Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 4 (2008) 1576–1582
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Brief communication

Improved attachment of mesenchymal stem cells on super-hydrophobic TiO_2 nanotubes

Sebastian Bauer [a,1], Jung Park [b,1], Klaus von der Mark [b], Patrik Schmuki [a,*]

[a] Department of Materials Science, WW4-LKO, Friedrich-Alexander-University of Erlangen-Nuremberg, Germany
[b] Department of Experimental Medicine I, Nikolaus Fiebiger-Center of Molecular Medicine, Germany

Received 11 January 2008; received in revised form 31 March 2008; accepted 7 April 2008
Available online 25 April 2008

Abstract

Self-organized layers of vertically orientated TiO_2 nanotubes providing defined diameters ranging from 15 up to 100 nm were grown on titanium by anodic oxidation. These TiO_2 nanotube layers show super-hydrophilic behavior. After coating TiO_2 nanotube layers with a self-assembled monolayer (octadecylphosphonic acid) they showed a diameter-dependent wetting behavior ranging from hydrophobic ($108 \pm 2°$) up to super-hydrophobic ($167 \pm 2°$). Cell adhesion, spreading and growth of mesenchymal stem cells on the unmodified and modified nanotube layers were investigated and compared. We show that cell adhesion and proliferation are strongly affected in the super-hydrophobic range. Adsorption of extracellular matrix proteins as fibronectin, type I collagen and laminin, as well as bovine serum albumin, on the coated and uncoated surfaces showed a strong influence on wetting behavior and dependence on tube diameter.
© 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

Keywords: Titanium-dioxide nanotubes; Self assembled monolayers; Super-hydrophobic; Cell adhesion; Mesenchymal stem cells

1. Introduction

Cell adhesion on biomedical devices is a fundamental factor for the integration process of a biomaterial after implantation [1]. Understanding such cell adhesion processes is critically important for the development of biomaterials in the field of tissue engineering and regenerative medicine. Implant materials have to be optimized, especially regarding their surface characteristics such as chemistry, topography, surface energy and morphology [2,3]. Surface wettability is recognized as a critical factor for cell behavior [4]. A considerable number of studies have indicated that cells tend to attach better to hydrophilic surfaces than to hydrophobic surfaces [5–7]. Contradictory reports argue that cells adhere better on intermediate hydrophobic surfaces with contact angles of around 70° [8–10]. These conflicting results may be due to the use of different materials, different surface topographies and especially different surface chemistry applied to alter the wetting behavior. Up to now investigations on cell adhesion and proliferation on surfaces with different wetting behavior have only been reported for contact angles ranging from approximately 0 to 120°. In contrast to these previous reports, here we address the super-hydrophobic range of surfaces with contact angles higher than 120° using coated TiO_2 nanotube layers.

Recent studies using ever-improving methods have indicated that the fabrication of ordered TiO_2 nanotube layers can be achieved by anodization of titanium in an adequate electrolyte [11–13]. Those findings generated considerable scientific interest owing to the broad range of applications of TiO_2 [14]. Furthermore, ordered oxide nanotube layers can also be grown on biomedical alloys [15–19]. In a previous work we showed that different nanotube diameters in the sub-100 nm scale [20] have a dramatic influence on the vitality of mesenchymal stem cells [21]. Earlier work showed that these tube layers can be surface modified to achieve a super-hydrophobic behavior [22,23].

* Corresponding author. Tel.: +49 9131 8527575.
E-mail address: schmuki@ww.uni-erlangen.de (P. Schmuki).
[1] Both authors contributed equally to this work.

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.04.004

In the present work we report on the cell response of mesenchymal stem cells to strongly hydrophobic and super-hydrophobic surfaces obtained via organic modification of TiO_2 nanotubes with self-assembled monolayers of octadecylphosphonic acid (ODPA). Contact angles between 108 and 167° were obtained by different tube diameters in the sub-100 nm region, i.e. the degree of wettability was altered while all other physiochemical properties at the surface remained constant.

2. Materials and methods

2.1. TiO_2 nanotube formation

Titanium foils (99.6% purity, Advent Ltd.) were used as substrates for the anodic growth of TiO_2 nanotube layers. For anodization an electrochemical cell with a three-electrode configuration was used. Platinum gauze served as a counter electrode and a Haber–Luggin capillary with Ag/AgCl (1 M KCl) electrode was used as a reference electrode. Electrochemical experiments were carried out with a high-voltage potentiostat (Jaissle IMP 88-200 PC). A 1 M H_3PO_4 (Merck) solution with the addition of 0.3 wt.% HF (Merck) was used as electrolyte. Nanotube formation was achieved at potentials ranging from 1 to 20 V at room temperature. After electrochemical treatment, the samples were rinsed with deionized water. For morphological characterization of sample surfaces, a field emission scanning electron microscope (S-4800, Hitachi) was used. All samples were cleaned by autoclaving before cell plating.

