Selective Attachment of Mammalian Cells and Polystyrene Microbeads to Functional Amine Patterns Defined by Perfluoroalkyl Silane Surfaces

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Surface modification technologies have been widely utilized for the investigation of the effect of surface properties on selective attachment of cells and cell functions.[1] Recently, hydrophobic surfaces modified with organosilanes such as dichlorodimethyl silane and perfluoroalkyl silane have been shown to form a resistant barrier for cell attachment owing to their low surface energy.[2] Moreover, they provide a chemically inert background and prevent the cross-contamination of liquid droplets on different spots. These characteristics have the potential to be utilized in the application of spot synthesis and array-type cell-based assays. To date, there have been a few reports on the formation of amine patterns defined by hydrophobic silane layers for the spatial organization of cells.[3] However, several problems, such as the use of the limited types of organosilane for the cell-adhesive patterns, complicated deep UV irradiation for the patterning, and possible contamination of patterns from organosilane coupling still remain to be overcome. Therefore, it is necessary to develop a new patterning technique that extends the versatile surface chemistry and eliminates the contamination problem.

Here, we present a novel and simple surface modification method for preparing amine-spacer patterns defined by perfluoroalkyl silane layers and their feasibility for the directed attachment of human retinal pigment epithelial cell line[4] (ARPE-19) and functional polystyrene microbeads. In particular, we have devoted special efforts to solving the contamination problem, which is generated from the conventional photolithography and organosilane coupling method. As illustrated in Figure 1, our strategy is based on the two-step silanization with a simple photoresist (PR) process and then subsequent diamine coupling. To simplify the PR lift-off process, we have designed a home-made rubber stamp for PR printing directly on the substrate in a short time without any further treatment. Formation of isocyanate layers using isocyanatopropyl triethoxysilane (IPTS) on the substrates makes it possible to introduce a variety of amino functional spacers on the surface via urea linkages. In this study, we employed hydrophilic diamine spacers such as 4,7,10-trioxa-1,13-tridecanediamine (TEG-diamine) and Jeffamine ED-600 (PEGdiamine) for the creation of amino-terminal groups that can serve as charged domains promoting cell adhesion, as well as the starting point for the versatile surface modification. Here, we investigated two methods for preparing the fundamental amine-spacer patterns defined by the perfluoroalkyl silane.

As illustrated in Figure 1 (Method 1), the PR was patterned on the acid-treated glass substrate by stamping. Thereafter, perfluoroalkyl silane layers (*1H,1H,2H,2H*-perfluorodecylmethyldichlorosilane, 17F) were formed on the exposed glass surface of the patterned substrates. Subsequently, after the removal of the PR on the substrates with acetone, the substrates were reacted with IPTS to produce patterned reactive sites. However, we found that the 17F layers were contaminated by IPTS coupling and the following amination process, so that substrates were no longer resistant to cell adhesion. In general, functional silane compounds having

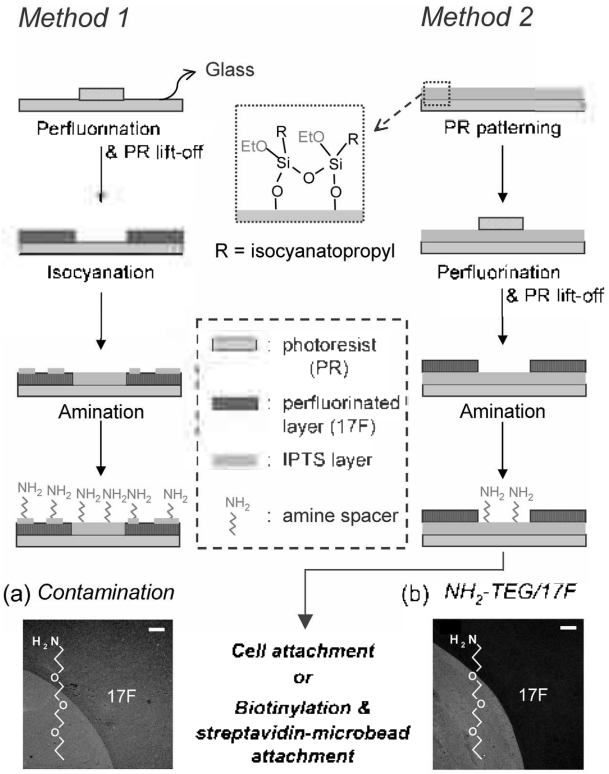

Figure 1. Schematic view of two methods for preparing an amine-spacer pattern defined by perfluorinated layers. (a, b) Fluorescence images of the FITC conjugated amine spacer (*NH2-TEG*) patterned substrates. Scale bar: 100 μm.

