Controlling Cell Adhesion through Dynamic Ligand Presentation

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Statement of Purpose:
The engineering of polymer surfaces or matrices that are capable of controlling cell adhesion has been widely explored. In nearly all of these works, the polymer chains (and ligands) are chemically attached to the underlying substrate or network, and therefore these systems are inherently static. By contrast, the cellular micro-environment is dynamic and remodeled by biochemical actions and biophysical forces. Borrowing this concept from Nature, we created polymer films by an interfacial self-assembly process, whereby individual chains can exhibit lateral or in-plane diffusive motion. Our intent is to manipulate the synergistic effects of film dynamics and ligand density to achieve *selective* cell recognition, and possibly, separation.

Methods: The amphiphilic block copolymer 1,2-polybutadiene-b-poly(ethylene oxide) (PBd-PEO, MW=10kDa) was obtained from PolymerSource, Inc. The peptide RGDS was prepared by solid-phase synthesis and coupled to the PEO block using NHS chemistry. Fibrinogen and Bovine Serum Albumin (BSA) were from Sigma. Fluorescein-labeled lipid (DHPE) was obtained from Invitrogen. Mouse 3T3 fibroblasts were cultured in DMEM medium with 1-10% fetal calf serum. Assembly of monolayer and bilayer films (supported on glass cover slips) was performed by Langmuir-Blodgett and Langmuir-Schaeffer techniques [1]. Protein adsorption studies were conducted by incubation with buffered solutions of 1-10 mg/mL for 1-24 h. The thickness of the adsorbed protein layers after a fixed time was determined by ellipsometry [1]. Cell adhesion studies were conducted with an initial density of $2x10^3$ cells/mL at 37°C and 5% CO_2, and monitored over time by microscopy. FRAP experiments employed fluorescein-labeled DHPE lipid mixed with the block copolymer solution at a molar ratio of 1:10. This mixture was used to create the topmost layer of the bilayer film. Photobleaching of the fluorescent lipid is induced by excitation with focused UV source for 0.5-5 min.

Results:
Fluorescence recovery after photobleaching qualitatively verifies the dynamic character of our films. Preliminary experiments show complete recovery of the initial fluorescence intensity within 2 hours (data not shown), demonstrating lateral diffusive motion. Therefore, the RGD ligands (tethered to the PEO blocks) will also be dynamically presented, and their local density will be subject to cell remodeling. Extensive bleaching causes cross-linking of the PBd block and irreversibly arrests the film dynamics. This crosslinked film is an important control surface. Protein incubation assays show that adsorption of fibrinogen and BSA is substantially reduced on bilayer films as compared to monolayer films [1]. This result is due primarily to the hydrophilic character of the bilayer film, via its high PEO content. Therefore, *plain* bilayer films serve as a non-fouling background to begin adding specific interactions.

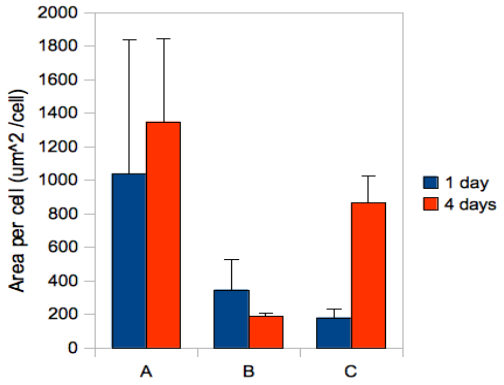

Figure 1. Projected cell area of 3T3 fibroblasts on various surfaces over time. (A) TCPS, (B) plain bilayer film, (C) RGD-presenting bilayer film.

Our cell adhesion study with anchorage-dependent cells (3T3 cells) allowed us monitor changes in spreading behavior and cell density. Comparing the projected cell areas after 1 day and 4 days, the RGD-presenting bilayer films show substantially more cell spreading than plain bilayers and eventually approach that of TCPS (**Figure 1**). Estimating the RGD-labeled concentration as 5 mole%, the RGD density is about 10^4 #/um^2, well in the range of prior studies. However, given the strong increase in spreading over the time, it is likely that the 3T3 cells are actively clustering RGD ligands to achieve a higher local density than what is initially presented. Clearly, exploring different initial RGD densities and polymer molecular weights will shift the time-scale of the cellular response. Importantly, cell viability by the erythrosin B dye does not show any adverse effects.

Conclusions:
The polymer bilayer films constructed are shown to be both dynamic and non fouling, presenting a unique platform for examining questions of cell behavior. Here we have shown that dynamic presentation of RGD ligands allows control over cell spreading and cell density in a time-dependent manner. Future work will focus on the quantification of film dynamics with FRAP on fluorescently-tagged polymers. Cell *migration* on these surfaces should display fundamentally different behavior from prior work in this field. Finally, by using a wider range of cell types we can test the possibility of using *biophysical* features to recognize and separate cells.

References: [1] Lerum RV and Bermudez H. ChemPhysChem 2010;11:665-669.

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