Tannin/glycosaminoglycan-based Polyelectrolyte Multilayers Improve the Endothelialization of TiO_2 Nanotubes

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Statement of Purpose: Cardiovascular diseases, often initiated by atherosclerosis, have become the leading cause of death worldwide.[1] A common treatment for atherosclerosis is the implantation of stents, which are often made of metals such as titanium. However, this procedure is often at risk of complications like thrombosis or restenosis.[2] This usually happens since the implant is not yet covered with an endothelium cell layer, which induces the thrombus formation. Therefore, a rapid and complete endothelialization is crucial for the success of cardiovascular implants. In this work, we propose a surface modification on titanium to enhance endothelialization of cardiovascular implants. TiO_2 nanotubes are fabricated, and the surfaces are further modified with polyelectrolyte multilayers (PEMs) using a tannin derivative (tanfloc) and the glycosaminoglycans heparin and hyaluronic acid. Previous study in our lab showed that these modified surfaces present anti-thrombogenic properties;[3] however, the endothelization ability of these surfaces has never been investigated.

Methods: The titanium surface was first modified to make TiO_2 nanotubes (NT) via an anodization process. NT was then modified with PEMs using tanfloc (TA) as polycation, and heparin (HP) or hyaluronic acid (HA) as polyanions. The surfaces were characterized using scanning electron microscopy (SEM), water contact angle measurements, and X-ray photoelectron spectroscopy (XPS). Human Microvascular Endothelial Cells (HMVEC) were seeded on the surfaces and their viability was investigated after 1, 3 and 5 days using an Alamar Blue assay. The adhesion and proliferation of the endothelial cells on the surfaces were also investigated using SEM and fluorescence microscopy. The HMVEC differentiation on the surfaces was further investigated after 7 and 10 days using immunofluorescence to determine the presence of specific marker proteins, such as von Willebrand factor (vWF) and vascular endothelial cadherin (VE-cadherin). A migration assay was also performed after a confluent monolayer was obtained on the samples.

Results: SEM results show that all surfaces present uniform and vertically oriented nanotubes, and that the surface modification with PEMs do not change the nanotube topography. XPS indicates the successful modification of NT surfaces with PEMs and contact angles results show that all surfaces are hydrophilic. The viability study indicates that TA/HP significantly increase the percentage of Alamar Blue reduction after 5 days of HMVEC culture (Figure 1). Adhesion and proliferation studies show that NT surfaces modified with TA had significant higher number of cells after 5 days in comparison with unmodified NT. TA/HP also shows significant higher expression of the proteins vWF and VE-cadherin (Figure 2). In addition, both surfaces modified with TA promoted enhanced HMVEC motility compared with unmodified NT surface.

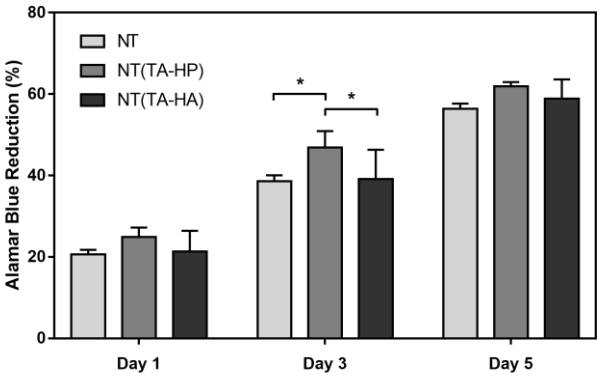

Figure 1. HMVEC viability on different surfaces after 1, 3, and 5 days of cell culture (* indicates $p < 0.05$).

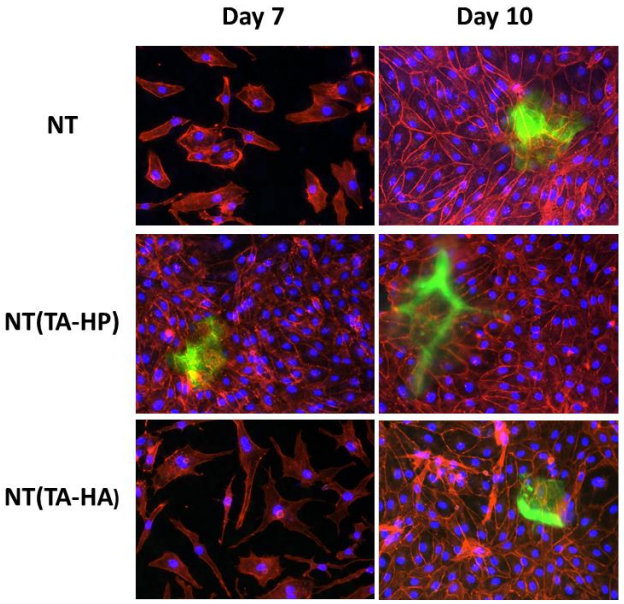

Figure 2. Representative fluorescence microscopy images of HMVEC immunostained with wWF.

Conclusions: The results show that tanfloc-based PEMs on NT enhance the endothelial cell growth and viability as well as the cell migration, which is crucial for the successful endothelialization of cardiovascular implants. In addition, TA/HP PEMs on NT also enhance the differentiation of HMVEC through significant higher expression of the proteins vWF and VE-cadherin, which is necessary for health vasculature. Therefore, the tannin/glycosaminoglycan-based PEMs showed improved endothelialization of TiO_2 nanotubes, thus being a promising approach for cardiovascular implants.

References:

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