Materials Science and Engineering C 44 (2014) 38–43

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

# Improved cell activity on biodegradable photopolymer scaffolds using titanate nanotube coatings

S. Beke [a,*], R. Barenghi [b], B. Farkas [a], I. Romano [a], L. Kőrösi [c], S. Scaglione [b], F. Brandi [a,d]

[a] Nanophysics, Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova, Italy
[b] IEIIT, National Research Council (CNR), Via De Marini 6, 16149 Genova, Italy
[c] Department of Biotechnology, Nanophage Therapy Center, Enviroinvest Corporation, Kertváros u. 2, H-7632 Pécs, Hungary
[d] Istituto Nazionale di Ottica, CNR, Via G. Moruzzi 1, 56124-Pisa, Italy

ARTICLE INFO

*Article history:*
Received 10 March 2014
Received in revised form 30 March 2014
Accepted 1 July 2014
Available online 9 July 2014

*Keywords:*
Laser photocuring
Scaffolds
Titanate nanotubes
Tissue engineering
Cell culturing
Excimer laser

ABSTRACT

The development of bioactive materials is in the premise of tissue engineering. For several years, surface functionalization of scaffolds has been one of the most promising approaches to stimulate cellular activity and finally improve implant success. Herein, we describe the development of a bioactive composite scaffold composed of a biodegradable photopolymer scaffold and titanate nanotubes (TNTs). The biodegradable photopolymer scaffolds were fabricated by applying mask-projection excimer laser photocuring at 308 nm. TNTs were synthesized and then spin-coated on the porous scaffolds. Upon culturing fibroblast cells on scaffolds, we found that nanotubes coating affects cell viability and proliferation demonstrating that TNT coatings enhance cell growth on the scaffolds by further improving their surface topography.

© 2014 Elsevier B.V. All rights reserved.

## 1. Introduction

Tissue engineering aims to repair and replace lost or damaged tissues by inducing a specific cellular response according to the chemical–physical cues provided by the implanted materials [1]. Ideally, the scaffold design is aimed at reproducing all required signals at macro-, micro- and nanoscales to foster and direct cellular attachment, proliferation, and desired differentiation towards specific cell phenotypes. In this respect, nanoscale surface properties are fundamental to enhance cell–substrate interaction [2,3], and nanostructures offer the possibility to further functionalize the substrates through a biomimetic approach [4–9].

Nanostructures, e.g. nanoparticles and nanotubes, can also be applied as drug delivery systems and thereby be a potential matrix for various therapeutic inductions. Their volume can be filled with chemicals, drugs, and biomolecules [10]. Surface nanostructures can also be used for more efficient and precise nano-sized delivery compared to conventional approaches [11–15].

Nanotubular titania ($TiO_2$) surfaces have been recently proposed as alternative architectures to enhance the interaction between the implant and living matter or biological species. In particular, it was demonstrated that titanium oxide nanotubes enhance growth rates and bone forming ability as well as accelerate osteogenic differentiation of mesenchymal stem cells [11,15]. Moreover, it was shown that titanium oxide-based materials adopted as bone implants might affect in vivo cell adhesion, osteointegration, and finally, bone regeneration [11,16]. For these reasons, they have been widely investigated as bioactive substrates to improve the osteoconductivity of orthopedic implants, finally enhancing the apposition of bone from existing bone surfaces and stimulate new bone formation [17,18].

Besides their chemical composition, high attention has been paid to the nanotubes' diameters which may influence the functionality and the activity of osteoblasts [11,19,20]. In particular, as the nanotube diameter increases, the osteogenic biochemical activity also increases, reaching the best values on 100 nm-diameter nanotubes [11,19,20]. By varying the diameter sizes, the location and spacing of transmembrane integrins change, thus cytoskeletal tensions in the actin filaments and in the adhering cells are affected differently.

Although both the nature of cell adhesion and the degree of cytoskeletal tension affect the cell response, the precise role of nanotopography on the adhesion, morphology, and differentiation of cells has not been established yet [11].

