Photo-initiated Grafting of N-maleic acyl-chitosan Enhances Endothelial Cell Adhesion and Function on PLA Surface

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Statement of Purpose: Vascular graft surface properties significantly affect adhesion, growth and function of endothelial cells (ECs). Poly(lactic acid) (PLA) has been widely used in tissue engineering because it is readily formed into desired shapes with good mechanical strength and controllable degradation timescales. However, lack of a cell adhesion motif in PLA leads to unfavorable cell adhesion and proliferation on the surface, and is a major limitation for its application as a vascular graft. Photo-initiated surface-grafting-polymerization offers great promise in immobilizing desirable biomacromolecules on material surfaces without compromising bulk properties. N-maleic acyl-chitosan (NMCS) is a novel biocompatible amphiphilic derivative of chitosan with double bonds, which allows it to be grafted on a polymeric surface under ultraviolet (UV) photo initiation. The purpose of this study is to graft the gelatin/NMCS (Gel/NMCS) complex, mimicking the properties of natural extracellular matrix (ECM), on the surface of PLA, with the aim of improving the compatibility of the synthesized PLA with ECs.
Methods: Chitosan powder was supplied by Lianyungang Biologicals Inc., China, which has a deacetylation degree of 90% and viscosity average molecular weight of 20KD. The NMCS was prepared using chitosan and maleic anhydride following our previous publication [1]. Surface-graft-polymerization of NMCS and gelatin was performed under UV irradiation after the PLA film was photooxidized with UV irradiated in hydrogen peroxide solution. The material surfaces were characterized by X-ray photoelectron spectroscopy (XPS) and water contact angle. The human umbilical vein endothelial cells (HUVECs) were seeded on the films at a density of 4,000 cells/cm^2 and cultured at 37 °C in 5% CO_2. The cell proliferation and morphology were examined by DNA assay and scanning electron microscopy (SEM), respectively. Immunocytochemistry staining was used to detect endothelial cell specific markers CD31, vWF, and acetylated low-density lipoprotein (Ac-LDL). After 7 days culture, both unmodified PLA and Gel/NMCS-PLA films with HUVECs were placed in a parallel plate flow chamber, and subjected to media and blood flow to detect the effect of shear stress on HUVEC retention as well as platelet adhesion. Nitric oxide (NO) and prostacyclin (PGI2) secretion by HUVECs grown on PLA films were quantified using a total nitric oxide kit and a 6-keto Prostaglandin F1α kit, respectively.
Results: XPS characterization and water contact angle confirmed that the PLA surface was successfully immobilized with the Gel/NMCS complex, which in turn enhanced its hydrophilicity. The influence of the Gel/NMCS modified PLA (Gel/NMCS-PLA) surface on HUVEC adhesion, proliferation, function, as well as its antithrombogenicity was examined extensively. The HUVECs on the Gel/NMCS-PLA film exhibited greater spreading and flattening than that on unmodified PLA films. The cells on modified PLA surface also expressed more structured intercellular molecules CD31 and vWF, and elevated acetylated low-density lipoprotein (Ac-LDL) uptake compared to the unmodified PLA surface. In addition, cell retention was 1.6 times higher on the Gel/NMCS-PLA surface after perfusion at a physiological shear stress of 5 dyn/cm2 over 24 hours. The endothelialized surface on the modified surface maintained the cell ability to modulate and adapt their metabolic properties to the physiological physical signals as indicated by the up-regulation of NO and prostacyclin PGI_2 production. Platelet adhesion observation further showed that less protein adhered on the modified PLA surface, indicating its improved blood compatibility as compared to unmodified PLA.
Conclusions: Complex of gelatin and NMCS was successfully immobilized onto PLA through surface-graft-polymerization initiated by UV photochemical method. The wettability of PLA film was improved due to the introduction of hydrophilic gelatin and NMCS. The ECs grown on Gel/NMCS-PLA exhibited better adhesion, greater spreading and faster proliferation of ECs than that on unmodified PLA. The cells on Gel/NMCS-PLA surface expressed more extensive endothelial cell marker CD31, vWF and up-took more Ac-LDL than unmodified PLA surfaces. In addition, the cell retention ratio is approximately 2 times higher on the Gel/NMCS-PLA surface after perfusion at a physiological shear stress of 5 dyn/cm2 over 24 hours. The endothelialized surface on the Gel/NMCS-PLA also has a better blood compatibility than that on the unmodified PLA. These results indicated that immobilization of the complex of Gel/NMCS on PLA with the surface-grafting-polymerization initiated by UV irradiation is an effective surface engineering method to enhance endothelium function for vascular tissue engineering.
References:
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