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Double-chimera proteins to enhance recruitment of endothelial cells and their progenitor cells

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ABSTRACT

Background: Enhanced attraction of selective vascular reparative cells is of great importance in order to increase vascular patency after endovascular treatments. We aimed to evaluate efficient attachment of endothelial cells and their progenitors on surfaces coated with mixture of specific antibodies, L-selectin and VE-cadherin, with prohibited platelet attachment.

Methods: The most efficient conditions for coating of L-selectin-Fc chimera and VE-cadherin-Fc chimera proteins were first determined by protein coating on ELISA plates. The whole processes were repeated on titanium substrates, which are commonly used to coat stents. Endothelial progenitor cells (EPCs) and human umbilical vein endothelial cells (HUVECs) were isolated and characterized by flow cytometry. Cell attachment, growth, proliferation, viability and surface cytotoxicity were evaluated using nuclear staining and MTT assay. Platelet and cell attachment were evaluated using scanning electron microscopy.

Results: Optimal concentration of each protein for surface coating was 50 ng/ml. The efficacy of protein coating was both heat and pH independent. Calcium ions had significant impact on simultaneous dual-protein coating ($P<0.05$). Coating stability data revealed more than one year stability for these coated proteins at 4 °C. L-selectin and VE-cadherin (ratio of 50:50) coated surface showed highest EPC and HUVEC attachment, viability and proliferation compared to single protein coated and non-coated titanium surfaces ($P<0.05$). This double coated surface did not show any cytotoxic effect.

Conclusions: Surfaces coated with L-selectin and VE-cadherin are friendly surface for EPC and endothelial cell attachment with less platelet attachment. These desirable factors make the L-selectin and VE-cadherin coated surfaces perfect candidate endovascular device.

1. Introduction

Despite of undoubted beneficial applications of stents in the treatment of acute and chronic vessel obstructions, in-stent restenosis is the great obstacle for obtaining optimal vessel patency [1]. In-stent restenosis is mainly due to the proliferation and migration of smooth muscle cells to the intima and is diminished using drug-eluting stents but at the expense of enhanced in-stent thrombosis [2,3]. Therefore, to prevent this dismal event, long-term dual-anti platelet therapy is warranted, which is associated with greater risk of bleeding and its cessation predisposes the patient to stent thrombosis [3,4].

Novel therapeutic strategies are based on targeting smooth muscle cells to prevent their proliferation, prevent platelet activation and promote attraction of endothelial cells and endothelial progenitor cells (EPCs). This multi-step approach should be occurred simultaneously to obtain optimal vessel patency with both diminished in-stent restenosis and thrombosis. In this context, some technical developments like ex vivo cells seeding have improved this field, but poor local accumulation of infused cells has limited their application [5]. Stents coated with monoclonal anti-CD34 antibodies also known as EPC-capture stents provide better vessel patency without the need for dual anti-platelet therapy [6]. But capturing cells expressing CD34 means recruitment of whole CD34 positive cells toward implantation site. CD34 is a marker of mast cells, eosinophils, hematopoietic and mesenchymal stem cells [7,8]. Some of these recruited cells might exert disadvantageous effects on the outcome of the deployed stent. Mesenchymal stem cells are major contributors of neointimal hyperplasia and mast cells promote

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inflammation at the implantation site [9–11]. Indeed, applied humanized murine antibodies might elicit immune response which might lead to the promoted inflammation due to antigen–antibody reactions.

One of the selective markers of the recruitment of endothelial and their progenitor cells is L-selectin (CD62L). Its ligand is expressed on the surface of endothelial cells. It improves vascular EPC homing within sites of selectin ligand expression by mechanisms typical to leukocyte adhesion [12–16]. CD34 which is present on the surface of both endothelial and EPCs serves as a ligand for L-selectin [17]. So, cell trapping and capture using L-selectin protein attracts CD34-expressing cells carrying L-selectin ligand. Interestingly, not all CD34-positive cells express L-selectin ligand which makes this strategy a specialized cell orientation [18]. CD34 on the surface of hematopoietic stem/progenitor cells (HSPCs) has not been reported as a ligand for L-selectin [19]. L-selectin protein entraps endothelial and EPCs which harbor ligand of L-selectin on their surface [20]. In this way, no inflammatory reaction would occur and just endothelial cells and their progenitors will attract to the surface of implanted stent. L-selectin is not a "homing receptor" for mesenchymal stem cells which is a point toward prevention of restenosis [21].

