POSITIONING AND ORIENTATION OF ADHERENT CELLS IN A MICROFLUIDIC CHIP USING THE MICRO PATTERNING OF A PARYLENE-C FILM

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Abstract: A new method for the positioning and orientation of adherent cells on a culture substrate is presented. We demonstrate the ability of a micro patterned parylene-C film deposited on a fused silica substrate to position, isolate and/or orientate cells. Such features are crucial for the development of future biodevices for the analysis and treatment of single-cell or organized cell tissues. In particular, our method is advantageous for controlling the orientation of the cells within an organized tissue while being exposed to an electrical field. The developed method does not require any chemical treatment of the cells or any additional surface modification and is suitable for integration into a microfluidic system.

1 INTRODUCTION

This paper reports a novel method, based on parylene-C patterning, for on-chip cell positioning. This new approach finds various applications such as i) the orientation of cells on biodevices ii) the study of cell motility.

Classically, cell positioning can be achieved thanks to different methods. Different works report on the use of specific (as Fibronectin) or non-specific (as poly-L-lysine) attachment biomolecules (Ruiz, 2009 and Vogt, 2003). These biomolecules are commonly patterned on the culture substrate by microcontact printing process. Another solution consists of using a high magnetic field (up to 10 T) value during cell growth. In these conditions, cell orientation is aligned in the same direction as magnetic field (Umero, 2001). Nevertheless, the efficiency of this kind of method for adherent cells requires (i) the application of a strong magnetic field and (ii) long exposure times (Hiroko, 2000).

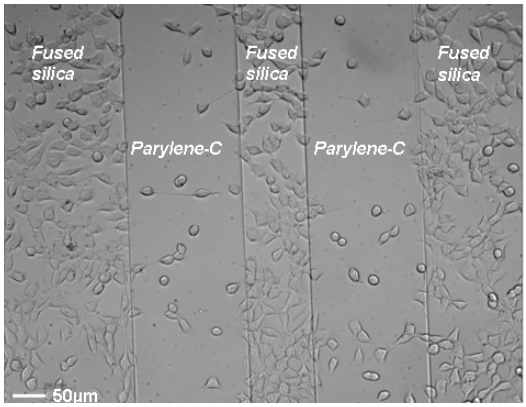

Figure 1: Photograph of NIH-3T3 EWS/fli fibroblasts cultured on a substrate of fused silica covered with two repulsive lines of parylene-C.

In parallel, the use of parylene-C polymer in biodevices has encountered an increasing interest due to its biocompatibility, transparency, conformity properties and long term stability (Shin, 2003 and

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Osaki, 2009). Some current cell-patterning approaches use parylene-C as a stencil to achieve patterns of cells (Wright, 2007 and Tan, 2009). In these cases, the parylene-C film is peeled-off after the cells have grown on the substrate in area delimited by etched windows. This method is also used to pattern proteins on a glass substrate, with various high resolution shapes (Atsuta, 2007).

Nevertheless, these approaches are not directly compatible with integration in a functional microfluidic chip as (i) the peeling of the parylene film inside a fluidic chamber or channel is not permitted (ii) the adherent cells progressively spread out from the initial patterns once the parylene stencil is removed.

Consequently, alternative methods are required to reach cell patterning techniques well adapted to the use in biodevices including microfluidic systems.

We show in this paper that differences in surface properties of parylene-C versus fused silica, combined with the use of specific nanosized or microsized patterns is capable of inducing precise cell patterns, as well as the orientation of cells towards specified directions. Our protocol does not require the removal of the parylene-C film after the cell culture, as the micro-patterned parylene-C film functionalizes the biochip surface on which cells are directly grown. The method benefits from several parylene properties like biocompatility, biostability, chemical inertness and hydrophobic nature.

Previous works have already demonstrated that the nature of the substrate, as rigidity for example, influences the cell movement (Lo, 2000). Here, we show that the difference in cell adhesion affinity between parylene-C and a fused-silica substrate provoques cell migration towards fused silica surfaces and induces their adhesion on the silica surface (Figure 1). The paper highlights that the cells are repulsed from small parylene structures, both in the case of dots or micro-holes as long as the size of the patterns is an order of magnitude lower than the cell size.

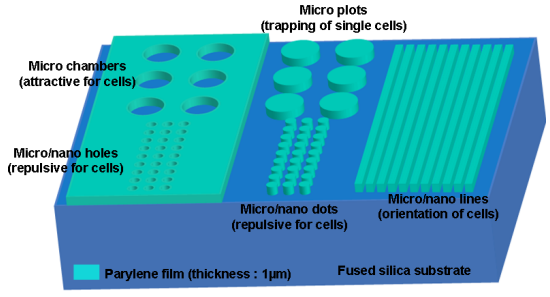

Figure 2: Schema of the patterned parylene film onto the fused silica substrate (arbitrary scale).

Moreover, the patterning of different shapes -lines or plots - within the parylene film (Figure 2), leads to a preferential orientation of cells on the substrate (typical result on figure 8).

