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Effects of polydopamine functionalized titanium dioxide nanotubes on endothelial cell and smooth muscle cell

Si Zhong[a,c,1], Rifang Luo[b,c,1], Xin Wang[b,c], LinLin Tang[b,c], Jian Wu[a,*], Jin Wang[b,c,*], Runbo Huang[d], Hong Sun[b,c], Nan Huang[b,c]

[a] School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China
[b] School of Material Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China
[c] Key Lab of Advanced Technology of Materials of Education Ministry, Southwest of Jiaotong University, Chengdu 610031, China
[d] School of Manufacturing Science and Engineering, Sichuan University, Chengdu 610065, China

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ABSTRACT

Previous investigations have demonstrated that TiO_2 nanotubes (NTs) with particular structure cues could control the behavior of different types of cells, including endothelial cells (ECs) and smooth muscle cells (SMCs). Besides, polydopamine (PDA) modified surfaces were reported to be beneficial to increase the proliferation and viability of ECs and meanwhile could inhibit the proliferation of SMCs. The TiO_2 nanotubes (NTs) were functionalized with polydopamine (PDA) (PDA/NTs) to study the synergetic effect of both nanotopography (NTs) and chemical cues (PDA) of TiO_2 nanotubes on the regulation of cellular behavior of ECs and SMCs. The PDA-modified TiO_2 nanotubes were subjected to field-emission scanning electron microscopy (FE-SEM), X-ray photoelectron spectroscopy (XPS), and water contact angle (WCA) analysis. *In vitro* cell culture tests confirmed that, comparing with flat titanium (Ti) and TiO_2 nanotubes, PDA/NTs surface synergistically promoted ECs attachment, proliferation, migration and release of nitric oxide (NO). Meanwhile, the PDA/NTs performed well in reducing SMCs adhesion and proliferation. This novel approach might provide a new platform to investigate the synergistic effect of local chemistry and topography, as well as the applications for the development of titanium-based implants for enhanced endothelialization.

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1. Introduction

Vascular stents or grafts are commonly used to treat coronary artery disease, which is one of the leading causes of death in the world. However, these treatments are usually associated with major complications such as restenosis due to the thrombosis formation and smooth muscle cell (SMC) proliferation. These complications delay the endothelialization process and remain challenging clinical problems. Injury and dysfunction of endothelial cell (EC) occur in response to the acute vessel-wall injury caused by implantation. Therefore, vascular devices that can either suppress inflammation and SMCs proliferation or promote endothelialization are highly desired [1–3].

Surface properties of biomedical devices can affect the cell–material interactions. A number of approaches have been reported to regulate cell–material interactions via regulating the topography or the local chemistry of the material surface. Textor found that cell activity of primary human osteoblasts after 24 h was significantly higher on the polymer/HA composites than on the polymer films [4]. According to Spatz, after immobilization of adhesion-promoting RGD motifs endothelial cells adhered more readily to the nanostructured Teflon surface [5]. Ratner found that pre-adsorption of albumin, Y-globulin or fibrinogen markedly affected subsequent adhesion of chick embryo muscle cells to the surfaces of polymeric materials [6]. As reported by Dufrêne, on rough substrata, the adsorbed collagen molecules no longer formed aggregated structures on the hydrophobic surfaces, which indicating that the supramolecular organization of the adsorbed layer is also controlled by topography of materials except for chemistry [7]. All these interesting findings indicated that the feasibility of local chemistry and topography will affect cell–material interactions. However, few researches had concentrated on both factors to achieve desired properties.

Studies on topography showed that TiO_2 nanotubes could lead to favorable cellular responses because these nanotubes

* Corresponding authors at: Southwest Jiaotong University, School of Material Science and Engineering, Key Lab of Advanced Technology of Materials of Education Ministry, The 2nd Ring road, 111, Chengdu, China.
Tel.: +86 28 87634148/+86 15 928510155; fax: +86 28 87600625.
E-mail addresses: lrifang@126.com (R. Luo), Wujianeer@sina.com (J. Wu), jinxxwang@263.net (J. Wang).
[1] Joint first authors.

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could restrict the size of SMC but not EC at a certain structure. Brammer reported that nanotube structure exhibited enhanced endothelialization, much increased extracellular matrix formation, and substantially raised level of nitric oxide/endothelin ratio [8]. Moreover, Peng found that the nanotubular surface significantly enhanced EC proliferation and secretion of PGI_2, the surface also decreased SMC proliferation and increased expression of smooth muscle α-actin [2]. These studies showed that TiO_2 nanotubes had great potential in stent or other vascular applications via structure cues. However, chemical inertness is the main weakness of this material when placed in contact with biological systems, so surface modification is a possible solution to this problem [9].

Bioactive molecules like adhesive peptides (RGD), vascular endothelial growth factor (VEGF), have been reported to accelerate re-endothelialization of grafts by regulating cell–material interactions *in situ*. Among those diverse chemical cues, polydopamine (PDA), a bio-inspired coating material, has been widely investigated as an ad-layer [10–14]. Lee et al. recently announced that surface functionalization of PDA attenuated the *in vivo* toxicity and the inflammatory responses to the implanted materials [15]. Moreover, Lynge found that myoblast cells showed a better adherence and proliferation when glass substrate was modified with PDA coating [16]. Additionally, Ku found that when coated with PDA, polycaprolactone (PCL) nanofiber scaffold exhibited highly enhanced adhesion and viability of HUVECs [17]. Shin deposited PDA on the surface of biocompatible poly(L-lactide-*co*-ε-caprolactone) (PLCL) elastomer and found enhanced EC adhesion [18]. Besides all these interesting findings, our previous study demonstrated the effect of PDA on reducing the proliferation of SMCs [19,20]. Phenolic groups were the key factor to influence SMCs proliferation [21], and polydopamine, as a catechol containing material, might also present similar effect *via* its catechol oxidation (inducing ROS), binding SMCs' ECM proteins and thus affect integrin expression [22–24]. The researches mentioned above showed that PDA components not only enhance EC functions, but also to a certain extent reduce SMC functions, which had great potential to be used as vascular biomaterials to enhance the endothelialization process.

