the surface is highly hydrated and presents an environment more similar to the in vivo situation [1]. Depending on the dissociation behavior of the hydroxyl groups, an electrostatic double layer develops at pH values differing from the isoelectric point (iep). This surface charge may have a strong influence on specific interaction with inorganic and bioorganic constituents of the plasma and extracellular fluid, respectively. For example, in vivo and in vitro apatite nucleation is favored on a hydrolyzed surface with a negative charge able to attract Ca^{2+} from the body fluid [16,17]. Though adsorption of proteins on a material surface is a complex process, electrostatic interactions with a charged surface are among the factors that were reported to control structural stability and conformational changes of the protein structure [18,19]. A high binding affinity for adhesion proteins from the extracellular matrix (collagen, laminin, fibronectin) containing RGD for transmembrane integrin receptor activation is envisaged in order to mediate the adherence of bone-forming osteoblast cells as well as vascular-tissue-forming endothelial cells [20,21]. The amount of adsorbed protein often shows a maximum near the iep of the protein where lateral and intramolecular repulsion of adsorbed protein reaches a minimum [22]. Minimization of protein adsorption triggered by a pronounced difference of iep or electrostatic repulsion might be of particular interest in order to reduce biofilm formation and the adherence of pathogen entities such as colonizing bacteria which may induce infections and trigger inflammatory responses of the immune system [23].

In the present work the effect of surface topography variation on the surface chemical behavior of nanoporous AAO layers was explored. AAO layers with a total porosity of ~10% and pore diameters varying from 15 to 40 nm were prepared by electrochemical oxidation of an Al-seed layer in $C_2H_2O_4$ solution. Zeta potential and iep were derived from streaming potential measurements. The cell response on AAO was analyzed and correlated to the variation of surface chemistry caused by different nanopore topography.

2. Experimental procedure

2.1. AAO formation and nanotopography

AAO coating layers were formed on zirconia-toughened alumina ceramic (Biolox®delta, CeramTec, Plochingen, Germany). Due to its superior biomechanical properties and wear resistance this bioceramic is widely used for total hip and knee arthroplasties. Rectangular bars ($12 \times 9 \times 2\ mm^3$) were ground and finally polished with 3 μm diamond suspension prior to AAO coating fabrication. The specimens were coated with a high-purity Al (99.999%) film (seed layer) of 0.5 μm thickness by sputtering (Leybold L400Sp, Dresden, Germany) in argon (5×10^{-3} Pa). The Al-coated samples were in contact with silver paste to a Cu back-plate serving as a working electrode. A Pt foil served as counter-electrode. The Al-seed layer was anodized in 0.22 M $C_2H_2O_4$ using a two-electrode setup operating under constant a potential condition until all the Al was converted to AAO. Samples dedicated to current transient measurements were anodized using an O-ring cell setup with an exposed area of 20 mm^2. Current transients were recorded with a high-voltage potentiostat (Jaissle IMP 88-200 PC, Waiblingen, Germany). Different anodization voltages ranging from 20 V up to 60 V were applied at room temperature in order to vary the pore topology. Samples dedicated to streaming potential measurements were anodized using a dip-in method, as described in previous work [12]. The exposed area of the sample was kept constant at 108 mm^2. After anodization the back-sides of the samples were rinsed carefully in acetone and in distilled water and leached in 0.1 M H_3PO_4 for 2 min in an ultrasonic bath. During leaching, dissolution occurred, resulting in a moderate pore widening. All solutions were prepared from reagent-grade chemicals and distilled water.

Nanoporosity topology of AAO layers was derived from scanning electron microscopy (SEM) micrographs (FE-SEM S 4800, Hitachi, Japan). Applying image analysis software (Image J 1.43u, National Institute of Health, Bethesda, MD) mean values of the pore diameter d_p, and the interpore distance (cell size) d_c were derived, Fig. 1. Assuming hexagonal close packing arrangement of cylindrical pore channels the total porosity fraction f_p was calculated from [24]:

$$f_p = \frac{\pi}{2\sqrt{3}}\left(\frac{d_p}{d_c}\right)^2 \tag{1}$$

and the total number of pores occupying the surface area of 1 μm^2 (areal pore number density) n_p (with d_c given in nm) is expressed by:

$$n_p = \frac{2 \cdot 10^6}{\sqrt{3} d_c^2} \tag{2}$$

Compared to a pore-free alumina layer, formation of nanoporosity causes an increase in surface area ΔS compared to initial surface area S_{Al} [25]:

$$\frac{\Delta S}{S_{Al}} = \frac{S_{AAO} - S_{Al}}{S_{Al}} = \frac{2\pi d_p h_{AAO}}{\sqrt{3} d_c^2} \tag{3}$$

where h_{AAO} is the AAO layer thickness. h_{AAO} was measured on SEM micrographs of cross-sectional cuts perpendicular to the coating layer.

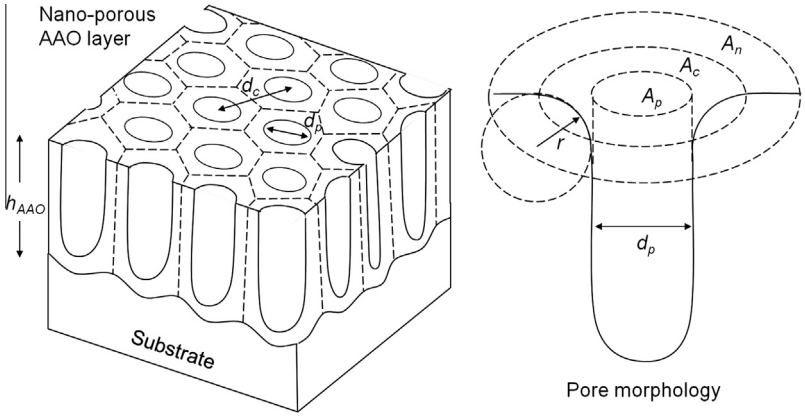

Fig. 1. Layer microstructure and single pore morphology of nanoporous AAO.