2 EXPERIMENTAL SETUP

2.1 Fabrication Process

The chip is fabricated using classical photolithography process for micropatterning the parylene-C. In case of thin film Parylene-C layer (1µm), we used Shipley S1818 photoresin as a masking layer. So first, a uniform layer of parylene-C, 1µm thick is deposited on a 2-inch diameter fused silica substrate using a Specialties Coating Systems Labcoater. The deposition is made by Chemical Vapor Deposition (CVD) after a silanization step, which ensures a good adhesion of the film. Patterns defined by a standard photolithographic process using Shipley S1818 positive photoresin and are subsequently etched through the parylene-C layer by oxygen plasma Reactive Ion Etching (Figure 3). For thicker parylene layers (above 3µm), aluminum layer (200nm) is used as interlayer mask since S1818 is etched proportionally to parylene. To finish, the biochip is sterilized under UV light to ensure that no contamination appears during the cell culture.

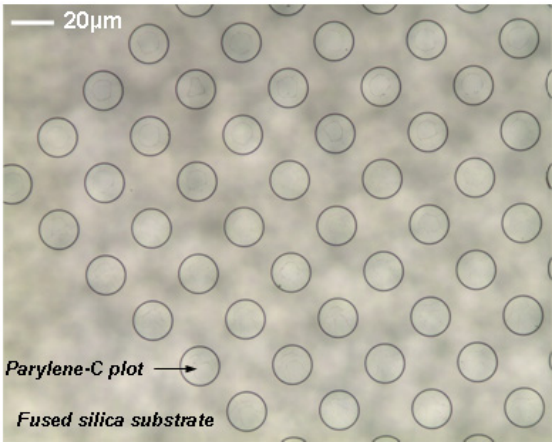

Figure 3: Photograph of a 1µm thick parylene-C film after patterning.

2.2 Material and Methods

Biological tests have been performed using NIH-3T3 EWS/fli fibroblasts which present a high motility (Figure 4). These cells have been specially

chosen for their good properties of adherence and migration.

First, cells are grown in standard culture wells using Minimum Essential Medium supplemented with 10% fetal bovine serum and 10% streptomycin at 37°C in a humidified 5% CO_2 - 95% air incubator.

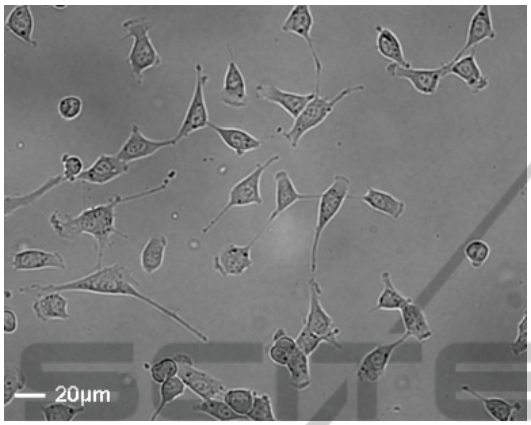

Figure 4: Photograph of a classical NIH-3T3 EWS/fli fibroblast culture.

Then, cells are collected and counted in order to be cultured within the biochip with the convenient concentration.

The chip is firstly placed into a Petri dish and immersed in the culture media. Then, approximately 200 000 cells are added into the media on the surface of the chip. A few hours are necessary to achieve cell adhesion. Cell behaviors such as migration, adhesion and orientation are then monitored during several days, using time-lapse microscopy, for further analysis of the impact of the micro patterned surface on their behavior.

3 BIOLOGICAL RESULTS

Biological experimentations led on these types of micro patterns pointed out several phenomena, which could be used advantageously for cell positioning or orientation once integrated in biodevices.

First, we show that it is possible to trap cells in specific areas of the biochip as shown in Figure 5 (to compare to the control experiment figure 4), thanks to the migration and preferential adhesion of cells towards fused silica surfaces versus parylene-C areas.

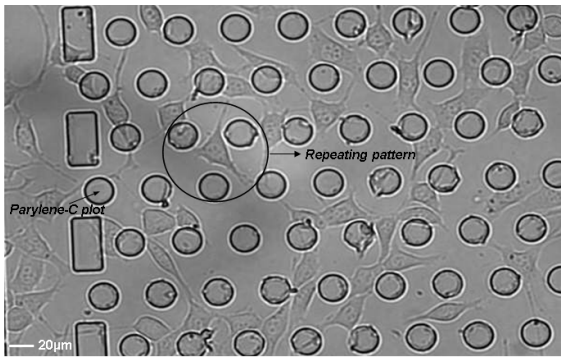

Figure 5: 20µm Diameter dots made of parylene arrayed with a 20µm step on a fused silica substrate. Photograph of cell patterning after 82 hours in culture.

Plots arrayed with a distance comparable to the cell diameter induce single cell patterning (which is the case on Figure 5).

