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Cell proliferation assays on plasma activated SU-8

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Abstract

The photoresist SU-8 is a common material for the fabrication of polymer microfluidic systems. This leads to the question, how cells respond to SU-8 as a substrate. Here, we investigate the effects of oxygen plasma activated SU-8 on cell culturing by means of cell proliferation assays performed on SU-8 coated glass chips. The assays revealed an increase in cell proliferation on activated SU-8 surfaces as compared to untreated SU-8 surfaces. We conclude that the observed increase in proliferation is most likely caused by a change in the surface chemistry.

Key words: SU-8, MRC-5, biocompatibility, cell growth, plasma activation, hydrophilicity

1. Introduction

The photoresist SU-8 - an EPON based epoxy resin - has proven to be favorable for microfabrication and 3D-lithography due to its excellent capabilities in lithography and its exceptional chemical stability.[1] It is used in soft lithography and for the fabrication of integrated microfluidics, such as lab-on-a-chip systems,[2] microreactors,[3] or sensors.[4] Polymeric microfluidic systems have also been suggested for biological and medical analysis,[5,6] since microsystems are small and, thus, reduce the required fluid volumes. Possible applications include channel systems for the measurement of visco-elastic properties of body fluids,[7,8] highly complex micro total analysis systems, and lab-on-a-chip systems for DNA amplification, and analysis. Implanted systems for health monitoring and drug delivery are also under discussion.[9] While silicon used to be the most important mate-

rial for the production of such systems, polymeric materials and in particular SU-8 have gained importance, since polymers are cheap to process. However, the native SU-8 surface is hydrophobic. Hence, to use SU-8 in passive microfluidics, it has to be activated. There are wet-chemical processes as e.g. with ceric ammonium nitrate [11] and plasma processes e.g. with oxygen plasma.[10]

For biomedical applications, biocompatibility of the material is essential. To assess the biocompatibility of untreated SU-8, cell culture experiments have been carried out. One study following ISO 10993 as well as successful implantation with different surfaces gave evidence that SU-8 might not be fully biocompatible although the study showed that the toxicity of SU-8 has only a minor impact [12]. Another study examined the effect of SU-8 surfaces – both untreated as well as treated with ceric am-

monium nitrate – on carcinoma cells and their genetic information. While cell proliferation decreased in case of the untreated SU-8, the treated SU-8 showed cell growth comparable to that on polystyrene cell culture flasks. Nevertheless, analysis of the genetic material revealed changes in the DNA for one of the wet chemical treatments [13,14]. For plasma activated SU-8 surfaces, biocompatibility still needs to be addressed. Therefore, the present study focuses on cell proliferation on oxygen plasma treated SU-8 in comparison to the untreated surface.

2. Materials and Methods

SU-8 10 as acquired from MicroChem Corp. (Newton, MA) was used with the developing agent SU-8 Dev 600 (MicroChem). All liquids and gases used in the lithographic process (solvents, developing agent, and process gases) were chosen according to clean room standards.

A wafer of soda-lime glass was cleaned consecutively with acetone, isopropanol, ethanol, and pure water in a spin coater. The wafer was heated to 200 °C afterwards and kept at that temperature for ten minutes to remove water from the surface. Immediately after cooling the wafer to room temperature, SU-8 was applied at 500 min⁻¹ for ten seconds in order to dispense the resin. This procedure was followed by spinning for 30 seconds at 5000 min⁻¹ to bring the resin to a final thickness of one micrometer. After ten minutes at rest, the wafer was softbaked for 60 s at 65 °C and another 300 s at 95 °C. Consecutively, it was exposed to UV-light (i-line) for 40 seconds in a MJB55 mask aligner (Süss Microtech, Garching, Germany). A post-exposure bake (65 °C, 60 s followed by 95 °C, 60 s) preceded the development of the wafer in SU-8 developer (300 s). Then the wafer was flushed with isopropanol and dried with clean nitrogen. After processing, the wafer was cut into chips of 1×1 cm².

