Controlling and Probing Protein Orientation / Conformation for Biomaterial Applications

Matthew Bernards, Michelle Maganis, and Shaoyi Jiang.
Department of Chemical Engineering, University of Washington.

Statement of Purpose: In order to develop biomaterials that integrate more readily into the body, work must be done to understand the influence that biomaterials have on the orientation and conformation of adsorbed proteins. Previous work in our group[1] has shown that charged self-assembled monolayers (SAMs) can be used to influence protein orientation, based upon the cellular adhesion levels and cell spreading on various surfaces. This work attempts to further probe the influence that biomaterial surfaces have upon the orientation and conformation of adsorbed proteins. Specifically we examine the influence of charged SAMs on the orientation/conformation of vitronectin (VN) and the influence of collagen and hydroxyapatite (HAP) on the orientation and conformation of bone sialoprotein (BSP) and osteopontin (OPN) specifically bound to these substrates.

Methods: The primary characterization method that is used in all of this work is the measurement of in vitro cellular adhesion levels and cell spreading in each of these systems. Before cell studies were completed, protein adsorption levels were quantified in order to assure that there are equal amounts of adsorbed protein in each of the individual cases that are compared. VN adsorption was quantified on charged SAM surfaces by atomic force microscopy (AFM) in order to assure that complete monolayers were obtained on both surfaces. Due to surface roughness factors, AFM could not be used to quantify the adsorbed amount of protein on either collagen or HAP in the other systems. Instead, protein adsorption isotherms were obtained using I^{125} protein radio-labeling. VN orientation/conformation was probed using bovine aortic endothelial cells (BAECs) and the adhesion levels and spreading in each of the cases was examined by light microscopy. BSP and OPN studies were completed with MC3T3 osteoblast-like cells due to the relevance of these proteins in bone. Cellular adhesion and spreading on collagen substrates was quantified by light microscopy. On HAP substrates adhesion levels were quantified with an MTT cell assay and spreading was examined by scanning electron microscopy (SEM).

Results / Discussion: Upon completion of protein adsorption under carefully controlled conditions, AFM images indicate that a complete monolayer of individual VN molecules could be obtained on both positively and negatively charged surfaces (Figure 1, a-b). When cell studies were completed on these surfaces, it was found that the cellular adhesion levels on positively charged SAMs were significantly greater than the cellular adhesion levels on negatively charged SAMs (Figure 1, c-d). These results indicate that preferential orientation of individual VN molecules can be obtained on positively charged surfaces to promote cell binding to the surface.

The results of the studies on BSP and OPN adsorbed to collagen indicate that when equal amounts of each protein

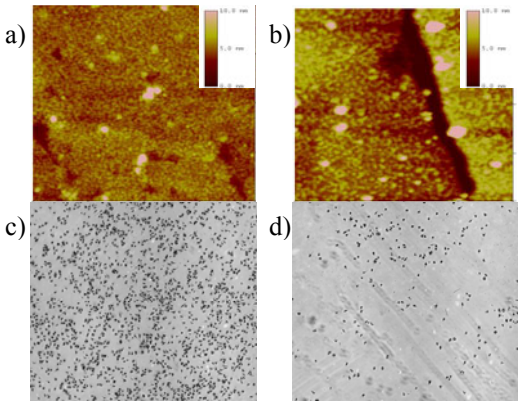

Figure 1: 1 µm x 1µm AFM images of VN adsorbed to (a) positive and (b) negative SAM surfaces showing a complete monolayer of adsorbed protein and 4x light microscopy images of the subsequent cellular adhesion to the (c) positively and (d) negatively charged surfaces.

are specifically adsorbed onto the collagen surfaces, similar amounts of cellular adhesion is obtained, and these are noticeably greater than the adhesion levels to collagen alone. Additionally, the results show that the cells are more spread on the collagen substrates with adsorbed BSP. Finally, the highest level of cellular adhesion and spreading occurs in the case where the collagen substrate is exposed to both OPN and BSP. These results show that OPN and BSP bind to different locations on the collagen structure.

In the studies of BSP and OPN adsorbed to HAP substrates, the MTT assay indicates that there is no significant difference in the cell adhesion in either of the two cases and the negative control (no protein). SEM images of the cells on the HAP substrates indicate that the surface roughness of the substrates leads to difficulties for cell adhesion, and this outweighs any benefits that the adsorbed proteins provide.

Conclusions: Based on the results obtained to date, it has been seen that protein orientation/conformation plays a significant role in cellular adhesion to biomaterial surfaces and that it is possible to control this effect. In studies with VN it was seen that positively charged surfaces oriented VN such that cell adhesion was promoted and that negatively charged surfaces had the opposite effect. Studies also show that cellular adhesion to collagen substrates can be greatly improved when either OPN or BSP is adsorbed to the surface first, and even further improved when both proteins are adsorbed. Finally, it appears that cellular adhesion to HAP substrates is not influenced by adsorbed proteins regardless of their orientation/conformation. This is due to surface roughness which limits cell-substrate contact.

References: 1. Liu L. et al. J Biomed Mater Res 74A: 23-31, 2005.