Arrays of titanium dioxide nanotubes with different diameter and surface chemistry influence C2C12 skeletal myoblast adhesion and differentiation

Giada G. Genchi1,2, Ioannis Liakos3, Virgilio Mattoli1 and Tejal A. Desai2

1Istituto Italiano di Tecnologia, Center for Micro-BioRobotics @SSSA, Viale Rinaldo Piaggio 34, 56025, Pontedera, Italy
2University of California, San Francisco, Department of Bioengineering and Therapeutic Sciences, 1700 4th Street, 94158, San Francisco, California, USA
3Istituto Italiano di Tecnologia, Smart Materials, Nanophysics Department, Via Morego 30, 16163, Genoa, Italy

giada.genchi@iit.it, tejal.desai@ucsf.edu

Abstract

TiO2 nanotubes (NTs) have attracted huge interest in several fields of nanotechnology, recently finding also application in the biomedical research [1]. Here, we propose the use of TiO2 NT arrays for C2C12 skeletal myoblast culture, and the array binding with a cell-adhesive protein to improve implementation in biological environments.

Arrays with different nanotube diameters and surface chemistry were obtained by anodization (4, 20, and 40 V), annealing (400°C, 1 h), and silanization with an alkaline 20% APTES solution (4 h, 80°C). Incubations with a 5 mg/ml genipin solution (1 h, 37°C) and with an acidic 50 μg/ml laminin solution followed. Surface morphology and chemistry were then investigated with SEM and XPS. SEM demonstrated open-top nanotubes and different nanotube diameters (10, 50 and 100 nm) were achieved, and a thin conformal layer of laminin was bound to the surfaces (Figure 1a,b). XPS demonstrated anatase phase was achieved in annealed samples, and silane/protein were bound (the Ti2p peak neatly decreased, whereas the Si2p and N1s peaks appeared) in laminin coated samples.

For proliferation studies, cell adhesion and viability were investigated in terms of focal adhesion clustering, live/dead cell number and metabolism. Focal adhesion clustering was improved on coated substrates (mostly on 10 nm diameter NTs), whereas no dead cells were found on all substrates. Cell metabolism decreased on laminin coated arrays.

For differentiation studies, myoblast fusion into myotubes and myotube width were assessed, demonstrating that the 10 nm diameter nanotubes promoted the formation of wider myotubes in laminin coated arrays as an evidence of better cell differentiation (Figure 1c,d).

Our results thus support the use of TiO2 NT arrays with skeletal muscle cells. Upon suitable modification, these arrays may prove excellent smart interfaces for addressing cell response, and eventually for cell stimulation [2].

References


Figure 1: Scanning electron microscopy images of 10 nm diameter nanotube arrays, without (a) and with laminin coating (b). Fluorescence microscopy image of TRITC-phalloidin and DAPI stained C2C12 cells after 3-day differentiation on 10 nm diameter nanotube arrays without (c) and with laminin coating (d). Arrows evidence the higher width of differentiated cells on laminin coated substrates.