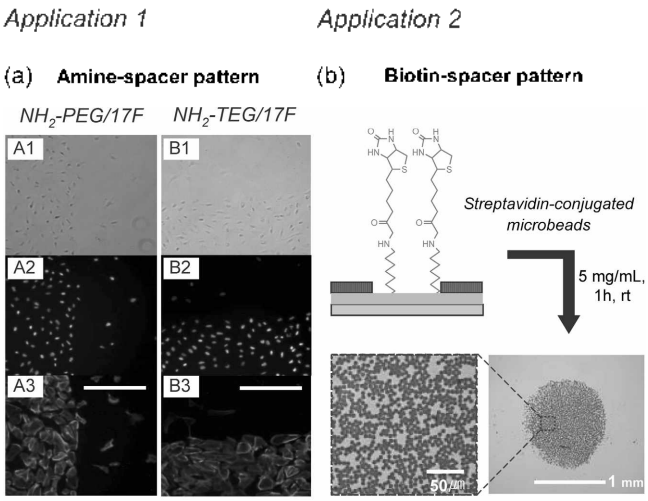

Figure 2. (a) Optical micrographs (A1,B1), and fluorescence micrographs (A2,A3; B2,B3) of the patterned cells over the same region on the designated amine-spacer patterns, scale bar indicates 100 μm, (b) self-assembled streptavidin-conjugated microbeads on the biotinylated amine-spacer patterns.

more than one alkoxysilyl or chlorosilyl group readily form multilayered networks on substrates and in solution.[5] Accordingly, the preformed silane layers (17F) on the substrates were contaminated by the oligomers resulting from the subsequent IPTS coupling.

These results prompted us to modify the surface modification method (Method 2) to solve the previously described contamination problem. In contrast to Method 1, isocyanate layers were formed in advance on the substrates by immersing the cleaned glass slides in a solution of 5% (v/v) IPTS in chloroform for 24 h at 50 °C. In this method, the key point was that the perfluoroalkyl silane layer (17F) could thoroughly cover the exposed isocyanato alkylsilane layers. After PR patterning, the substrates were immersed in a solution of 1% (v/v) 17F in *iso*-octane for 20 min at room temperature. After removal of the PR on the substrates by rinsing with acetone, the resulting substrates (*IPTS/17F*) were treated with the amine spacers in aqueous solution for the grafting of amine-functional spacers on the exposed isocyanate patterns affording the patterned substrates: *NH_2-TEG/17F* and *NH_2-PEG/17F*. The fluorescence images of FITC-conjugated substrates demonstrated that amine-spacer patterns were formed properly on the substrates (Figure 1b). In comparison with the patterns prepared by Method 1, the substrates showed the discrete patterns with low background signals ($S/N = 5.5$-7.9).

Figure 2(a-b) shows the patterning of ARPE-19 cells on the amine-spacer patterns after one day of culture *in vitro*. In order to see the attachment of cells more clearly, F-actin and Hoechst 33342 staining were performed in parallel. It was clearly observed that the cells were selectively attached to the amine-spacer patterns. Most of the ARPE-19 cells on the amine-spacer patterns showed strong attachment with spreading, whereas those on the perfluorinated surface were sparse and only loosely attached to the surface.

In order to investigate the applicability of the amine-spacer patterned substrate for the patterning of microbeads, we firstly biotinylated the prepared patterns and then assembled the microbeads conjugated with streptavidin on the patterns using the biotin-streptavidin affinity binding.[6] Figure 2b shows images of the monolayered microbeads on the biotinylated patterns. The resultant functionalized microbead patterns can provide higher surface area for the biochemical reaction, and are expected to be exploitable in the study of focal adhesion of the cells after introduction of the biotinylated ligands (*eg.* peptides, proteins) onto the microbeads.

Therefore, we suggest that the functional amine patterns defined by perfluoroalkyl silane surfaces, which arrange cells for the selective attachment and maintenance of intracellular signaling, can be applied for cell-based assays and chips. In addition, the developed amine patterns hereby could provide a tool for the modulation of the cell-substrate interaction and the assembly of microbead patterns for biochemical screening. Furthermore, well-organized attachment of the RPE cells on the patterned substrate may find use in the proper formation of blood retinal barrier *in vitro*.

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