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Effect Of Surface Activated Poly(dimethylsiloxane) On Fibronectin Adsorption And Cell Function

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ABSTRACT

Cell function on biomaterials may depend on surface chemistry and concentration (as well as conformation) of protein molecules. To understand the interplay between these two effects, fibronectin (Fn) was physi-sorbed on a smooth, activated poly(dimethylsiloxane), films spun cast on silicon wafers. Contact angle goniometry, ellipsometry, Atomic force microscopy and Rutherford backscattering spectrometry were used to characterize the nanoscale roughness and thickness of the films. The films were activated by exposure to 30 min ultraviolet ozone radiation. Water contact angle measurements indicated higher hydrophobicity (> 100°) prior to surface activation. Tapping mode AFM scans showed that the activation process produced a rougher substrate (Ra > 0.50 nm). Fibronectin surface coverage after incubating PDMS in 2.5μg/mL of Fn was significantly higher than on non-activated surface, possibly due to favorable hydrophobic interactions between PDMS and Fn. To investigate the effect of surface activation on MC3T3-E1 osteoblast-like cells, cell spreading on PDMS and activated PDMS (30 min) coated with 2.5 μg/mL Fn was studied. Cells plated on the activated Fn-coated PDMS, for 15 min, in DMEM (with serum) showed higher cell attachment. Cell spreading after 72 h plating was clearly favored on the hydrophilic substrates as well. The increase in cell area is attributed to favorable conformational changes in absorbed Fn molecules on these substrates.

INTRODUCTION

The surface characteristics of materials like, their topography, chemistry or surface energy plays an essential role in osteoblast adhesion to biomaterials.
One area of focus in this study has been in fabricating a smooth polymer substrate with a protective oxide of nanoscale roughness, after exposure to ultra violet ozone radiation. The resistance of the oxide formed due to UV radiation and coupled with increasing hydrophilicity to protein binding provides the platform for cell-substrate interactions. Protein adsorption is significantly improved by surface treatment of polymers prior to attachment. The polymer backbone is readily excited by UV laser irradiation to cause cleavage of the polysilane [1]. UV exposure of the polysilanes then leads to formation of an etch-resistant SiO_xH mask and a highly reactive silicon oxide film [2]. Uniformity of surface modification with a corresponding uniformity of adhesion is thus achieved. X-

ray photoelectron spectroscopy (XPS), Rutherford backscattering (RBS), Auger electron spectroscopy (AES) and scanning electron microscopy (SEM) have been used to study properties of these polysilanes. However, data on the use of atomic force microscopy (AFM) technique to study the surface morphologies of these polymers is scanty.

Enhancement of cell function on these polymer surfaces pre-coated with Fn has been reported [3,4], and this is attributed to the denatured conformations of the Fn molecules. Techniques such as fluorescent labeling, radiolabeling, X-ray photoelectron spectroscopy, XPS, and time-of-flight secondary ion mass spectrometry (ToF SIM) have been used to determine total amount of protein adsorbed on such substrates [5, 6]. The protein-surface interaction mechanisms are crucial since accumulation of proteins on biomaterial surfaces plays a critical role in determining the biocompatibility of tissue-implant interface. There is no available data however, that discusses the effect of surface activation on Fn surface density (Γ) and conformation, although Fn conformational changes on hydrophobic polymer surfaces have been investigated. This study utilizes polymer surface activation to control Fn adsorption, conformation and the cell-biomaterial interactions. The objectives are to fabricate and characterize poly(dimethylsiloxane) substrates; understand the effect of polymer surface activation on Fn adsorption; and cell function on activated /non-activated Fn-coated polymer substrates.

EXPERIMENTAL DETAILS

Silicon (Si) wafers (102 cm^2) were sliced into squares (12 mm x 12 mm), rinsed with toluene and dried under nitrogen stream. Thin films of polydimethylsiloxane (PDMS) were then spun cast onto the Si substrates. The PDMS films were exposed to UV/Ozone radiation for times ranging between 10 to 70 min in order to oxidize the surfaces. The films were characterized using contact angle goniometry (to determine wettability), ellipsometry (thickness measurements), Atomic force microscopy (AFM) and Rutherford backscattering spectrometry (RBS). AFM employing tapping mode method was used to analyze the substrates before and after Fn coating. Beams of $^4He^{++}$ were utilized to obtain backscattering spectra of PDMS films containing C, O and Si elements.

Different concentrations (0, 2.5 and 20 μg/mL) of Fn were adsorbed on both the activated and non-activated PDMS. Human osteoblast cells (MC3T3-E1) that expressed several osteblastic markers, *in vitro*, and attaches to a variety of ECM proteins were grown in DMEM supplemented with 10 % FBS and 2 % PSF. Cell attachment was measured, at 600 nm wavelength, by plating 2.0 x 10^5 cells/mL onto the Fn-coated PDMS films for 15 min. Selective labeling of F-actin was performed to study the cytoskeleton organization of the cells on the Fn-coated PDMS films. The MC3T3-E1 cells were plated for 15 min, 1h, 24 h and 72 h, stained overnight in rhodamine-conjugated Phalloidin 548, at 4^oC, for F-actin and analyzed for cell spreading. Phalloidin staining provided convenient labels for identifying actin in fixed cell preparations. Confocal laser-scanning microscope was used to scan extent of cell spreading and cytoskeleton formation.

DISCUSSION

The physicochemical surface characteristics of the poly(dimethylsiloxane) and surface densities of the adhesion protein, Fn, affected the function of MC3T3-E1 osteoblast cells. Contact angle measurements on films before Fn coating showed lower contact angles at high UV/Ozone irradiation times. This signified a gradual decrease in surface hydrophobicity of the PDMS. There was a near linear correlation between contact angles and thickness of films (Fig. 1).

