Colloids and Surfaces B: Biointerfaces 111 (2013) 134–141

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Interaction of preosteoblasts with surface-immobilized collagen-based nanotubes

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ARTICLE INFO

Article history:
Received 21 December 2012
Received in revised form 21 May 2013
Accepted 22 May 2013
Available online 29 May 2013

Keywords:
Collagen
Nanotube
Biointerface
Nano/micro topography
Cell–material interactions

ABSTRACT

In a previous work, we demonstrated the successful use of electrophoretic deposition (EPD) to immobilize collagen-based nanotubes onto indium-tin-oxide-coated glass (ITO glass), leading to the creation of biointerfaces with protein-based chemistry and topography [1]. In this work, we present a first study of preosteoblasts behavior in contact with surface-immobilized collagen-based nanotubes. Changes in cell morphology after their interaction with ITO glass modified with collagen-based nanotubes were studied using fluorescence microscopy and compared to those observed on virgin ITO glass as well as on ITO glass on which a collagen layer was simply adsorbed. Scanning electron microscopy (SEM) was used to study interactions of cell filopodias with the deposited nanotubes. Cytotoxicity of these biointerfaces was examined as well in short term cultures, using Alamar blue assay.

Cells showed particular morphologies on ITO glass coated with nanotubes compared to virgin ITO glass or collagen adsorbed layer on ITO glass. High resolution SEM images suggest that apart from cell morphology, length and thickness of filopodias seem to be significantly affected by surface modification with collagen-based nanotubes. Moreover, nanotube-coated ITO glass did not show any obvious cytotoxicity in short term culture, opening new perspectives for the surface modification of biomaterials. We show the versatility of the proposed surface modification procedure by tailoring biointerfaces with a mixture of micro- and nanometer-scale collagen-based tubes.

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1. Introduction

Surface modification of biomaterials is widely applied to improve their functionality. In order to achieve predictable cell responses from biomaterials, different surface modification strategies have been developed and evaluated over the last few decades [2–4]. These strategies may alter a variety of surface properties, including topography, chemical composition, wettability, electrical potential, mechanical behavior, etc. [5]. The spatial distribution of topographical or chemical cues, at the micro- and more recently at the nanometer scale, was moreover shown to affect cell adhesion, morphology, migration, proliferation, gene expression and differentiation [6–11]. These cues may be brought at the interface through the immobilization of nanoparticles. A comprehensive review on the use of nanoparticles for tissue engineering applications has been recently published by Zhang and Webster [4].

Such surface modifications are especially useful for orthopedic materials [5,11] with the aim to improve cell–material interactions and thereby promote integration with the surrounding bone tissue [12]. Surface modification of orthopedic implants is generally achieved either by anodisation to introduce micro/nano features [13] or by coating with ceramic particles of interest such as hydroxyapatite or bioglass [2]. More recently, the use of polymer-based particles, the polymer being of natural or synthetic origin, has been explored as well to enhance osseointegration [5]. The combination of hydroxyapatite and collagen in biointerfacial coatings, both being natural components of bone, may open the way to novel biomimetic strategies [4,14]. However, little is known regarding the appropriate structure and composition to be tailored for the success of such composite biomimetic interfaces.

Recently, we have synthesized collagen-based nanotubes using layer-by-layer assembly within the nanopores of a polycarbonate membrane used as a template [15,16]. The dimensions of these nanotubes can be precisely controlled through the choice of the template: their outer diameter is indeed equal to the pore diameter, while their length is equal to the template thickness. We have then shown that electrophoretic deposition (EPD) can be used to immobilize collagen-based nanotubes onto indium-tin-oxide-coated glass (ITO glass), leading to the creation of original

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http://dx.doi.org/10.1016/j.colsurfb.2013.05.035

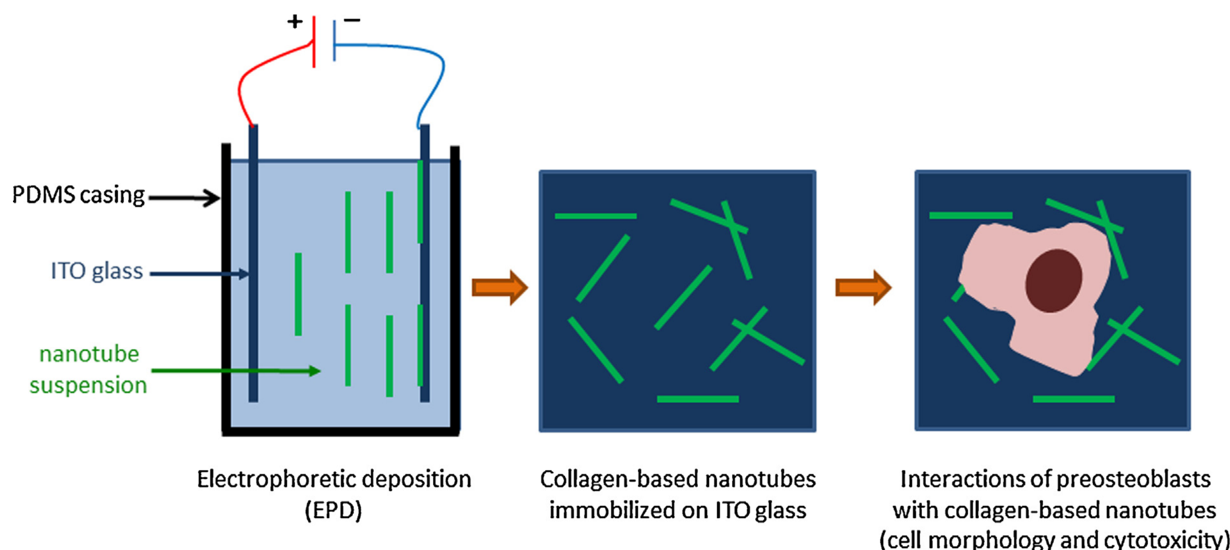

Fig. 1. Schematic representation of the research strategy.