In this work, we functionalized TiO_2 nanotubes with PDA to investigate the effects on cellular behavior of ECs and SMCs affected by nanotopography and chemical cues. This research also aimed at enriching the study of mussel chemistry on vascular cellular behavior.

2. Experimental

2.1. Materials

Titanium foils (0.25 mm thickness, 99.5%) were purchased from Alfa Aesar (Tianjin, China). Glycerol, sodium chloride (NaCl), Tris (hydroxymethyl) aminomethane (Tris-base) and glutaraldehyde were obtained from Jinshan Chemical Reagent (Chengdu, China), NH_4F, 3,4-dihydroxyphenylalanine (dopamine) were supplied from Sigma-Aldrich (U.S.A). Reagents for cytocompatibility test were provided from professional manufacturers which were mentioned in the Experimental Section. Other reagents were local products of analytical grade.

2.2. Preparation of samples

2.2.1. TiO_2 nanotube fabrication

Titanium foils were sequentially ultrasonicated in acetone, ethanol and distilled water, respectively (10 min × 3 times), then dried under nitrogen flow. For anodization, titanium foils were used as the positive electrode, whereas platinum foil was employed as the negative electrode. A solution mixture of glycerol and water (4:1, vol) containing 0.5 wt% NH_4F and 0.35 wt% NaCl was used as electrolyte. Then the reaction was conducted at a voltage of 25 V at room temperature for 4 h. After electrochemical treatment, all samples were rinsed and then thermally treated at 450 °C before use.

2.2.2. Preparation of polydopamine coatings

Dopamine solution was prepared by dissolving dopamine hydrochloride (0.5 mg/mL) in Tris-base buffer (10 mM, pH = 8.5). After cleaning, samples were all cut into $1 \times 1\ cm^2$ as substrates, then immersed in above dopamine solution for 12 h at room temperature. The substrates were then ultrasonically washed for 5 min in water (three times) to remove the nonattached polydopamine and dried under nitrogen. Samples were labeled as Ti, NTs and PDA/NTs, which were short for Titanium, TiO_2 nanotubes and PDA modified TiO_2 nanotubes, respectively.

2.3. Surface characterization

The crystal structure and surface morphology of the nanotube arrays were analyzed by X-ray diffractometer (XRD) and field-emission scanning electron microscopy (FE-SEM). The surface chemical composition was measured by X-ray photoelectron spectroscopy (XPS, Perkin Elmer 16PC) with a monochromatic Al *K*α excitation radiation ($h\upsilon$ = 1486.6 eV). The C1s peak (binding energy 284.7 eV) was used as a reference to calibrate binding energies. The contact angle was measured by static drops, using DSA100 (Krüss, Hamburg, Germany) with the method depicted by the manufacturer at 25 °C, in 60% relative humidity.

2.4. ECs culture and viability assay

Human umbilical vein endothelial cells (HUVECs) were digested by collagenase II from newborn umbilical cord. Following isolation, HUVECs were cultured in M199 media (Hyclone Company) with 15% fetal bovine serum (FBS, Sigma, US), then cells were cultured in a humidified incubator containing 5% CO_2 at 37 °C. Cells in passage three were used. To investigate the cell proliferation behavior affected by different samples, ECs were seeded on the surfaces at a density of 5×10^4 cells per sample, then incubated at 37 °C in M199 culture media with 15% FBS supplemented 1 mL 20 μg/mL endothelial cell growth supplement (ECGS). After incubated for 2 h, 1 day and 3 days, samples were, respectively, taken out and washed before fixed the adherent cells with 2.5% glutaraldehyde for 1 h. For actin immune staining, cells were Rhodamine-phaloidin treated (50 μL per sample) for 20 min in the dark. As for nucleus immune staining, DAPI (50 μL per sample) was used per the same method for 5 min. After that, samples were visualized by fluorescent microscopy (Zeiss, Germany).

2.5. Apoptosis or necrosis of HUVECs

For cell apoptosis or necrosis array, a cell-permeable reagent acridine orange (AO) in combination with a plasma membrane-impermeable and DNA-binding dye propidium iodide (PI) were used to identify apoptosis or necrosis of HUVECs. AO excites green fluorescence while PI gives red fluorescence when they inserted into DNA. Only AO can cross the plasma membrane of natural cells. However, both dyes are absorbed by late apoptotic and necrotic cells and show a predominant orange fluorescence. In detail, HUVECs at a high density of 5×10^4 cells per sample were seeded. After cultured for 1 and 3 days, the cells were immune stained with a 1:1 mixture of AO (100 mg/mL) and PI (100 mg/mL) for 5 min at 37 °C, and then inspected in a fluorescence microscope immediately. The criteria for identification are as follows: (a) vital cells: green intact nucleus; (b) early apoptosis: dense green areas

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