Cell Adhesion Micropatterning by Plasma Treatment of Alginate Coated Surfaces

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Air plasma treatment, coupled to a masking technique, was used to promote micropatterned cell adhesion onto a cell-adhesion-resistant alginate coated surface. L-929 mouse fibroblasts were successfully confined into 50 μm diameter cell-adhesive areas patterned inside a cell-resistant layer. The plasma treatment performed, albeit very mild, destroys the molecular architecture of the hydrophilic polysaccharide coating, leading to an enhancement of protein adsorption and hence of cell-adhesion. Both the cell-adhesion-resistant and the cell-adhesive regions are hydrophilic, yet they show a completely different behavior towards cells. Thus, they are a very interesting subject in the study of interfacial interactions in aqueous media, and, in particular, on the mechanisms of bio-adhesion at hydrophilic surfaces.

KEY WORDS: Micropatterning; cell adhesion; wettability; alginate; polysaccharides; surface modification; plasma.

1. INTRODUCTION

Micropatterning cell adhesion by surface microfabrication techniques is becoming more and more popular in cell culture practice.¹ This approach enables the direct visualization of the effect of surface properties on cells function and it allows spatial control of the cellular micro-organization. These characteristics offer interesting opportunities both for basic studies²–⁴ and technological applications.⁵–⁷

Surface micropatterning as related to cell adhesion can be achieved in several different ways.¹ Micropatterning with cell adhesion or cell growth factors takes advantage of specific biomolecular effects. Surface topography micropatterning or micropatterning of regions with different chemico-physical properties are probably the most widely studied approaches. Concerning surface physico-chemical properties, literature contains several interesting examples. Hydrophilicity/hydrophobicity are among the most commonly exploited properties for surface

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patterning. In particular, the Matsuda’s groups presented interesting results on photo-micropatterning hydrophilic (polyacrylamide) non-adhesive regions on tissue culture-polystyrene (TCPS) or hydrophobic (polystyrene) regions on a cell-resistant polyvinylalcohol (PVA) substrate.\(^{8-10}\) As to plasma treatment, several interesting papers have discussed the patterning of cell adhesion on PS surfaces patterned by means of oxygen plasma treatment PS.\(^{11,12}\)

Most literature studies exploit wide differences in surface physico-chemical properties to micropattern cell-adhesion. Typically, one region is hydrophobic, while the other is more hydrophilic: the water contact angle of PS is about 90 degree, while that of plasma treated PS (TCPS) usually ranges from 40–60 deg. The adsorption of proteins that control cell adhesion is markedly affected by substrate hydrophobicity and by the nature and density of functional groups, as well known.\(^{13}\) In this paper, we are interested in micropatterning cell adhesion by more subtle modifications of surface properties and structure. In particular, cell-adhesion-resistant surfaces can be obtained by the covalent linking of hydrophilic polysaccharides, such as dextran, hyaluronan, and alginate.\(^{14-18}\) These same cell-resistant surfaces can be made cell-adhesive by a very simple air plasma treatment.\(^{19}\) In our work, we use very mild treatment conditions, that produce only minor effects on the chemistry of the surface layer yet they have dramatic effects on the outcome of the cell-surface interaction. The purpose of this work is to show that this same approach can be exploited, using conventional masking techniques, to obtain micropatterned surfaces containing cell-resistant and cell-adhesive domains. Contrary to the quoted literature examples, where the chemico-physical nature of the different domains is very different, in the present case both regions show high wettability. In more precise terms, both the cell-adhesive and the cell-resistant interface are extensively involved in Lewis acid-base interactions with the interfacing water molecules and the aqueous phase containing the cell suspension.\(^{19,20}\) We believe that surfaces like these can be an interesting tool in the study of cellular response at hydrated surfaces and, more in general, in the study of the role of hydration effects on the biological response to solid surfaces.\(^{21}\)

2. EXPERIMENTAL

2.1. Materials

For cell adhesion and XPS measurements, surface modification was performed on 5 cm diameter bacteriological-grade PS Petri dishes (Corning). XPS analysis confirms that the surface is clean and free from significant contamination, as shown by the overall surface composition, the detailed analysis of the shape of the C1s peak and of the valence band of the samples. Tissue Culture Polystyrene (TCPS) Petri dishes (Corning) were used as a control in cell adhesion experiments. Allylamine and “low viscosity” sodium alginate \(M_w \approx 20000\), from *Macrocytis pyrifer*a were from Sigma-Aldrich. Sodium alginate (AA) is composed of