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2D spatially controlled polymer micro patterning for cellular behavior studies

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ABSTRACT

A simple and effective method to functionalize glass surfaces that enable polymer micropatterning and subsequent spatially controlled adhesion of cells is reported in this paper. The method involves the application of laser induced forward transfer (LIFT) to achieve polymer patterning in a single step onto cell repellent substrates (i.e. polyethyleneglycol (PEG)). This approach was used to produce micron-size polyethyleneimine (PEI)-patterns alternating with cell-repellent areas. The focus of this work is the ability of SH-SY5Y human neuroblastoma cells to orient, migrate, and produce organized cellular arrangements on laser generated PEI patterns.

1. Introduction

Manipulating and guiding the cellular adhesion and migration for the formation of various cellular networks *in vitro* is of high interest both in medical basic research and for biotechnological applications.

By defining the design and the direction of cellular outgrowth and connectivity on artificial surfaces it offers potential application in the design of prostheses and implants (i.e. which contain grafted cells) or in the creation of novel biosensors or microfluidic devices. This selective surface modification at the micrometer-scale represents the necessary step to direct cellular adhesion and growth into patterns, implying various types of modifications, from chemical surface modification [1,2] to topographical surface modifications [3,4] or combinations of both [5].

Material characteristics are an important issue, especially when they are used in special environments. It is known that cell adhesive coatings do not maintain their surface properties in a physiological environment. One approach to enhance cell adherence on a material that should act as cell adherent coating is the utilization of the possible electrostatic interaction between positively charged amino-groups and negatively charged phospholipids in the cell membrane. Collagen, fibronectin, laminin, or synthetic polymeric cations, such as polylysine or polyornithine, have also been used as attachment promoting factors in numerous studies [6–9]. However, one of the main drawback in the use of the most polymers is the presence of peptide-like amide linkages onto a synthetic polymer backbone which would imply dehydration process and a lost in the structural, mechanical and biological properties of the polymer [8–11]. An interesting alternative is polyethylenimine (PEI) which can avoid the presence of this type of linkage in the polymer backbone. Its electrostatic properties and moreover the chemical stability promotes a stable binding between cell membranes and negatively charged surfaces (e.g. mica, silicon dioxide) [12–14].

Methods such as microlithography or micro-contact printing have been used as tools to produce topographical and chemical patterns. The main drawback of these methods is related to contaminations, a limited number of materials that can be used for obtaining patterns, and the application of masks and stamps. During the last years, lasers proved to be useful tools for fast and accurate patterning of various sensitive materials [15,16], such as DNA [17], proteins [18–21], cells [22], or synthetic polymers [23,24]. In conventional laser-induced forward transfer (LIFT), a transparent substrate is coated with the material to be transferred, i.e. the donor, and placed close to a receiver substrate. The laser beam passes through the donor substrate on a precise spot of the film and results in its deposition on the receiver. In order to avoid any damage of the sensitive transfer material by the laser irradiation, a sacrificial layer or *dynamic release layer* (DRL) can be used [25,26]. The aim of this paper is to obtain two dimensional spatially controlled PEI micro patterns using DRL assisted LIFT and to study the adhesion and cellular behavior of SH-SY5Y human neuroblastoma cells on these patterns.

2. Materials and methods

The transfer was achieved using a single pulse from a XeCl excimer laser (Compex, Lambda Physik, 308 nm, 30 ns). A square mask with variable aperture was applied to utilize a homogeneous