2.2. Surface wettability

Before surface modification with self-assembled monolayers (SAMs) the samples were washed in acetone, ethanol and ultrapure water each for 10 min, followed by exposure to ultraviolet (UV) light using a high-power Hg lamp (UV irradiation 15 W cm^{-2}) for 30 min for cleaning purposes. Attachment of SAMs to TiO_2 nanotubes was achieved by soaking the samples for 24 h in tetrahydrofuran (Roth) containing 150 μM of ODPA ($C_{18}H_{37}PO(OH)_2$) (97%, Aldrich). The samples were then removed, subsequently washed with tetrahydrofuran and ultrapure water, and dried under nitrogen gas flow and then at 70 °C overnight. Wettability of the different surfaces was characterized by contact angle measurements under equilibrium conditions with a contact angle setup from Orbisphere Laboratories (water droplet volume ~14.1 μl).

2.3. Mesenchymal stem cell culture

Rat mesenchymal stem cells were isolated from fresh bone marrows from femurs of 4-week-old Wistar rats as described previously [24]. Cell isolation and expansion was performed as described in our previous work [21]. Selected clonal cells were further expanded in medium containing 60% Dulbecco's modified Eagle's medium with low glucose (Gibco BRL) and 40% MCDB-201 (Sigma), supplemented with 1 × insulin–transferrin–selenium (Sigma), 1 × linoleic acid–bovine serum albumin (Sigma), 10^{-9} M dexamethasone (Sigma), 10^{-4} M ascorbic acid 2-phosphate (Sigma), 100 units of penicillin, 1000 units of streptomycin (Gibco), 10 ng ml^{-1} epidermal growth factor (Sigma), 10 ng ml^{-1} platelet-derived growth factor-BB (R&D Systems), 1000 units ml^{-1} rat LIF (Chemicon) and 2% fetal calf serum (FCS; Hyclone Laboratories). The expanded cells showed multipotent potential to differentiate into multiple mesenchymal lineages, including osteoblasts, chondroblasts, adipocytes, myoblasts, myofibroblasts and endothelial cells, in differentiation experiments in vitro.

2.4. Cell adhesion and proliferation assay

GFP-labeled mesenchymal stem cells were plated on titanium surfaces with cell densities of 5000 cm^{-2}. For cell adhesion experiment 24 h after cell plating non-adherent cells were washed with phosphate-buffered saline (PBS) and adherent cells were counted at 6 different areas (1280 × 1024 pixels), each sample depicted under a fluorescent microscope (2× magnification). Additional cell adhesion and proliferation were quantified 24 h and 3 days after cell plating using cell proliferation reagent WST-1 (Roche) according to the manufacturer's instructions. For SEM observation cells were fixed with 2.5% glutaraldehyde solution (Merck, Germany) overnight at 4 °C. Samples were rinsed in PBS solution, dehydrated in a series of acetone (60, 70, 80, 90 and 100%) and critical point dried (CPD 030, Balzers). Cell count and cell spreading analysis were each evaluated by 10 individuals and values were combined to produce one mean and standard deviation for each specimen.

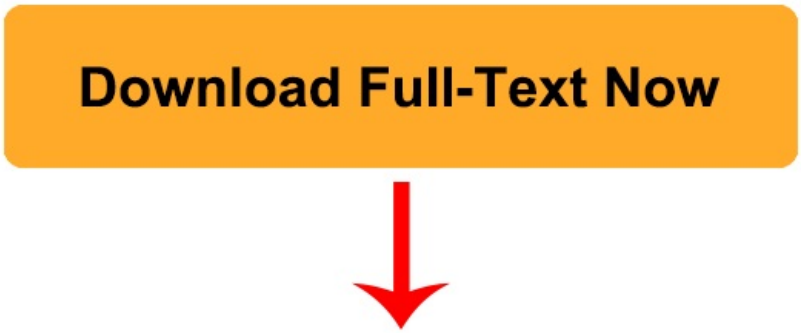
2.5. Immunocytochemistry

For immunocytochemistry, cells grown on nanotubes for 3 days after plating with a cell density of 5000 cm^{-2} were rinsed in PBS and fixed with 2% paraformaldehyde in PBS at RT for 10 min. After fixation, cells were permeabilized with 0.2% Triton X-100 in PBS for 2 min, washed with PBS and incubated with antibodies of mouse monoclonal anti-paxillin (signal transduction). Cell nuclei were stained blue with DAPI (Roth). Cell images were taken using an Axiovert 2000 ApoTome microscope with AxioCam digital camera and AxioVision software (Zeiss). Fluorescence images were employed to evaluate cell sizes using Photoshop 7.0 software (Adobe).

2.6. Protein absorption assay

To evaluate the interaction between extracellular matrix (ECM) proteins and nanotube surfaces type I collagen, laminin and fibronectin were plated with concentrations of 10 μg ml^{-1} on unmodified and organically modified sur-

ID	Title	Pages
1592	Improved attachment of mesenchymal stem cells on super-hydrophobic TiO2 nanotubes	7



http://fulltext.study/article/1592

FullText.study

Categorized Journals
Thousands of scientific journals broken down into different categories to simplify your search

Full-Text Access
The full-text version of all the articles are available for you to purchase at the lowest price

Free Downloadable Articles
In each journal some of the articles are available to download for free

Free PDF Preview
A preview of the first 2 pages of each article is available for you to download for free

http://FullText.Study