

The Relationship between Fibronectin Conformation and Osteoblast Cell Morphology On Iodine Containing Tyrosine-derived Polycarbonate Blends

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Introduction: Iodine is routinely incorporated into amorphous polymeric materials to increase the X-ray contrast (Yang, Y. Y. *Biomaterials*. 2008, 29, 1901-1911), but can cause distinct physico-chemical changes in the material which in turn can have a variety of biological implications, including changing the nature of the adsorbed protein layer. To assess influence of iodine incorporation of the cell-biomaterial interactions including protein adsorption, a blending strategy was designed to minimizing the amount of iodine necessary within the polymers for X-ray based imaging. In this contribution, 2D thin films of poly(desaminotyrosyl tyrosine ethyl ester carbonate), p(DTE carbonate), its iodinated analog p(I₂-DTE carbonate), and discrete blends (25:75, 50:50, 75:25, 90:10) mass ratio are used as model systems to study the impact of iodine on the amount and conformation of fibronectin adsorption and the resulting morphology of MC3T3-E1 fibroblast cells.

Materials and Methods: Synthesis and characterization of polymers were reported previously (Fiordeliso, J. J. *Biomater. Sci.-Polymer E*. 1994, 5, (6), 497-510). Flow coating was used to prepare thin films (Stafford, C. M. J., *Rev. Sci. Instrum.* 2006, 77, (2)). The thin films were characterized by atomic force microscopy (AFM), contact angle, and X-ray photoelectron spectroscopy (XPS) measurements to elucidate their surface morphology, surface energy, and surface chemical composition. MC3T3-E1 P10 & P14 cells were cultured on the thin polymer films coated on glass and incubated for 4 h before cell fixing/staining. The adsorption of Fn was monitored via Quartz Crystal microbalance (QCM). The morphology of adsorbed Fn on p(DTE carbonate) and p(I₂-DTE carbonate) films were imaged using Fluid AFM in PBS. Changes in the protein secondary structure on these polymers was monitored via FTIR measurements.

Results: Cultured MC3T3-E1 cells spread area do not linearly increase with polymer iodine content as illustrated in Figure 1. Alternatively, subtle differences between different compositions exist in which cells spread more on the 50% by mass p(I₂-DTE carbonate) blend than they spread on both the 25% and 75% by mass p(I₂-DTE carbonate) blend. In addition, no significant statistical differences were found for the cell area on both homopolymers. The Fn adsorption affinity to the thin films was measured by the QCM measurements in which Fn affinity to adsorb on the thin films follows a similar pattern as the cell area up to 75% by mass p(I₂-DTE carbonate) blend. The measured affinity of Fn was twice as high on p(I₂-DTE carbonate) than on p(DTE carbonate).

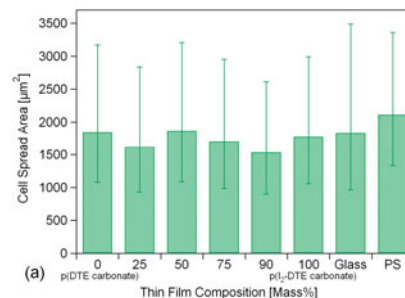


Figure 1. Cell area as a function of thin film composition

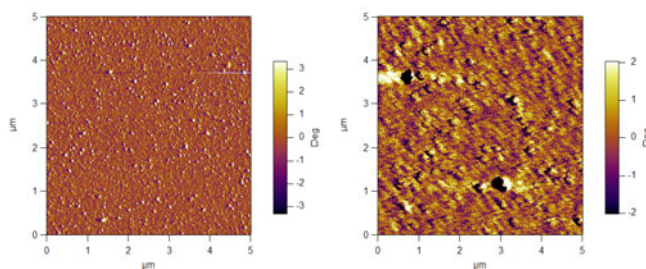


Figure 2. Fluid AFM phase image of Fn Morphology on (a) p(DTE carbonate) and (b) p(I₂DTE carbonate) in PBS buffer.

The morphology/conformation of Fn on both the polymers have subtle differences as shown in Figure 2. Fn tends to adsorb with larger blubs on p(I₂DTE carbonate) than on the p(DTE carbonate). Fn secondary structure from FTIR data demonstrate the subtle difference on the homopolymers and emphasize the impact of the polymer composition on the Fn conformation at the interface.

Conclusions:

MC3T3-E1 cells morphological data as well as QCM data of Fn adsorption show that the frequency shift of Fn adsorption follows the same trend as the cell spread area up to 75% by mass p(I₂-DTE carbonate). Although no significant statistical differences were observed for cell area between the homopolymers, Fn shows higher affinity to adsorb on p(I₂-DTE carbonate) than on the p(DTE carbonate) surface as demonstrated by twice the frequency shift. These data show that there is a subtle relation between Fn adsorption/conformation at the interface and cell area.