Manipulating protein structure and distribution on surfaces to control endothelial cell behavior

Lingyun Liu[1], Buddy Ratner[1, 2], E. Helene Sage[3], Shaoyi Jiang[2]
Department of Bioengineering[1], University of Washington, Seattle, WA 98195
Department of Chemical Engineering[2], University of Washington, Seattle, WA 98195
Hope Heart Program[3], Benaroya Research Institute at Virginia Mason, Seattle, WA 98101

Statement of Purpose: Type I collagen is a major extracellular matrix (ECM) component of most mammalian tissues, which plays both structural and signaling roles in many aspects of cell behavior. A better understanding of the relationship between cell behavior and collagen structure will provide fundamental information for a better understanding of the wound healing process and facilitate the development of biomaterials. Collagen I is also one of the most commonly used natural materials for tissue engineering scaffolds. When collagen matrix is in contact with the environment near implant sites, the outer-most surface structure of a collagen scaffold will affect how cells respond to collagen. In this work, we controlled the surface structures of collagen I on various self-assembled monolayer (SAM) surfaces with well-controlled surface properties. All of the collagen films with controlled structures were investigated to determine their influence on the endothelial cells cultured on them. In addition, we investigated the effect of a surface-bound fibronectin gradient on the alignment of endothelial cells, which is expected to provide helpful cues for the design of new materials with better angiogenesis. Controlling the structures and distribution of proteins on surfaces allows a better control of cell-material interactions and opens the way to biomaterial and tissue engineering applications.

Methods: Collagen films with different surface structures were formed on -NH_2, -COOH, -CH_3 and -OH terminated SAMs on gold and characterized by tapping mode AFM. The -NH_2, -COOH, -CH_3 and -OH terminated SAMs were prepared following the standard procedure. Neutralized solutions of native type I collagen (BD Biosciences, Bedford, MA) were prepared by diluting into PBS (10mM, 138mM NaCl, 2.7mM KCl) and adjusting pH to 7.4 with NaOH. The concentrations of collagen I used in this study were 3mg/ml, 1mg/ml and 0.15mg/ml. Alkanethiol-treated gold-coated substrates were immersed into neutralized collagen solutions and incubated at 37°C for 48 h. After incubation, the samples were slowly lifted out of the gelled collagen solutions and rinsed by water to remove all loosely adhered gel and salts. The samples were then dried by N_2, and kept in a desiccator overnight before tapping-mode AFM experiments. Bovine aortic endothelial cells were cultured on all of the collagen films with controlled structures in serum-free medium. Cell properties such as the attachment, spreading, proliferation and viability were investigated.

The gradient of fibronectin was generated by the cross diffusion of $HS(CH_2)_{15}COOH$ and $HS(CH_2)_{11}OH$ on gold substrates followed by NHS/EDC activation and chemical immobilization of fibronectin. The gradient was characterized by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). An endothelial cell assay was then performed on the fibronectin gradient.

Results / Discussion: For protein structure, AFM results show that changing surface chemistry as well as bulk collagen concentration will affect collagen structure significantly. On each type of SAM surface, there is a general trend that as collagen bulk concentration changes, collagen structures vary from no fibrils to micro-fibrils to fibers within the tested concentration range. It appears that fibers are more prominent on the –NH_2 and –CH_3 surfaces than on the –COOH and –OH surfaces. In general, the -OH surface is a relatively non-fibrous surface compared with other surfaces. Cell culture results show that the cell attachment increases but the cell spreading area decreases when the size and density of collagen fiber increase on the surface. We also examined the proliferation, viability, and protein synthesis (e.g., collagen I and fibronectin) of endothelial cells cultured on different collagen structures. For protein distribution, XPS and AFM results show that the fibronectin gradient is successfully generated on surfaces. The cell culture results show that bovine aortic endothelial cells cultured on the fibronectin gradient align parallel to the direction of the gradient while cells on the uniform fibronectin does not demonstrate a directional preference.

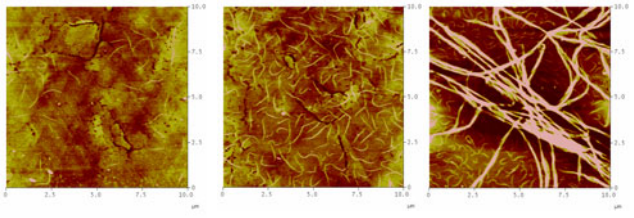

Figure 1. Collagen structures on the -NH_2 terminated SAM surface with the collagen bulk concentration of 3mg/ml, 1mg/ml or 0.15mg/ml characterized by AFM.

Conclusions: In this work, we manipulated the structures of type I collagen on different SAM surfaces. These structures were shown to affect endothelial cell behavior. We also demonstrated the effect of a surface-bound fibronectin gradient on the alignment of endothelial cells. Our findings are expected to provide helpful information for the design of new biomaterials and tissue engineering scaffolds for biomedical applications.

References:

1. Bain CD. J Am Chem Soc. 1989; 111: 7155-7164.
2. Chen SF. Langmuir 2003; 19: 2859-2864.
3. Elliott JT. Langmuir 2003; 19: 1506-1514.
4. Smith JT. Langmuir 2004; 20: 8279-8286.