For the experiments, the chips were grouped in three sets of four chips each. Two of the sets were treated with different doses of low frequency (40 kHz) oxygen plasma (Femto, Diener Electronic, Nagold, Germany), applying 25 W for 30 seconds (2.77 J/cm²) and 50 W for 120 seconds (22.2 J/cm²), respectively, at a pressure of 0.4 mbar. A third set remained untreated. In addition to these SU-8 chips, one test set was cut from a standard object slide. This set was treated with oxygen plasma of 50 W for 120 seconds and served as a control.

After surface treatment, all sets were autoclaved and rinsed with phosphate buffered saline (PBS) to provide sterile surfaces. All four sets of chips were seeded with the same amount of MRC-5 cells. This cell line is derived from human fibroblast like cells from fetal healthy lung tissue. It is commonly used in the quality assurance of cell culture devices. The

cells were cultivated in Eagle's Minimal Essential medium (EMEM) with Earle's BSS and 2 mM L-Glutamine (Invitrogen, Carlsbad, USA) together with 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 1.5 g/l sodium bicarbonate and 10 % fetal bovine serum. During cultivation the cells were stored at 37° C and 5 % CO₂. After three days of cultivation, the cells were fixed by submerging them into 4 % paraformaldehyde in PBS solution for 20 min. Finally, the chips were inspected with an optical microscope and the number of cells/mm² was counted in four areas at the center of each chip. A statistical one way analysis of the count variance with a Holm-Sidak test to a probability of p = 0.05 was carried out.

Surface roughness values were determined by atomic force microscopy (AFM) in tapping mode with a Dimension 3100 equipped with a NanoScope IV controller (Veeco Metrology Inc., Santa Barbara, CA) using silicon cantilevers (BS-TAP300, BudgetSensors, Sofia, Bulgaria).

3. Results

After several days of cell culturing, cell proliferation and cell morphology were investigated by phase-contrast light microscopy. Mainly solitary cells were found on the glass control. Cell agglomerations were very scarce on both surfaces, glass and untreated SU-8 (Fig. 1 a,b). The cells formed a nearly confluent layer on SU-8 surfaces treated with both low (Fig. 1 c) and high (Fig. 1 d) dose plasma. On all samples, the cells showed typical filamentous and vital appearance. A typical fibroblast morphology, as it is described in reference [15], with multiple cytoplasmic appendages interconnecting the cells was observed on the plasma treated surfaces. In contrast, the cells loosely covered the surface and remained solitary in most cases on the untreated SU-8 (Fig. 1 b).

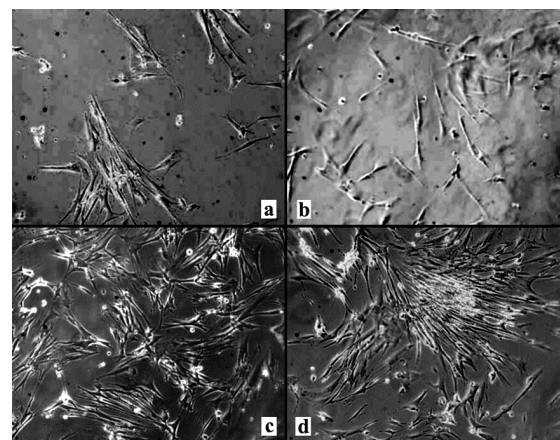


Figure 1. Representative images (10 ×) of MRC-5 cells after three days of cultivation on (a) plasma activated soda lime glass, (b) an untreated SU-8 surface, (c) an oxygen plasma activated SU-8 with a dose of 2.77 J/cm² and (d) 22.2 J/cm².

A quantitative analysis of the number of cells per unit area revealed a significant increase of the cell density on the plasma activated SU-8. As shown in Fig. 2, the density increased from about 50 cells/mm² on glass and 75 cells/mm² on untreated SU-8 to 260 cells/mm² and 350 cells/mm² on activated SU-8 subjected to a low and a high plasma dose, respectively. This observation indicates an increased cell proliferation on activated SU-8 in comparison to the untreated SU-8. The degree of cell proliferation on the SU-8 surfaces activated by a high and low plasma dose did not differ with statistical significance.

Surface roughness was determined by AFM prior to cell culturing. The results are summarized in Fig. 4. The untreated SU-8 surface was rather smooth with an root mean square (rms) roughness of 0.25 nm. Surface roughness is increased by the plasma treatment to 0.3 nm for the low plasma dose and 2.4 nm for the high plasma dose.

