CONTROL OF PROTEIN ABSORPTION AND CELL ADHESION: EFFECT OF POLYMER GRAFT DENSITY

Ying Mei,[1] Tao Wu,[1] Chang Xu,[1] Kurt J. Langenbach,[2] John T. Elliott,[2] Kathryn L. Beers,[1] Eric J. Amis,[1] Newell R. Washburn, Lori Henderson [1,]*

[1]Polymers Division and [2]Biotechnology Division
National Institute of Standards and Technology
Gaithersburg, MD 20899

Introduction

The control of protein absorption and cell adhesion presents a challenge for various biomedical and biotechnological applications.[1] Surfaces modified with non-fouling polymers such as poly(ethylene glycol) (PEG) are well-known to resist protein absorption and interfere with cell adhesion. Although PEG grafting density is believed to be an important factor to resist protein absorption, the experimental results are not always unambiguous. This is partially due to the fact that grafted PEG layers in many cases are not well characterized and result in poorly controlled grafting density.[1]

Our group and others have demonstrated that gradient techniques provide a fast and convenient tool for high throughput screening of polymeric surfaces over a spectrum of material parameters.[2] Similar to PEG, poly(2-hydroxylethyl methacrylate) (HEMA) is a well-established biomaterial, which can resist non-specific protein absorption and cell adhesion.[3] Although the effect of grafting density on protein absorption has been extensively studied, little has been done to further our knowledge on its effects on cellular response, which is critical to many cell-based biomedical and biotechnological applications such as tissue engineering. The goal of this research is to design combinatorial gradient libraries that elicit specific cellular responses as rapid screening tools. Here, we demonstrated the utility of a combinatorial method in preparation of a novel, cell adhesive protein coated poly(HEMA) gradient with variable grafting densities for the investigation of its effect on cell adhesion.

Experimental[*]

The details of the preparation and the characterization of poly(HEMA) gradients was available in reference 4. In brief, a self-assembled monolayer of octyltrichlorosilane (OTS) was established on a silica substrate by vapor evaporation. The polymerization initiator solution was slowly pumped into a test tube, which contained the OTS self-assembled monolayer (SAM) covered silicon wafer, to backfill the defects inside the OTS SAM and generate an initiator density gradient.[4] Surface-initiated atom transfer radical polymerization (ATRP) was employed to prepare poly(2-hydroxylethyl methacrylate) (HEMA) grafting density gradient surfaces.[5] The thickness of poly(HEMA) film and protein layer were measured by a variable angle spectroscopic ellipsometer (J. A. Woollam, Inc.).

Cell Culture and Cell Imagine Analysis: Substrates were incubated in fibronectin (FN) (25 mg/mL) for at least 5 h at 4 ℃ and rinsed with Dulbecco's Phosphate Buffered Saline (DPBS) before use. NIH-3T3 fibroblasts were maintained in Dulbecco's Minimum Essential Media (DMEM) supplemented with nonessential amino acids, glutamine, penicillin, streptomycin and fetal bovine serum, and maintained in a humidified 5 % CO_2 balanced-air atmosphere at 37 °C. Substrates were placed in four-well tissue culture polystyrene plates and NIH-3T3 cells were seeded on the substrates at (2000 cells/cm^2). Substrates were removed from the incubator after 8 h, and fixed for 24 h at room temperature. Cells were stained by Texas Red-C2-Maleimide and 4',6-diamidino-2-phenylindole (DAPI). Images were collected with 1 mm step sizes over the entire area of the gradient samples, and individual cell morphology and cell density were determined with image analysis software.

Results and Discussion

Synthesis and Characterization of Poly(HEMA) Grafting Density Gradients:

In this investigation, we were able to quantify specific biological responses of NIH-3T3 cells on tailored polymeric surfaces. A combinatorial method was employed to make a FN coated poly(HEMA) surface with variable amounts of FN. This was achieved by grafting poly(HEMA) onto silicon wafer, and essentially producing a conformational gradient with both a mushroom and brush regime (see Figure 1)[6].
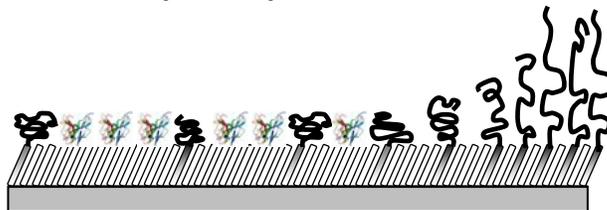

Figure 1. Schematic illustration of poly(HEMA) conformational change from "mushroom" regime to "brush" regime, and FN density gradient established by backfilling the open space between poly(HEMA) chains.

