Fabrication of Human Serum Albumin Nanofilms for Enhanced Hemocompatibility and Smooth Muscle Cell Response

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Statement of Purpose: Restenosis and thrombosis are two major clinical complications of endovascular stents and grafts. In this study, a novel bio-polymeric nanofilm made of Human Serum Albumin (HSA) grafted onto poly (glycidyl methacrylate) (PGMA) is proposed as a potential coating inhibiting smooth muscle cell (SMC) hyperplastic response as well as shielding fibrinogen adsorption and platelet adhesion.

Materials and Methods:Thermal properties of freeze-dried HSA were assessed by Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). Plasma treated nitinol (NiTi) discs were surface modified with PGMA film (Mn=176000g/mol) using 0.5% wt/vol PGMA solution in chloroform to produce an epoxy rich anchoring layer followed by adsorption of HSA using 5% wt/vol HSA solution in buffer. Samples were then annealed at 150˚C in a vacuum oven for 1 hour. Morphologies, thickness and swelling behavior of the protein films were studied with Atomic Force Microscopy (AFM), Ellipsometry and Quartz crystal microbalance. Binding between HSA and PGMA was confirmed by Fourier Transform Infrared (FT-IR) spectroscopy. Contact angle measurements were performed to evaluate surface energy of the films. Changes in secondary structure conformation of HSA post annealing were studied using CD-Spectropolarimetry.HSA grafted samples were incubated in Rhodamine-NHS labeled Human Plasma Fibrinogen (Fg) solution for 1 hour at 37˚C.Adsorption of Fg was assessed by measuring fluorescence at 525nm and 575nm. Adhesion forces between HSA and Fg were examined with (AFM) colloidal probe technique using a 20μm glass bead. Platelet-rich plasma isolated from fresh human blood was incubated on HSA films for 5 hours at 37˚C. Platelet adhesion was measured by lactate dehydrogenase (LDH) assay. Sprague Dawley rat aortic SMCs were cultured at passage 5 on HSA nanofilms to confluence for 72 hours in DMEM with 10% FBS and quantified by CyQuant cell proliferation by measuring fluorescence at 480nm and 520nm. The phenotype of SMCs grown on HSA films and controls, fixed with 4% formaldehyde and triton-X 100 was identified by immunofluorescent staining with antibodies against α-smooth muscle actin and smooth muscle myosin heavy chain-2.The nuclei were counterstained with 4', 6-diamidino-2-phenylindole (DAPI).

Results: The thickness of the PGMA (12.5±0.4nm) and HSA layer (90±4.5 nm) was determined through ellipsometry. The root mean square (RMS) roughness, evaluated for each 1x1 μm scan was calculated to be <1 nm for HSA films, indicating smooth layer formation and complete coverage. HSA films on silicon wafers displayed a significantly smaller adhesion force of 0.3±0.1nN/m (mean±SD; N=50 points) to human fibrinogen as compared to 8.7±0.4nN/m of pure PGMA. HSA grafted onto PGMA displayed a 31±0.2% loss in alpha-helix secondary structure post annealing.There was significantly less human plasma fibrinogen adsorption on HSA films (90±9.8ng/mm^2; $p<0.05$) as compared to bare NiTi (320ng/mm^2). LDH assay showed minimal human platelet adhesion (4.58±1.7%; mean±SD;N=5, $p<0.05$) on HSA films as compared to bare NiTi (34.4±2.4%).There was significantly less rat aortic SMC proliferation (37 ± 1.8%; mean±SD; n=5, $p<0.05$) measured on HSA nanofilms for 72 hours as compared to bare NiTi (148±9.3%). SMC's grown on HSA films showed intense staining for α-actin and myosin heavy chain-2 as compared to SMC's cultured on bare NiTi which displayed weak staining. Hence, SMCs grown on HSA films retained their contractile phenotype as compared to SMC's grown on bare NiTi which attained synthetic morphology.

Conclusions: Beyond a critical point of unfolding (>34%), human albumin has been shown to undergo a change in the structural orientation which results in adhesion of platelets on albumin even though it has no amino acid sequences to bind to platelet receptors [1]. HSA film on PGMA anchoring layer retains most of its secondary structure conformation which prevents the adsorption of the adhesive protein fibrinogen on the albumin adsorbed surface and minimizes platelet adhesion. HSA film also prevents the hyperplastic response of smooth muscle cells which play a major role in the progression of neo-intimal hyperplasia and eventually restenosis. Thus, HSA grafted on PGMA could be used as a potential coating for vascular devices.

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