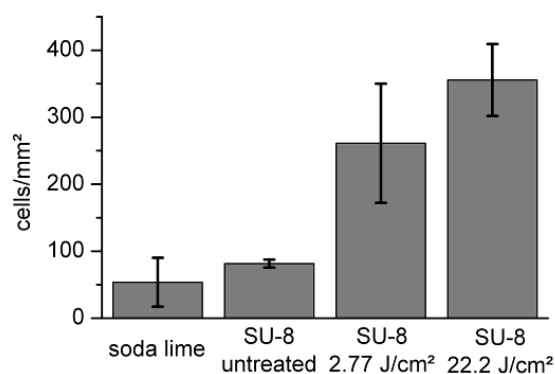


Figure 2. Average and standard deviation of cells counts on surfaces as indicated (n = 16 each).

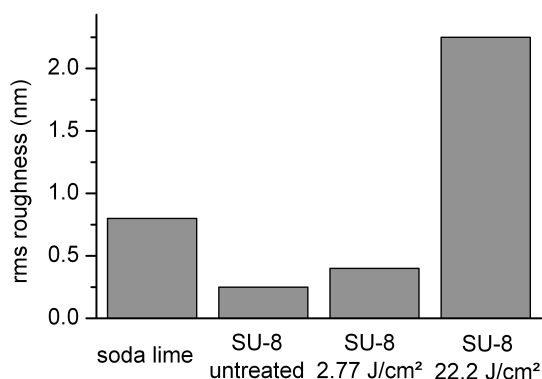


Figure 3. The rms surface roughness of the substrates prior to cell culture.

4. Discussion

For the interpretation of the results it is important to recall that the SU-8 solution contains an antimony salt as photo acid generator. Oxygen plasma treatment of processed SU-8 surfaces not only activates the surface, but also preferentially etches the hydrocarbon components of the polymer. Thus, antimony is increasingly accumulated at the surface [10] and might, thus, influence cell proliferation. Antimony and its compounds are suspected to be toxic, but its toxicity and carcinogenic potency of antimony is still under discussion.[16,17] Several articles describe the clastogenicity and elucidate the effects of antimony to different organisms [16-19]. Despite the enrichment of antimony at the surface of plasma treated SU-8 (up to 2.6 at.%) [10], we did not observe a decreased cell proliferation. This observation can be explained as a combined effect of several aspects. Firstly, antimony could either be chemisorbed at the surface or washed away during the sample preparation. Both processes would reduce the amount of antimony available for the cells. Secondly, the Sb(V) compounds which we found in SU-8 [10] are reported less toxic than Sb(III) compounds [16]. As a word of caution it should be mentioned that it still remains to be tested experimentally, to which extent the interaction with SU-8 might provoke carcinogenic mutations although reference [16] suggests that mutations are not dominant in the case of Sb(V).

The change of the chemical surface properties and the resulting presence of charges could contribute to the increased proliferation on plasma treated SU-8 [20-22]. Our earlier XPS measurements on plasma activated SU-8 showed an increased density of polar carboxyl groups [10], which also cause surface charges in aqueous solution. Thus, the increased wettability of oxygen plasma activated surfaces correlates with an improved cell attachment. Another important factor might be the surface roughness. A correlation between roughness, wetting, and cell adhesion has been reported by several authors [21-23]. However, no increase of cell proliferation could be found on surfaces with a submicrometer roughness for various substrates (PMMA, titanium, hydroxyapatite). Cell adhesion was only enhanced for roughnesses in the order of several micrometers [23,24]. This is in agreement with our observation that there was no statistically significant difference in the cell proliferation on SU-8 surfaces after low and high dose plasma treatment although the rms roughness differs by nearly one order of magnitude.

5. Conclusion

The results indicate that oxygen plasma treatment enhances cell proliferation on bare SU-8. Cell proliferation on plasma etched SU-8 surfaces significantly exceeds that on untreated SU-8 surfaces. The most probable reason for the enhanced cell growth is the altered surface chemistry due to the plasma

treatment. Concluding, the surface activation by oxygen plasma treatment can be a valuable step in processing SU-8 surfaces for applications, where biocompatibility and, hence, cell adhesion is of interest.

Acknowledgements

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