In this study, we employed a recently developed method to prepare a grafting density gradient.[4] The grafting density could be evaluated by the polymer film thickness from the following equation: $\sigma = h\rho N_A/M_n$, where h is the polymer film thickness, ρ is the density of polymer, N_A is Avogadro's number, and M_n is the relative number average molecular mass of the polymer.[6] Ellipsometry was used to measure the film thickness of the gradient poly(HEMA) as a function of position, and the grafting density was calculated from the equation above. The thickness measured by ellipsometry and the corresponding grafting densities of a gradient sample were summarized in Figure 2. A linear increase in film thickness ranging from 18 Å to 75 Å was found across the gradient library, which corresponded to the grafting density from 0.01 chain/nm^2 to 0.083 chain/nm^2. The fitting of X-ray reflectivity profiles (data not shown) confirmed this grafting density library covered a broad range from the "mushroom" regime to the "brush" regime.[4] (Figure 1)
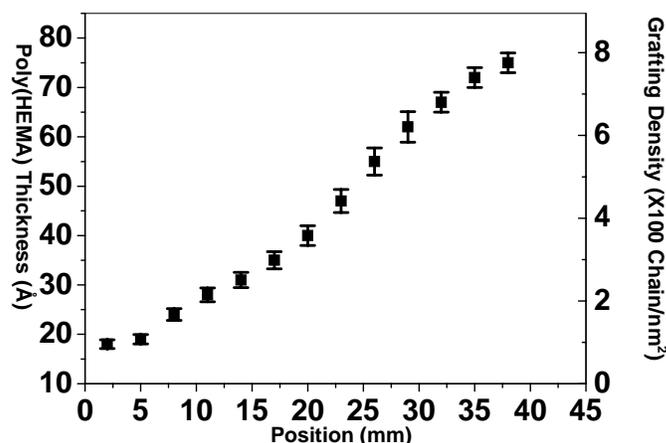

Figure 2. The plot of poly(HEMA) thickness and grafting density versus position on the silicon wafer and exposure time of OTS covered silicon wafer in initiator solution. The error bars denote the experimental uncertainties.

Fibronectin Adsorption on the Grafting Density Gradient Substrate.

The thickness of the FN layer was subsequently measured across the gradient to determine it's density. Figure 3 clearly showed that we were able to produce a well-characterized gradient with respect to the polymer film thickness and the FN density using the backfilling protocol. The results also show an inverse relationship where the FN thickness at the surface decreases with increasing poly(HEMA) thickness at 40 Å. This latter is due to the

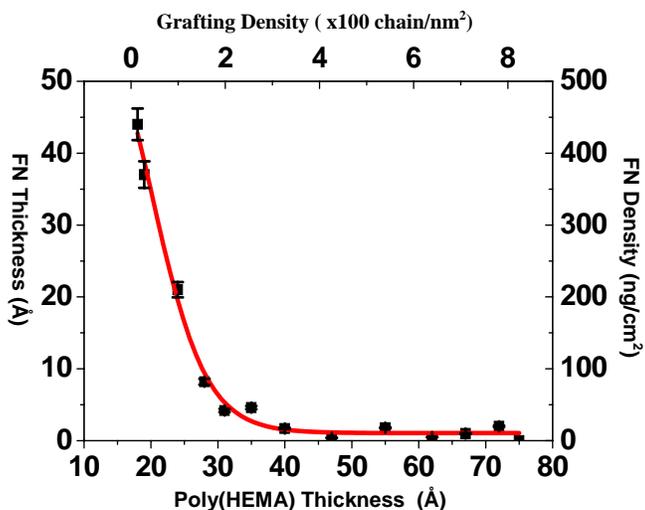threshold limit of detection for FN absorption. Note that this occurs at a poly(HEMA) graft density of 0.0358 chain/nm^2

Figure 3. The plot of FN density versus poly(HEMA) thickness and grafting density.

Cellular Response on a FN-Poly(HEMA) Density Gradient

Cell adhesion and spreading experiments were performed with the fibroblast 3T3 cell line to investigate the effect of FN density on cellular response.[7] The results of cell adhesion and spreading after 8 h cell culture on FN density gradient were shown in Figure 4. As the FN density increased from (0 ± 5) to (90 ± 5) ng/cm^2, the number of adherent cells increased from (1 ± 0.3)/frame to (10 ± 1)/frame. In addition, the cell area increased from (400 ± 50) μm^2 to (1400 ± 100) μm^2. The details of quantitative analysis of cell adhesion and spreading based on an automatic fluorescence microscope will be presented at the conference.
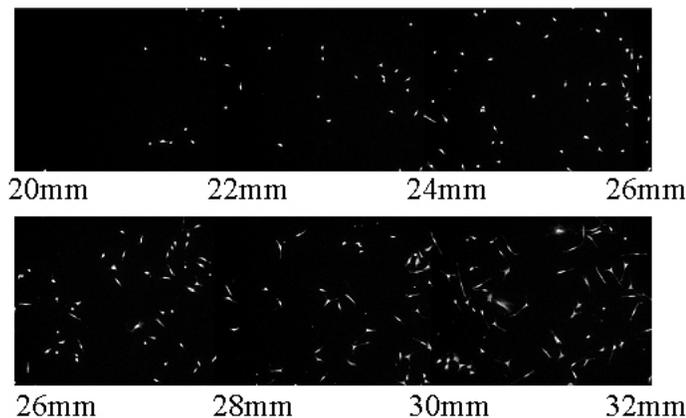

Figure 4. Membrane staining for fibroblast NIH-3T3 cells seeded on FN pre coated poly(HEMA) grafting density gradient.

Conclusions:

This investigation was designed to evaluate cellular responses of novel gradient materials using combinatorial preparation methods. The example presented herein demonstrate the ability to regulate cell adhesion through a rationale design of a poly(HEMA) based material. By varying the chemistry, morphology and functionality of this film, the number of adherent cells and their corresponding shapes varied significantly. The poly(HEMA) grafting density gradients were prepared by combining "controlled" free radical polymerization with gradient technology. The backfill technique was effective in strategically positioning the FN between poly(HEMA) chains. As a result, the cells varied in number and shape with respect to the FN density.

Thus, we conclude that by fine-tuning the grafting density, the cell adhesion and cell morphology could be manipulated.

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