biointerfaces [1]. In the future, such biointerfaces could be further developed by adding functional components inside the nanotubes, or by co-immobilization of nanotubes with different dimensions and, or compositions. Moreover, while the conducting properties of ITO are here only used to immobilize nanotubes by EPD, they could also be exploited to deliver electrical signals to adherent cells.

In this paper, we present a first study of MC3T3 preosteoblasts behavior in contact with surface-immobilized collagen-based nanotubes, with the aim to demonstrate the possibility to use such biomimetic interfaces for the control of cell–material interactions. Cell morphology (spreading and shape) was evaluated in short term cell culture on ITO glass modified with collagen-based nanotubes using EPD, and the obtained response was compared to the one observed on virgin ITO glass as well as on ITO glass previously treated by collagen adsorption. The effect of surface modification on cell attachment and type of interactions with the substrate was illustrated by studying changes in developed cell filopodias. Alamar blue assay was performed to evaluate cell viability and metabolic activity. This research strategy is illustrated in Fig. 1. Thereby, we wish to show the absence of obvious cytotoxicity of the created biointerfaces, and to evidence the potential of these biointerfaces to trigger particular cell behaviors, which would open interesting perspectives in the field of biomaterials science and tissue engineering. Finally, we show the versatility of the proposed surface modification procedure by tailoring biointerfaces with a mixture of micro- and nanometer-scale collagen-based tubes, and suggest ways to exploit these new materials further.

2. Materials and methods

2.1. Materials

Poly(sodium-p-styrenesulfonate) (PSS, Mw ~70 kDa) was purchased from Acros Organics. Poly(fluorescein isothiocyanate allylamine hydrochloride) (Flu-PAH, Mw ~15 kDa), sodium acetate, acetic acid, dichloromethane, dimethylformamide (DMF), isopropanol, hexamethyldisilazane (HMDS), paraformaldehyde, glutaraldehyde solution, cytotoxicity assay (Alamar blue) kit, sodium pyruvate solution (100 mM), Tween 20, Triton X-100 were purchased from Sigma–Aldrich. Red-fluorescent COOH-coated polystyrene particles (Red-PSNp) with diameter of 100 nm were bought from Micromod Partikeltechnologie GmbH (Rostock, Germany). Collagen G from calf skin (COL, 4 mg/mL at pH 3.0) was bought from Biochrom AG, Germany. Polycarbonate track-etched membranes, supplied by it4ip (http://www.it4ip.be), were used as nanoporous templates. They had an average pore diameter of 500 nm or 3 μm, a respective thickness of 21 and 22 μm, and a respective pore density of 4×10^7 and 3×10^6 pores cm^{-2}. Indium-tin-oxide-coated borosilicate glass slides (ITO glass) of 20 mm × 20 mm (8–12 Ohms resistivity) were purchased from SPI Supplies/Structure Probe, Inc., PA, USA. Fetal bovine serum was purchased from Harlan, Loughborough, UK. α-MEM (Modified Eagles Medium (A1049001)), penicillin-streptomycin (penstrep) solution with 10,000 U/mL penicillin-G and 10,000 μg/mL streptomycin, and Alexa Fluor® 594 phalloidin were bought from Invitrogen (Merelbeke, Belgium). Primary anti-vinculin mouse monoclonal antibody and FITC-conjugated secondary anti-mouse goat IgG were purchased from Millipore (Overijse, Belgium). Vectashield Mounting Medium with DAPI was bought from Vector Lab (Burlingame, CA). MC3T3-E1 (subclone 14) preosteoblasts (product number CRL-2594™) were purchased from the American Collection of Cell Culture (ATCC-LGC Standards S.a.r.l., France).

2.1.1. Preparation of polyelectrolyte and collagen solutions for LbL assembly

All polyelectrolyte solutions were prepared using 100 mM acetate buffer (pH 4.7). PSS and Flu-PAH solutions were used at a concentration of 1 mg/mL in acetate buffer. Collagen solution was also prepared in acetate buffer at a concentration of 0.5 mg/mL. All solutions were freshly prepared before use.

2.1.2. Synthesis of nanotubes

Synthesis of nanotubes was carried out using layer-by-layer (LbL) assembly in polycarbonate track-etched membranes as reported previously [15]. Briefly, LbL assembly was performed by successive adsorption steps of COL (or Flu-PAH) and PSS in polycarbonate track-etched membranes with pore diameter of 500 nm or 3 μm. Membrane pieces of 13 mm in diameter were immersed in a polycationic solution of either COL or Flu-PAH. The polyanionic layers were built similarly, by immersing the membranes in a PSS solution. The immersion time for all polyelectrolytes was 30 min, except for COL in 500 nm pores, for which the immersion time was extended to 2 h. After each immersion step, samples were rinsed in pure acetate buffer for 4 min. When the desired number of bilayers was reached, membranes were cleaned on both faces using a cotton bud soaked with a NaCl solution at pH 12. Tubes

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