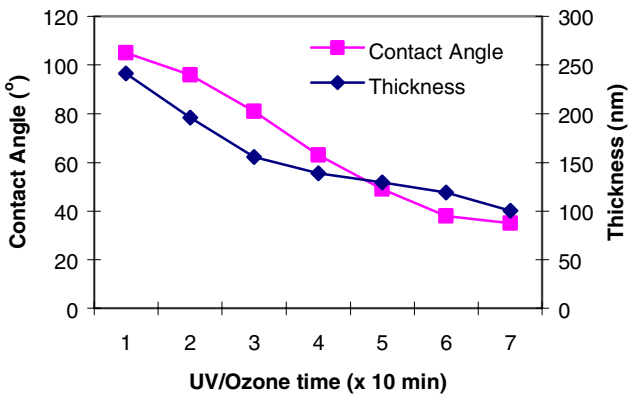

Fig 1: Film thickness of poly(dimethylsiloxane) is nearly linear correlated with the contact angle values measured on the surfaces.

Ellipsometry data (Fig. 1) showed that film thickness decreased as radiation time increased, which indicated surface oxidation of the elastomer by the slow removal of C and H. RBS analysis showed a loss of C with UV exposure. AFM data indicated a nanoscale smoothness (Ra ~ 0.20 nm) of PDMS prior to UV/Ozone radiation, comparable to Ra values on a smooth Si wafer surface. After 30 min of radiation, the Ra of PDMS increased to ~ 0.50 nm. AFM scan on activated surfaces with adsorbed Fn (2.5 μg/mL) showed increased roughness (~ 1.20 nm, indicating successful adsorption of Fn to hydrophilic surfaces. Ra value was slightly higher (Ra ~ 1.30 nm) on the hydrophobic surface (non-activated PDMS) after Fn adsorption.

Fn surface density (> 160 ng/cm^2) obtained on the hydrophobic substrate after incubation in 2.5 μg/mL Fn is comparable to data reported. As the surface hydrophobicity of polymer substrate decreases, Γ also reduces to 120 ng/cm^2 for a 120 min radiated PDMS. A plot of Fn surface density vs. radiation times (Fig 2) shows reduction in Γ decreases by nearly 4x with increasing radiation.

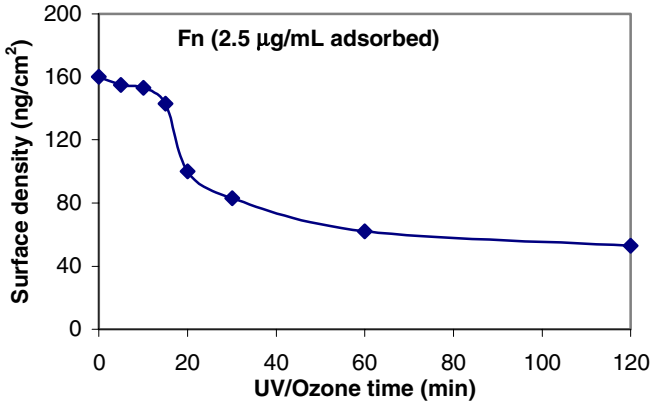

Fig 2: Protein surface density values as a function of UV/Ozone radiation times. The decrease in surface density is attributed to loss of surface hydrophobicity and therefore weakens the protein-surface attraction.

Cell function was generally enhanced on the 30 min-activated Fn-coated PDMS (hydrophilic) than the non-activated substrate. The 25 % increase in cell attachment on the hydrophilic substrate may be due to a favorable Fn conformation and strongly suggests the influence of surface hydrophobicity on cell-substrate interaction, in addition to concentration of the ECM protein. MC3T3-E1 cells transformed from rounded morphology to a discoid shape after 24 to 72 h of plating on these substrates. Organization of actin into microfilament bundles [7, 8] to form an actin cytoskeleton and spreading was similar on both the activated Fn-coated PDMS (hydrophilic) and hydrophobic PDMS up to 24 h, but was more enhanced after 24 on the activated Fn-coated PDMS compared to the hydrophobic PDMS, after 72 h (Fig. 3).

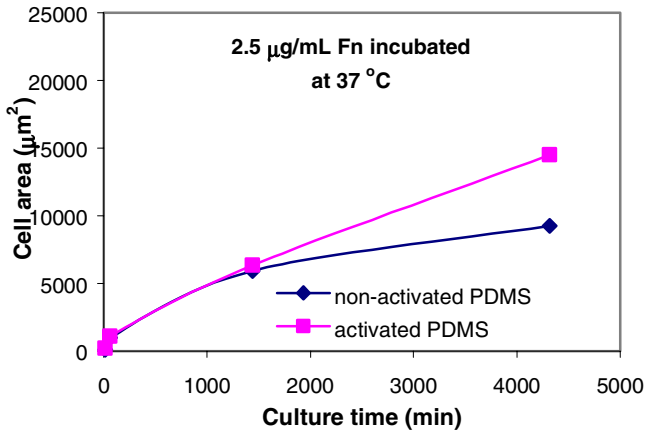

Fig. 3: Area covered by cells due to spreading on Fn-coated PDMS substrates. Greater spreading occurs on activated Fn-coated substrates (squares). PDMS was activated by 30 min exposure to UV/Ozone radiation.

CONCLUSIONS

Fn adsorption and conformation on a functionalized elastomer can be controlled by surface activation. Thus controlling the surface chemistry affects cell-substrate interactions and cellular functions. Data suggests the designing of other bioengineered compatible substrates that can incorporate RGD peptide-containing molecules such as Fn, will greatly enhance and upgrade cellular functions. Improved cell-biomaterial binding therefore, can be achieved by means of a combination of factors such as, surface activation and roughness in order to control protein adsorption.

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