Use of Nylon-3 Polymer Libraries to Characterize and Control Cell-Polymer Interactions

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Introduction

The use of synthetic materials that encourage cell adhesion avoids many of the limitations associated with natural materials, but such materials often require labor-intensive synthetic protocols. Nylon-3 copolymers are intriguing as prospective biomaterials because these polymers have a protein-mimetic backbone (β-amino acid residues) and can be assembled rapidly in functionally diverse forms. Moreover, the chemical features of nylon-3 copolymers are particularly amenable to performing a mechanistic investigation into how discrete, controlled changes in materials chemistry can control cell behavior, thereby yielding information that can be used to construct scaffolds with an optimized composition. We have previously demonstrated that nylon-3 polymer composition can alter cell adhesion (Lee et al., J. Am. Chem. Soc. 2009, 131:16779). In the present work, we describe how elements of the polymer structure regulate cell adhesion both in serum-containing and serum-free conditions; this information may be applied to the design of scaffold environments with predictable and tailored protein adsorption and cell adhesion profiles.

Methods

Wells were created on NHS-ester functionalized glass using silicone coverslips and then incubated with various nylon-3 polymer solutions. Suspensions of either untreated or proteinase-treated 3T3 fibroblasts were added to wells and incubated at 37°C for 1 d at a concentration of 1500 cells/well in DMEM containing 10% FBS or 2000 cells/well in serum-free medium. Cell viability and morphology were assessed via live/dead staining followed by whole slide scanning for fluorescence quantification. Protein adsorption assays were executed by incubating fibronectin (FN), vitronectin (VN), collagen (Coll) solutions or DMEM containing 10% FBS on polymer-functionalized surfaces followed by protein quantification with NanoOrange dye, or by incubating DMEM medium containing 10% FBS followed by protein-specific ELISAs to detect adsorbed individual proteins.

Results/Discussion

In serum-free medium, the extent of adhesion and spreading of 3T3 cells on nylon-3 polymers was dependent upon the subunit composition of the polymers (Fig. 1a). Treatment of cells with various enzymes immediately prior to cell seeding upon the polymers revealed that the mechanism of cell adhesion in a serum-free environment was likely the interaction of cell-surface FN and VN with the polymer substrates, with VN being adsorbed more efficiently than FN. The cell adhesion was linearly proportional to VN adsorption (R^2=0.63; data not shown). In turn, the adsorption of VN was governed by polymer composition, with (DM+CH) as the best combination, followed by (MM+CO) (Fig. 1c).

In serum-containing medium, both protein adsorption and cell attachment were regulated by subunit composition in a trend similar to that obtained in the serum-free condition. 3T3 cells also exhibited varied adhesion efficiencies on copolymers composed of the same subunits but in different ratios, reaching the highest level of adhesion when polymers contained 80% MM or DM subunit (Fig. 1d). Cell adhesion in 10% FBS conditions was also linearly proportional to the specific adsorption of VN from the serum solution (R^2=0.64; data not shown).

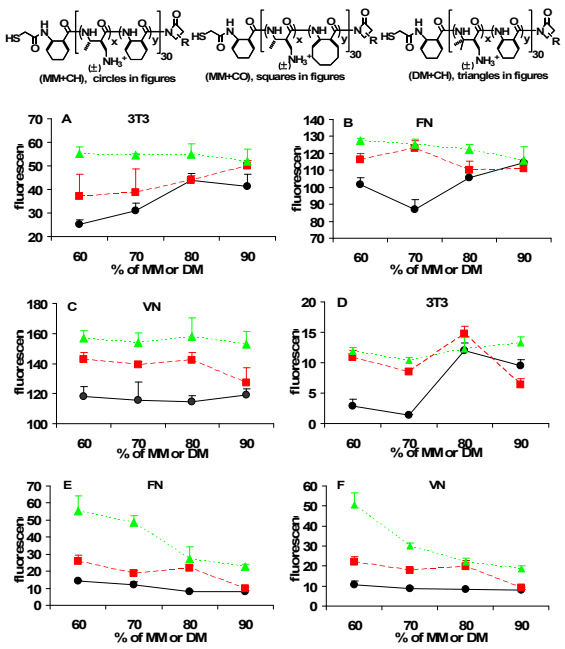

Figure 1. All polymers are random in subunit sequence and stereochemistry, composed of a cationic subunit (monomethyl (MM) or dimethyl (DM)) and a hydrophobic subunit (cyclohexyl (CH) or cyclooctyl (CO)). Data shown are attachment of 3T3 cells (1a, 1d), FN (1b, 1e), and VN (1c, 1f) to nylon-3 copolymers composed of subunits (MM+CH) (circles), (MM+CO) (squares), and (DM+CH) (triangles) in serum-free medium (1a-1c) or medium containing 10% FBS (1d-1f). Lines are drawn only to indicate the trends. Proteins were adsorbed from prepared solutions of single protein (1a-1c) or whole serum protein, or ELISAs for individual proteins adsorbed from DMEM containing 10% FBS (1d-1f). X axis represents MM or DM subunit content. Y axis is fluorescence intensity representing the amount of adsorbed protein or adherent cells.

Conclusions

Our results indicate that, in both serum-free and serum-containing environments, cell-polymer substrate interactions can be manipulated via relatively subtle, discrete variations of subunits within polymers. The creation of such polymer libraries to study cell adhesion may enable the development of highly tailorable materials with predictable and controllable interactions with cells.

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