Secondly, we demonstrate that cells are strongly repulsed from the areas covered with micro-dots made of parylene arrayed on fused silica substrate (figure 6.a.). In the same way, arrays of micro-holes etched in the parylene film, down to the fused silica substrate, induce the repulsion of cells (Figure 6.b). These assumptions are verified as soon as patterns characteristic sizes are equal or less than 5 µm.

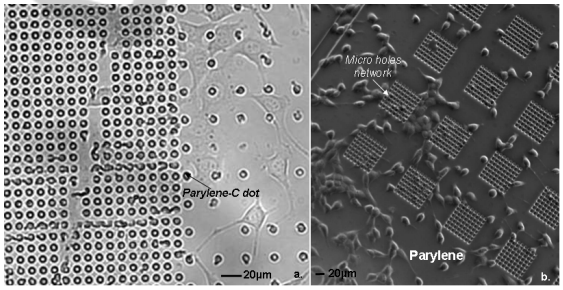

Figure 6: Cells are repulsed from arrays of micro-dots or micro-holes (a) 5µm diameter dots made of parylene, arrayed with a 5µm step on a fused silica substrate. Cell patterning after 82 hours in culture (b) 5µm diameter micro-hole etched in the parylene film down to the fused silica substrate, arrayed with a 5µm step. This network (8x8 holes) is itself arrayed with a 75µm step. Phase contrast image of cells patterning after 88 hours in culture.

Thirdly, we realized time-lapse imaging of cells cultured on the micro patterned substrate for 15 hours. This experiment allowed us to observe the migration of cells towards the fused silica and away from the parylene surfaces (as shown in Figure 7).

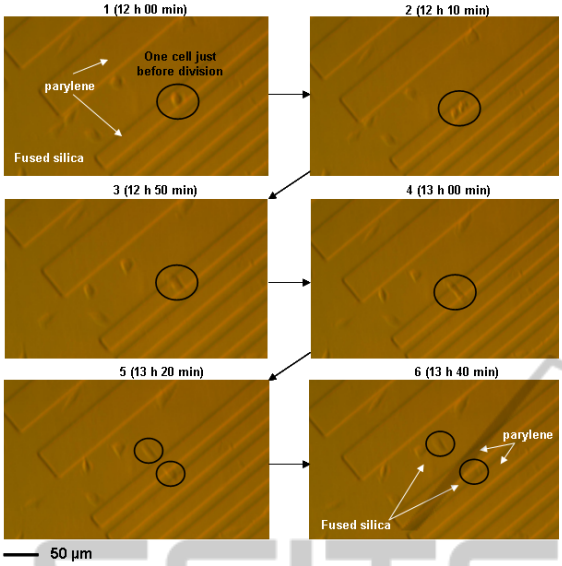

Figure 7: Images of cell division and migration over a span of 1h40min – cells are kept in sterile conditions under 5% $C0_2$ at 37°C – Migrating cells are dark circled. After 10 minutes dark circled cells divided. Then divided cells started to send their pseudopods towards fused silica areas where they finally migrate.

Finally, we show that the micro-patterning of parylene induces the orientation of cells on the substrate, as demonstrated on figure 8 where an array of thin lines (typical width between 1 to 3µm, spaced every 2µm) of parylene-C is performed on a fused silica substrate. As shown on Figure 8, cells orient themselves in parallel to the lines and grow along the parylene lines (phase contrast image, compared to a cell culture made on a non-patterned substrate shown in Figure 4).

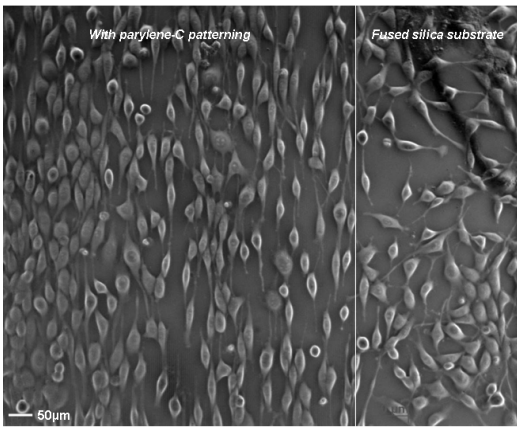

Figure 8: Phase contrast image of cells after 38 hours in culture. On the left side the cells orient themselves along 2µm spaced network of 2µm width parylene lines. On the right side of the images the cells cultured on a plain silica surface display a random orientation.

4 CONCLUSIONS

These results demonstrate the high capability of micro-patterned parylene film on fused silica substrate to position and orientate adherent cells. The developed technique can be easily adapted for use inside a microfluidic system making it very attractive in the biodevice field. It might be used advantageously in cell biochips where a physical, chemical, or electrical solicitation is applied to cultured cells, as these activations may be sensitive to the cell orientation (like electroporation chip for instance). In addition, the possibility to isolate cells may be very promising in the development of biodevices for single-cell analysis.

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