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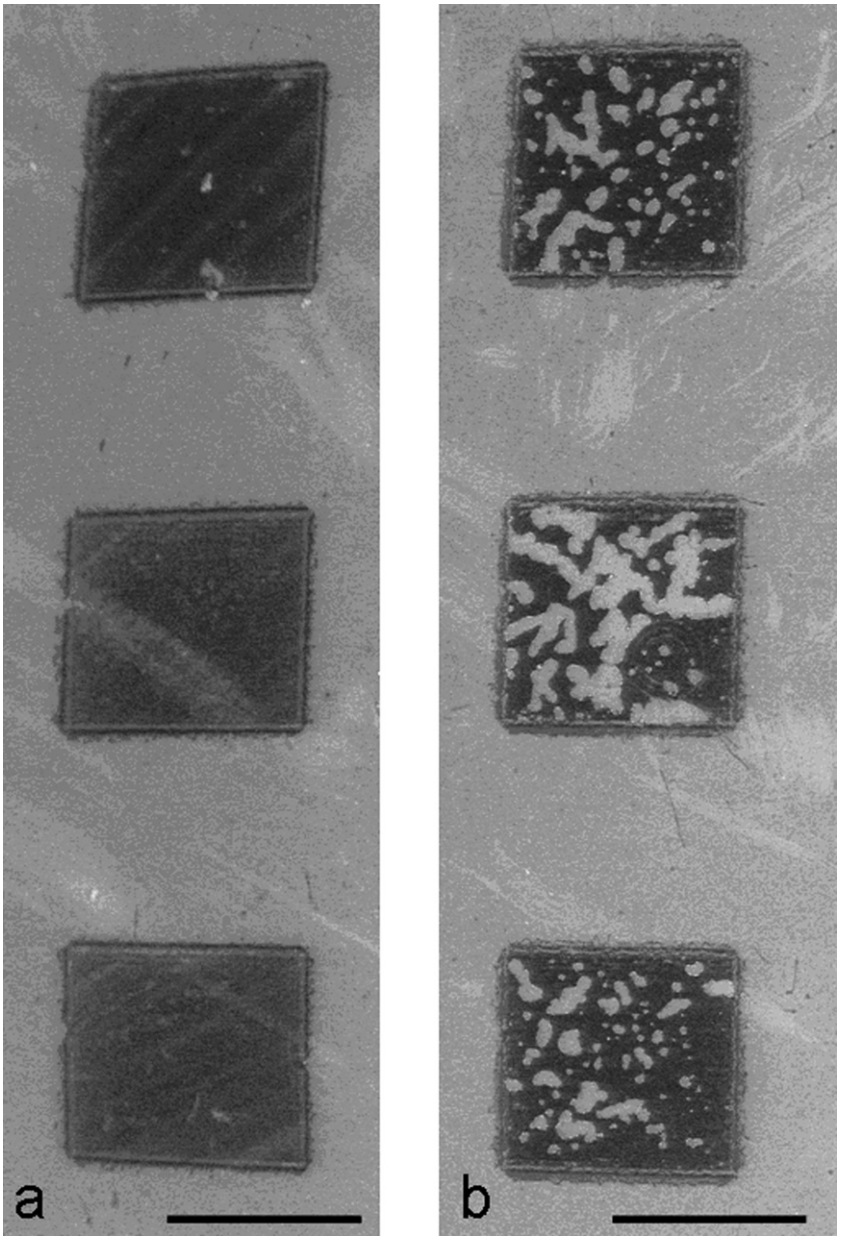

Fig. 1. SEM images of PEI pixels obtained with the high laser fluence of 520 mJ cm^{-2} (a) and with a laser fluence below 400 mJ cm^{-2} (b). The scale bar corresponds to 200 μm.

part of the beam, which was focused by a lens onto the backside of the donor film giving a spot size of 200–300 μm. The donor and the receiver substrates were placed in contact perpendicular to the beam, on a motorized translation stage.

The computer-controlled system allows creating a matrix of pixels for each sample, where the pulse energy and the distance between the PEI pixels (from 50 to 250 μm) are varied. Images were taken by an optical microscope (Zeiss Axioplan) coupled with a digital camera (Leica DC500) and by a Scanning Electron Microscope (SEM).

All transfer experiments were performed in air. To avoid direct laser damaging, an additional triazene polymer (TP) layer with a strong absorption at the applied laser wavelength of 308 nm, was applied as dynamic release layer [25–27].

The multilayer donor films were prepared by coating successively fused silica substrates with the TP and the materials to transfer (PEI). Films of the TP were prepared by spin coating from solutions in chlorobenzene and cyclohexanone (1:1, w/w) with final thicknesses of around 150 nm. PEI, 1.5% in ethanol was spin-coated on top of the triazene layer and thin films with thicknesses of about 100 nm were obtained.

The exact thickness of the films was determined by a surface profiler (Dektak 8000). The receiver substrates were glass plates coated with polyethyleneglycol (PEG) polymer. The coating protocol was the same as for the TP, and the used solution was 2% PEG in water.

Optical Microscopy images were used to analyze the morphology of the deposited pixels and of the adhesion of neural cells on the patterns.

To obtain the cell cultures, the receiver surfaces were washed gently twice with sterile SF-HBSS (Serum-free Hank's balanced salt solution), and placed in 6-cm Petri dishes. 5 ml of SH-SY5Y human neuroblastoma cell suspension in DMEM (Dulbecco's modified Eagle's medium) with phenol red, 10% FCS and 0.1% penicillin/streptomycin, were placed over the surface of the cells and kept in a CO_2 incubator at 37 °C.