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Evaluation of Cell Plating Efficiency on Polymer Surfaces for Organ-on-a-Chip Applications

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Summary:

Due to their many benefits, polymers have gained popularity recently, particularly in the biomedical industry. SU-8 and polydimethylsiloxane (PDMS) in particular have drawn attention for their advantageous qualities, such as chemical stability and biocompatibility. Proper cellular function in biological environments depends on effective cell-surface interactions. As such, in this work, a method of surface modification that involves coating Poly-L-lysine (PLL) and applying oxygen plasma treatment is reported which appears to be promising in improving the adhesion of HEK 293 cells to PDMS and SU-8 surfaces.

Keywords: Cell attachment, HEK 293 cells, Hydrophilic surfaces, PDMS, Poly-L lysine

Introduction

In recent years the usage of polymers has gained importance especially for biomedical applications. The studies suggest that the interest towards polymers such as Polydimethylsiloxane (PDMS), and SU-8 is caused by their biomechanical features such as chemical stability, transparency, biocompatibility.

PDMS, and SU-8 are widely used polymers in biotechnology. However, in biological applications cell-surface interactions are crucial for cells to function properly. Therefore, we propose a new surface modification method that enables attachment of HEK 293 cells to PDMS and SU-8 surfaces. In this study, PDMS and SU-8 surfaces were treated with oxygen plasma to make the surfaces hydrophilic then after sterilization the surfaces were coated with Poly-L-lysine (PLL). PLL is a synthetically produced compound composed of amino acid lysine. PLL is mainly utilized particularly for the cells that are hard to attach to surfaces for enhancing cell attachment. After the surface modifications, we observed that HEK 293 cells successfully adhere to treated PDMS and SU-8 surfaces.

Materials and Methods

To test the cell plating, PDMS and SU-8 were coated onto 4 inch glass wafers. In order to form a 50 µm-thick layer of SU-8, SU-8 3050 (Microchem. Corp., Westborough, MA, USA) was carefully applied onto the wafer surface and the substrate was spinned first at 500 then at 3000 rpm with a spin coater. Then, using a KLA Tencor profiler, the thickness of the SU-8 3050 resin, was verified as ~ 50 µm. To prepare the PDMS coating, the prepolymer base and curing base were carefully mixed at a 10:1 ratio for 10 minutes. The mixture was both spin-coated onto the wafer, and also the excess was poured into a plastic petri dish to create additional PDMS elastomers to act as reservoirs to contain the cells and culturing media. The prepared PDMS mixture was placed under a vacuum desiccator. The degassed PDMS mixture was then cured for 3 h at 70°C inside a vacuum oven. The SU-8 coated glass slide and PDMS samples were activated by subjecting them to oxygen plasma for one minute. PDMS and SU-8 coated wafer surfaces were sticked to pre-cut and cured PDMS pieces by gluing with drops of uncured PDMS and heating on a hot-plate.

For the culturing of HEK 293 cells, the cells were grown in DMEM (%10fbs+%1 pen-strep+%1l-glutamine) media at incubator. Before the culturing the media was removed then 1mL trypsin was added and waited for a minute at 37°C to detach the cells from the petri dish. Next, 4mL DMEM was added onto the cells. The cells were counted by using a cell counter device. After the determination of cell numbers, the cells were centrifuged at 300g for 5 minutes. Then the volume was determined according to the substrate. The new media was

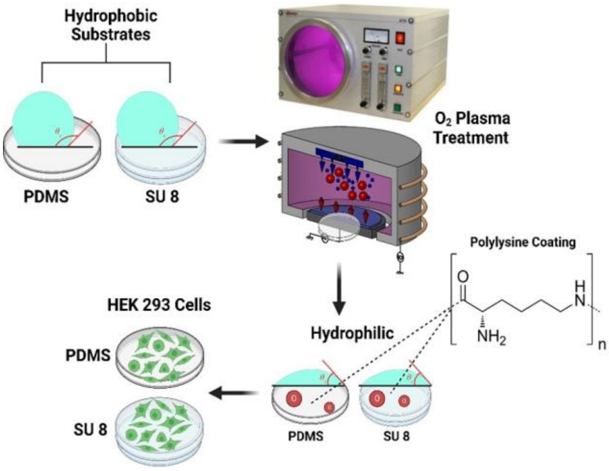

Fig. 1. Methodology diagrams of producing PDMS and SU-8 substrate and plating of HEK 293 cells on the substrates.

added accordingly as 30000 cell/cm^2. Next, the petridish was stirred smoothly and left inside the incubator at 37°C. The experimental procedure concerning cell plating on PDMS/SU-8 coated surfaces is summarized in Fig. 1.

Results

Table 1: Experimental design and results of two different substrates (X: failed attachment, ✓: sucussed attachment).

Polymers/Surface Modification	PDMS	SU-8
Bare Polymer	X	X
Poly-L-lysine Coating	X	X
O_2 Plasma Treatment + Poly-L-lysine	✓	✓

As seen in Table 1, in this study, PDMS and SU-8 materials were coated with poly-L-lysine without any O_2-plasma treatment, and with poly-L-lysine and with O_2-plasma treatment, and cell culturing was performed on these surfaces. As seen in Figure 2, cell attachment was successful only on the PDMS and SU8 materials that were O_2-plasma treated and coated with poly-L-lysine.

Fig 2. a, demonstrates the HEK 293 cell viability on standard cell culture plates as a control experiment. When the same cells were cultured on a PDMS substrate with poly-L-lysine which was non-treated via oxygen plasma in *Fig 2. b*, it was seen that HEK 293 cells were not alive and did not attach to the PDMS- poly-L-lysine substrate.

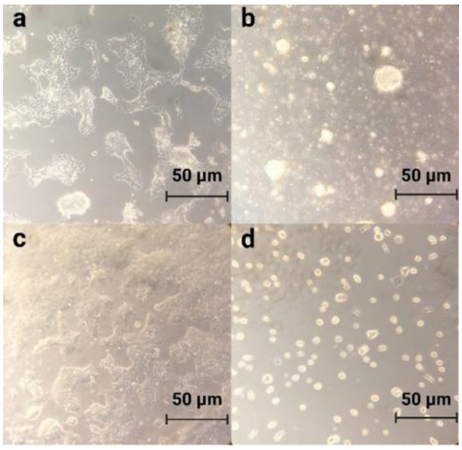

Fig. 2. ***a*** *control HEK 293 cells,* ***b*** *HEK 293 cells on PDMS with poly-L-lysine,* ***c*** *HEK 293 cells on oxygen plasma PDMS with poly-L-lysine, and* ***d*** *HEK 293 cells on SU8 coated plate with poly-L-lysine.*

In *Fig 2. c*, HEK 293 cells successfully clustered and remained viable on the PDMS substrate that was treated with O_2-plasma and afterwards coated with poly-L-lysine. Similar to *Fig 2. b, Fig 2. d* shows optical microscope images of SU-8 substrates without oxygen plasma treatment but with poly-L-lysine coating, where the HEK 293 cells do not attach to the substrates.

These results show that both oxygen plasma treatment and poly-L-lysine coating are needed to adjust the surface conditions for proper cell plating.

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