Controlling Protein Adsorption and Bone Cells Response to PDLLA by a Gas Plasma Surface Treatment

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Statement of Purpose: Plasma treatment technologies, such as Radio Frequency Glow Discharge (RFGD) have been used in a variety of applications for improving the biological response to biomaterial surfaces. By changing surface properties different interfacial processes, such as protein adsorption are affected. This, in turn influences cell mechanisms as division or endocrine and paracrine signalling pathways and ultimately the integration or rejection of biomaterials. In this study we merged the concepts of surface characterization and modification with protein adsorption and cellular response. Poly(lactic acid) is a recognized biodegradable material previously explored for several applications including bone replacement, fixation and TE scaffolding. RFGD ability to modify the chemistry and physics of poly(lactic acid) was analysed. Furthermore, the study also aimed to evaluate the response of proteins to modified surfaces and also how surface changes coupled to adsorption of proteins influences cell adhesion and proliferation.

Material and Methods: Poly[DL-Lactide] (PDLLA, Birmingham Polymers, Inc., USA) films were fabricated by casting of polymer-acetone solution under clean conditions. Briefly, surfaces were modified by injecting O_2 (15 psi) into a RFGD chamber (Harrick Scientific Corporation, USA) for 30s and plasma treatment initiated for 180s (Power: 100W, Frequency: 13.5 MHz). After UV sterilization, surface analysis was performed by means of contact angle, adhesion tension of water,[1] surface energy (Owens method[2]), Fourier Transform Infrared Attenuated Total Internal Reflection (FTIR-ATR), X-Ray Photoelectron Spectroscopy (XPS) and Scanning Electron Microscopy (SEM). Two different protein adsorption and cell kinetics studies were performed. In any of the cases, proteins and controls were incubated with characterized samples for 15 min at 37ºC and adsorption assessed by coupling the depletion method to the BCA assay (Pierce, USA). The first study was conducted using single protein solutions and complex systems: 1000 μg/mL of bovine serum albumin (BSA), 100 μg/mL of fibronectin (FN), 0.7 μg/mL of vitronectin (VN) and 1000 μg/mL of fetal bovine serum (FBS). Primary Fetal Rat Calvarial (FRC) cells seeded at $4x10^4$ cells/mL were incubated for 3h, 7, 9 and 14 days in αMEM containing β-glycerophosphate, L-ascorbic acid, and 10% FBS. Cells were trypsinized and counted. In a second study, protein solutions were prepared to 1% of their concentration in the human plasma:[3] 350 μg/mL of BSA, 4 μg/mL of FN, 3 μg/mL of VN and 1% (v/v) of FBS. MG63 osteoblast-like osteosarcoma cells ($4x10^4$ cells/mL) were incubated for 1, 4 and 7 days in DMEM with 10% FBS and proliferation assessed by WST-1 assay. Statistical analysis was performed by two-tailed t-test ($P<0.05$).

Results and Discussion: Water contact angle measurements showed that RFGD surface treatment significantly increased ($P<0.05$; $n>9$) surface wettability (from 75.4±2.6 to 59.3±1.6º), adhesion tension of the water (from 18.3±3.2 to 37.2±1.8 mN/m) and surface energy (from 42.7±2.3 to 50.0±1.8 dyn/cm). After plasma treatment FTIR-ATR changes in the C-H region (3000 cm^{-1} to 2850 cm^{-1}) were observed and XPS revealed an increase in the O1 and C-O-O. Morphological differences were observed by SEM. A solution depletion technique and BCA assay were used to calculate total protein adsorption. Results indicated that on gas plasma modified surfaces an adsorption increase of 5.8% and 15.3% was observed respectively for BSA and FN single solutions. In comparison to controls, the presence of BSA, FN and VN had a positive influence on FRC cell adhesion to gas plasma treated films as shown by higher cell numbers than those obtained for non-treated PDLLA. Also, FRC cell proliferation was decreased by days 9 to 14 in both controls and protein exposed surfaces. Interestingly enough, this is the time when others have shown an increase in alkaline phosphatase expression.[4] In a second study, the relative proportion of these proteins (BSA, FN, VN, FBS) in human blood was used. Although lower protein concentrations were used, MG63 cell adhesion on the different surfaces was consistent to that observed for primary cell cultures. In the presence of proteins, gas plasma treatment improves cell adhesion. In contrast, without proteins, there is no difference in MG63 cell adhesion on gas plasma treated or untreated films. From days 4 to 7 of culture, there was a decrease in OD for modified PDLLA films exposed to VN and FBS. Also, on day 7, gas plasma treated control surfaces presented higher OD than untreated ones. This proliferation delay on control surfaces was understood as a protein absence effect. It is expected that gene expression tests will provide new insights on the cell growth profiles.

Conclusions: Oxygen-based RFGD treatment was shown to modify the surface of PDLLA films. Treated surfaces resulted more hydrophilic, presenting higher surface energy, as well as by chemical and morphology different. PDLLA properties achieved by means of surface modification promoted, in turn albumin and fibronectin adsorption. Although cell adhesion was unaffected by the surface treatment itself, higher cell densities were observed when combining gas plasma modification with the different protein systems.

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