Another approach to construct a defensive functional endothelial layer is recruitment of neighboring endothelial cells and tightening of the junctions between desquamating endothelial cells. Extended

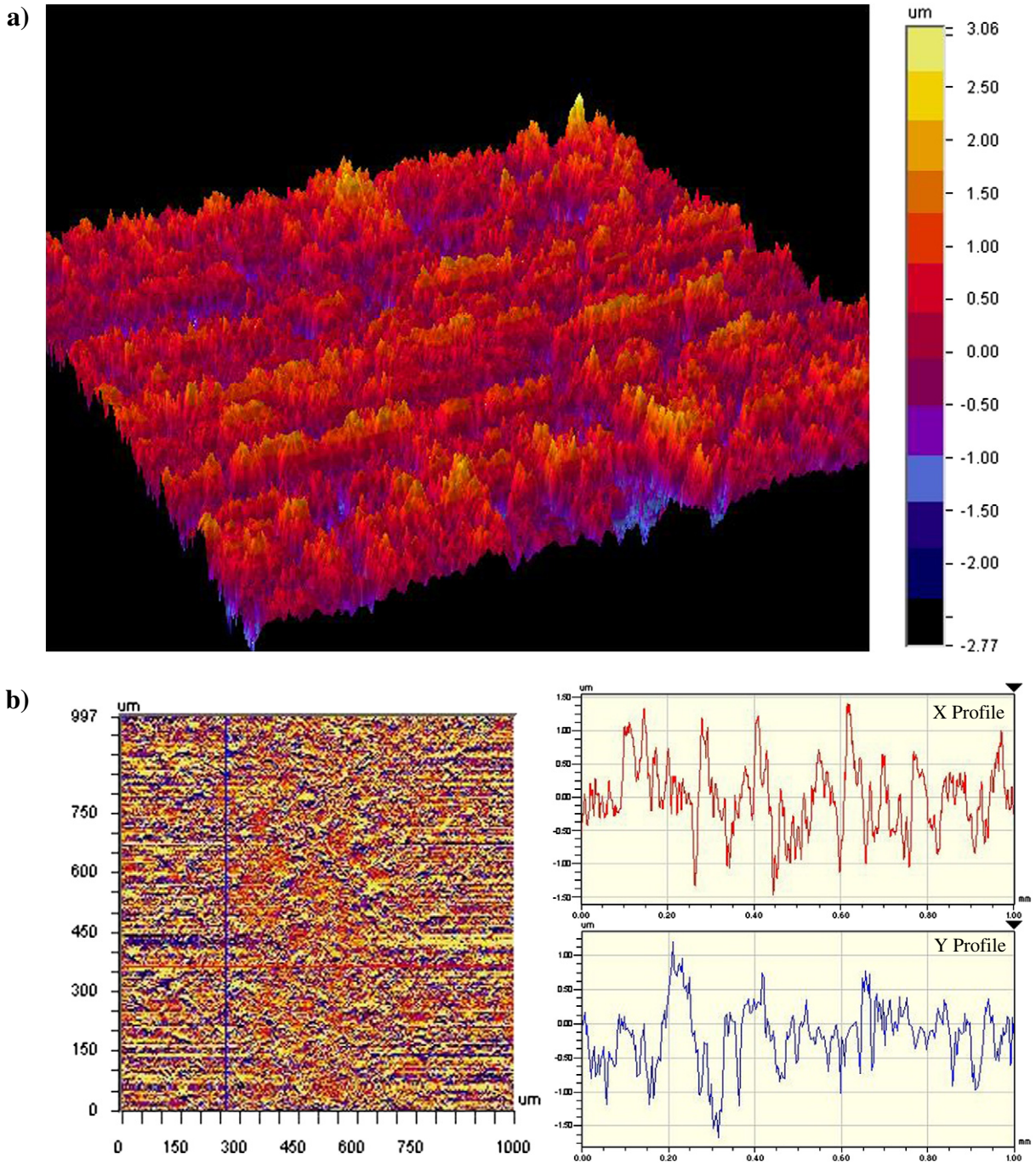

Fig. 1. AFM characterization of surface topography of commercial purity titanium sheet grade I as-received. (a) 3D AFM picture (5 μm × 5 μm); (b) the height profiles along the X (red line) and Y (blue line) scanning lines indicated in the 2D pictures.