$TiO_2$ nanotubes have the capability to change the adsorption of extracellular matrix (ECM) proteins, such as fibronectin, laminin, and bovine serum albumin and mesenchymal stem cell (MSC) attachment if subjected to a change in their wetting behavior [19]. In particular, nanotubes turn from super-hydrophilic (as prepared) to super-hydrophobic after the addition of a self-assembled

* Corresponding author.
*E-mail address:* szabolcs.beke@iit.it (S. Beke).

http://dx.doi.org/10.1016/j.msec.2014.07.008
0928-4931/© 2014 Elsevier B.V. All rights reserved.

monolayer (octadecylphosphonic acid) [11]. Differences in hydrophobicity were found to be diameter dependent, too. It is important to note that in the super-hydrophobic case the adhesion is diameter-independent, while in the case of super-hydrophilic surface the adhesion is diameter-dependent [11,16].

In this article, we report on cell culture experiments using fibroblast cells on bioactive composite scaffolds. The scaffolds are biodegradable photopolymer scaffolds functionalized with titanate nanotubes (TNTs). In a previous paper [21], we reported on the development of a rapid process to produce rigid biodegradable photopolymer scaffolds using excimer laser photocuring of a synthetic biopolymer: poly(propylene fumarate)/diethyl fumarate blend (PPF:DEF, 7:3 w/w). We also investigated high-resolution photocuring of PPF:DEF using a laser wavelength of 248 nm [22], and performed a comparative study between the two laser wavelengths at 248 nm and 308 nm [23]. We recently introduced our novel method, called mask projection excimer laser stereolithography [24], being a versatile and accurate technique to fabricate 3D scaffolds with controlled architecture. Its capability is showcased in that paper by a variety of mm-sized biodegradable scaffolds with a high spatial resolution well-suited for tissue engineering applications.

Among polymeric materials, PPF-based scaffolds display satisfactory properties in terms of biocompatibility, mechanical properties, sterilizability [25], and handling characteristics making them a promising alternative to traditional substitutes for autologous or allograft bones [26]. In [25] we presented the biodegradability of the material used for this study.

The combination of laser-produced PPF-based scaffolds and spin-coated TNT films was presented in a previous study [27] being a facile production of a novel material made of a PPF:DEF scaffold coated with additive-free TNTs. The structure and the morphological properties of the resulting hybrid scaffolds were discussed. Here we investigate the role of TNT functionalization on cellular activity at early period of cell culture in vitro.

## 2. Experimental

### 2.1. Synthesis of TNTs and polymer

The synthesis of TNTs was similar as described in [28]. 0.5 g of Degussa P25 $TiO_2$ was dispersed in 15 mL of 10 M aqueous NaOH solution and the resulting dispersion was transferred to a Teflon-lined autoclave. The autoclave was heated at 150 °C for 12 h (p ~ 4.7 bar) without stirring. After the hydrothermal reaction, the alkaline dispersion was washed with water until the pH decreased to ~11, and then 0.1 M aqueous $H_3PO_4$ solution was added to decrease the pH further to 6. The acid treatment was carried out for 12 h at room temperature and then the system was centrifuged. The obtained sediment was washed with water and then ethanol. The washing procedure with ethanol was continued via centrifugation at 12,000 rpm. The resulting supernatant (the stable TNT sol) was collected.

PPF was synthesized as reported in [29]. Briefly, a condensation reaction was conducted between fumaric acid and propylene glycol, with a molar ratio of 0.8. In a triple-neck flask with an overhead mechanical stirrer, a thermometer and a Barrette trap connected beneath the condenser, the reaction was conducted in 140 °C for 16–17 h and then in 180–190 °C for 4–5 h. During the first period of the reaction, water was collected as byproduct, then with an increasing temperature the unreacted propylene glycol and low molecular weight impurities were removed. After keeping the product at room temperature overnight, it was possible to purify it by rotary evaporation in $CH_2Cl_2$. Finally, PPF was blended with DEF in ratio 7:3 and 1 % photoinitator (Bapo) was added to the polymer resin.

