Acta Biomaterialia 8 (2012) 2639–2647

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

ECM spreading behaviour on micropatterned TiO_2 nanotube surfaces

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ARTICLE INFO

Article history:
Received 20 December 2011
Received in revised form 6 March 2012
Accepted 27 March 2012
Available online 30 March 2012

Keywords:
TiO_2 nanotubes
Mesenchymal stem cells
ECM
Micropatterning
Photolithography

ABSTRACT

By electrochemical anodization, highly ordered nanotubular TiO_2 structures were formed on titanium surfaces with diameters of 15 and 100 nm. In previous work we showed that 15 nm tubes strongly enhanced adhesion and vitality of many cell types, whereas on 100 nm diameter tubes the induction of apoptosis was observed. In the present work we produce mixed (15 nm contrasted with 100 nm) nanotube microstructures that combine highly defined micro- and nanostructures using a photolithographic approach to achieve a direct comparison of adhesion and spreading of mesenchymal stem cells on different diameter nanotubes present on a single surface. On these coupled different nanoscale surfaces mesenchymal stem cell adhesion is initially favoured on 15 nm tube areas but, with time, a gradient in cell number and shape to the "unfavourable" regions of the substrate (100 nm tubes) can be observed. This can be explained by cells on the "favourable" 15 nm regions that strongly produce and shed extracellular matrix onto the "unfavourable" locations. These findings contribute to the design of cell guiding surfaces, but also demonstrate the need for a long-range defined homogeneous order when studying cell behaviour on nanostructured surfaces.

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1. Introduction

In the field of biomedical materials research, especially considering cell/biomaterial interactions, many studies concerning micrometre-scale topographies have been conducted in the last decades [1–9]. More recently, nanoscale topographies have also been considered as promising surface cues for triggering cellular responses, see e.g. Refs. [9–12]. Our own studies with mesenchymal stem cells (MSCs) on highly ordered TiO_2 nanotube layers with different tube diameters ranging from 15 to 100 nm revealed that defined nanotopographies in the sub-100 nm range strongly influence cell functions such as adhesion, proliferation, activity and survival [13–15]. These nanotube layers can be created on Ti surfaces employing a simple but optimized electrochemical anodization treatment, enabling the formation of regular nanotopographies of adjustable dimensions with a high lateral resolution [16–20]. Experiments with MSC on ZrO_2 nanotubes and AuPd-coated TiO_2 nanotubes revealed that the geometrical effect of the nanotopography clearly dominates over surface chemistry effects [15]. The fact that other cell types, such as endothelial cells [21], osteoblasts/osteoclasts and hematopoietic stem cells [14], showed comparable responses to nanoscale geometries indicates that there seems to be a universal spacing constant of ~15 nm which leads to maximum cell adhesion, spreading and activity in adherent cell types, while nanotubes with larger diameters (100 nm) seem to inhibit cell adhesion inducing apoptosis.

However, conflicting results have also been reported, indicating superior cell adhesion and proliferation on 100 nm [22] or 80 nm [23] nanotubes as compared to nanotubes with smaller diameter (down to 30 nm), or reference "flat" surfaces. Moreover, in further work the differentiation of MSC was reported to be highest on such bigger diameters compared to smaller nanotubes and control surfaces [24]. In the present work we try to resolve this conflict [13,14,25] using defined model surfaces with both large and small diameter nanotube features in a single sample. This provides a direct comparison of cell adhesion and spreading depending on nanotopographies, allowing simultaneous observation of the cell behaviour on the two nanoscale surfaces. This eliminates experimental side-effects such as different pre-treatment conditions, varying cell seeding densities, and the use of different anodization protocols.

2. Materials and methods

2.1. Anodization and pattern formation

The formation of TiO_2 nanotube patterns was achieved using an adapted version of a three-step process reported previously, as

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http://dx.doi.org/10.1016/j.actbio.2012.03.040