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Minute changes in composition of polymer substrates produce amplified differences in cell adhesion and motility via optimal ligand conditioning

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Abstract

We explored the interplay between substratum chemistry of polymeric materials and surface-adsorbed ligand concentration (human plasma fibronectin) in the control of cell adhesion and cell motility. We found that small changes in the chemical composition of a polymeric substratum had different effects on cellular motility—depending on the concentration of preadsorbed fibronectin. We used two tyrosine-derived polyarylates, poly(DTD diglycolate) and poly(DTD glutarate), as substrata for the seeding of NIH-3T3 fibroblasts. The only compositional difference between the two test polymers was that one single oxygen atom in the polymer backbone of poly(DTD diglycolate) had been substituted by a methylene group in the backbone of poly(DTD glutarate), The two polymers had closely matched hydrophobicity and physical properties. Flat, spin-coated surfaces of these polymers were pretreated with different concentrations of human plasma fibronectin (0–20 μg/ml). After seeding with NIH-3T3 fibroblasts, we examined the adhesion and motility behavior of these cells. We found that NIH-3T3 fibroblasts migrated significantly faster on poly(DTD diglycolate), but only when the polymer surfaces were pretreated with intermediate concentrations of fibronectin. Only at these intermediate levels of ligand conditioning, did the presence of an extra oxygen atom in the backbone of poly(DTD diglycolate) relative to poly(DTD glutarate) (i) alter the overall organization/concentration of the fibronectin; (ii) weaken cell attachment strength and inhibited excessive cell spreading; and (iii) promote cell motility kinetics. These findings indicate that the biological effect of minute changes in substratum chemistry is critically dependent on the level of surface-adsorbed cell-binding ligands.

Keywords: Polyarylates; Surface chemistry; Fibronectin; Cell adhesion; Cell migration

1. Introduction

A major goal of tissue engineering is to design biomaterials that can specifically govern the functions of attached cells in order to ultimately promote tissue functionality and morphogenesis on implanted biomaterials. Many of the important functions of cells and tissues depend on the presentation of tissue-specific biologic molecules at biomaterial surfaces [1–5]. However, the effect of biomimetic surfaces on cell responses is not well understood [6–10]. Thus, cellular responses to an artificial substratum are difficult to predict in a physiologic environment such as that encountered post-implantation.

Several extracellular matrix proteins, including fibronectin, regulate cell adhesion and motility. Typically, these proteins contain specific peptide sequences (such as the RGD sequence), which bind to integrins with high affinity [11–13]. Fibronectin has been used as a model extracellular

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matrix protein to study cell–material interactions [14–19]. The spatial distribution and the three-dimensional conformation of surface-adsorbed fibronectin has been shown to regulate cell adhesion on biomaterial surfaces [20]. These highly specific interactions regulate how strongly cells adhere to surfaces and how fast they migrate across the surface [19,21,22]. A well known observation of particular relevance to this study is that the strength of cell adhesion to the substrate is an important factor in the regulation of cell motility [1,14,23,24]. Due to the complex nature of the three-dimensional structure of proteins, their adsorption to biomaterials surfaces depends on the hydrophobic/hydrophilic properties and the chemical composition of the surface [15,16,22,25,26]. This study investigates the interplay between the chemical composition of the substratum and cell attachment/cell motility as a function of the concentration of preadsorbed, cell-attachment ligands. In our study, human plasma fibronectin was the model ligand [27], and two tyrosine-derived polyarylates, poly(DTD diglycolate) and poly(DTD glutarate), were selected as test substrata. These two polymers have very similar chemical structures, differing only in the replacement of a single oxygen atom in the polymer backbone in poly(DTD diglycolate) by a methylene group in poly(DTD glutarate) (Fig. 1) [5,24,28]. Despite this change in the polymer backbone composition, the polymers have closely matched physiomechanical properties [5,24]. Particularly important to this study, the polymers also have similar hydrophobic/hydrophilic properties as determined by goniometry. We found that, at certain concentrations of preadsorbed fibronectin, NIH-3T3 fibroblasts were unaffected by the change in the chemical composition between poly(DTD diglycolate) and poly(DTD glutarate), while at other fibronectin surface concentrations, the two polymers elicited significantly different responses in terms of cell attachment and the speed of NIH-3T3 migration. Thus, intermediate levels of adsorbed ligands can make even minute changes in polymer substrates discernable to cells.

2. Materials and methods

2.1. Cell culture

Mouse NIH-3T3 fibroblasts were obtained from the American Type Culture Collection (ATCC; CRL-1658). Cells were thawed from a frozen cell bank, and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 4 mM glutamine, and 200 U/ml penicillin–streptomycin in a humidified incubator at 37 °C and 5% CO_2.

2.2. Substrate preparation

Poly(DTD diglycolate) and poly(DTD glutarate) (Fig. 1) were synthesized as described previously [5,28]. Glass coverslips (12 mm diameter, Belco Glass Company, Vineland, NJ) were sequentially cleaned as described before [29] and rinsed with ethyl acetate before being sonicated twice in a 2.5% (w/v) solution of poly(styrene silane) in methylene chloride. The coverslips were stored in an oven at 60 °C for 2 days and then in a glass vial until needed for spin coating. Thin polymer films were obtained by spincoating 1% (w/v) solutions, prefiltered via 0.45 μm syringe filters, of poly(DTD diglycoate) or poly(DTD glutarate) in methylene chloride onto coverslips. The polymer films were then coated with human plasma fibronectin

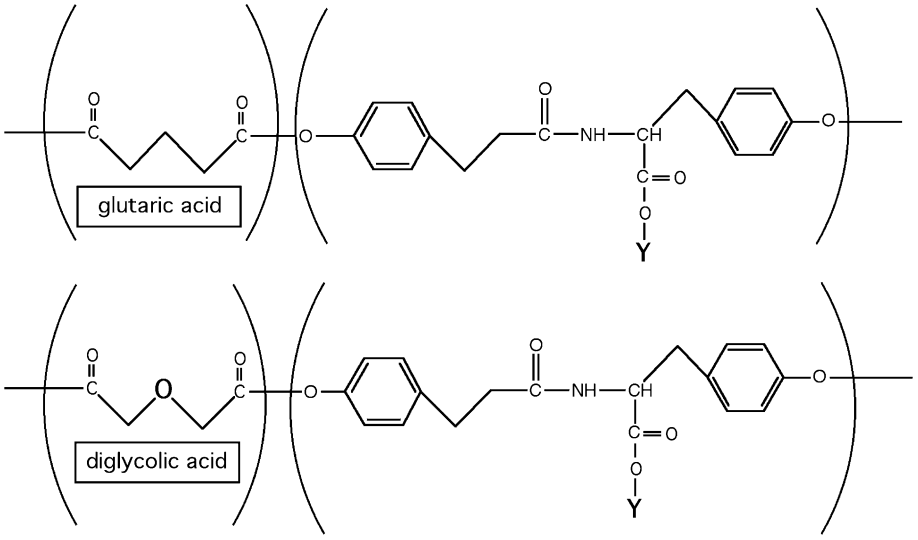

Fig. 1. Chemical structures of poly(DTD glutarate) (top) and poly(DTD diglycolate) (bottom). The two polymer structures are identical, with the exception of the replacement of one single methylene group (–CH_2–) in glutaric acid by an oxygen atom in diglycolic acid. Both polymers present highly hydrophobic surfaces. **Y** is the pendant group that stands for dodecanoyl.

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