The schematic of the experimental apparatus used for high-resolution photocuring experiments is shown in [21]. The light source is a XeCl excimer laser at 308 nm with laser pulse duration of 20 ns and repetition rate of 1–100 Hz (CompexPro 110). The mask image is projected on the target using a demagnification of 4.

### 2.2. TNT coatings on the scaffolds

TNT-coated scaffolds were prepared by a spin-coating method. 50 μL of 1% TNT ethanolic sol was dropped onto the scaffolds immobilized on a 1 cm-diameter quartz substrate rotated at 3000 rpm. The as-prepared layer was subsequently dried for 30 s in the spin-coater. In order to prepare multilayer films, the above deposition step was repeated five times.

### 2.3. Cell seeding and culture

Samples used for the preliminary cell studies were divided in two groups: polypropylene fumarate (PPF) scaffolds either coated or not coated with TNT. Glass slides, either coated or uncoated, were used as control. All scaffolds were irradiated overnight with a UV lamp to obtain high sterility before culturing.

Mouse fibroblast cell line (3T3) was expanded in a DMEM expansion medium supplemented with 10% fetal calf serum (FCS). The culture media was changed twice a week. When the cells became confluent and reached the required number, 3T3s were enzymatically detached with 0.05% trypsin and counted. Samples were sterilized under UV lamp for 90 min and placed in a 24-well plate for cell culture. Cells were then seeded onto the surface of samples, at a density of $5 \times 10^4$ cells/cm$^2$ and a concentration of 0.5 million/mL. Samples were cultured up to one week in the incubator in an atmosphere of 5% $CO_2$ allowing gas exchange in the reservoir at 37 °C. After 1 and 7 days of culture, samples were washed in buffer saline solution, and the cellular adhesion/orientation and proliferation, respectively, were investigated. All experiments were performed in duplicates.

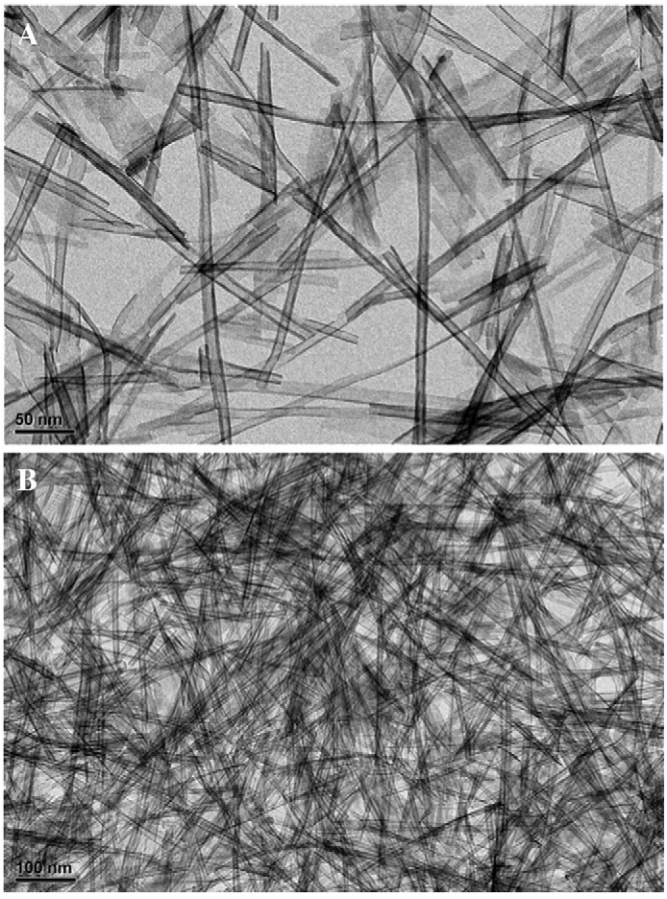


**Fig. 1.** TEM images of TNTs at two different magnifications.

Download English Version:

https://daneshyari.com/en/article/1428256

Download Persian Version:

https://daneshyari.com/article/1428256

Daneshyari.com