Reproducibility of automated protein microcontact printing with Innostamp40

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Introduction

Microcontact printing (μCP) is a soft lithography technique for the creation of micrometric and nanometric molecular patterns. When used for the production of living cell arrays, these patterns are composed of extracellular matrix (ECM) proteins and can be printed on glass slides, Petri dish, PDMS layers, hydrogels or other kind of materials and formats. The aim is to control the adhesive pattern of the cells to investigate key cellular mechanisms such as differentiation, migration, adhesion, polarity or mechanosensing. This technique is applicable to various types of living cells: stem cells, cancer cells, neural cells, immune cells, epithelial cells [1-8]; and allows biologists to create precise conditions of adhesion over a large population of cells under investigation.

Innostamp40 (Fig 1) is the most advanced instrument of μCP of Innopsys company. It allows the process of μCP to be automated and independent of the operator. The quality and homogeneity of the produced adhesive patterns are improved through a fine tuning of the force applied on the stamp which is totally controlled by magnetic actuation [9]. In addition, the attained reproducibility allows to create Cell MicroArrays (CMA) exhibiting single cell resolution while covering large dimensions (cm²).

This application note analyses the reproducibility of protein patterning produced with the Innostamp40.

Protein printing on glass substrate

Stamps, substrate and proteins

PDMS stamps are obtained by molding PDMS on 4” silicon wafers processed by photolithography with SU8 resist. The first layer of the stamp is made of conventional PDMS by mixing at 10:1 weight ratio, the silicon elastomer and its curing agent. The mixture is degassed for 30 min before pouring on the mold and curing at 60°C for 45 minutes. The second layer of the stamp is made of magnetic PDMS allowing actuation by the Innostamp40 head and the control of the applied pressure during the printing step. The magnetic PDMS is obtained by mixing conventional PDMS precursors and iron powder in a 1:1 weight ratio. After deposition on the top of the first pre-reticulated PDMS layer, curing is performed over night at 60 °C.

The substrate used is a glass slide; it is cleaned with acetone and isopropanol prior to air plasma treatment at a pressure of 0.5 mbar and a power of 50W for 1m30s.
Throughout the study, the ink used to investigate the reproducibility of the printed micropatterns was a solution of Cy3-streptavidin concentrated at (10 \( \mu g.mL^{-1} \) in PBS). The patterns investigated included 3 different shapes, with typical size from 10 to 50 \( \mu m \) and pitch from 10 to 100 \( \mu m \):

**Innstamp40 protocol**

The standard protocol with the Innostamp40 includes an inking step, a drying step and a printing step. The handling of the stamp between the steps is automatized by magnetic actuation.

During inking (Fig 3.b) a drop of ink is left 1 minute on the stamp surface, then dried under nitrogen flow (Fig 3.c). Magnetic contact printing is performed (Fig 3.d) as follows. The stamp is approached 1 mm above the printing area and then released. For 60 seconds, the Innostamp40 apply a constant magnetic force adjusted by the vertical position of the magnet under the substrate. During this contact time the patterns of proteins are transferred to the plasma activated glass slide. Finally, the stamp is slowly removed by magnetic handling (Fig 3.e).

**Figure 2 - Layout of the patterns:** triangles, squares and disks of different sizes in columns: 20, 30, 40 and 50 \( \mu m \) and different spacing between patterns, from top to bottom, 100, 50, 20 and 10 \( \mu m \), repeated for each shape.

**Figure 3 - \( \mu CP \) process diagram**

**Acquisition and Image processing**

Produced patterns are imaged with the scanner Innoscan1100 directly after printing (Fig 4). This scanner can reach a resolution of 0.5 \( \mu m^2/pixel \) with 3 different excitation wavelengths (488, 532 and 635 nm).
In this note, all the images were recorded with a resolution of 1 µm²/pixel and processed by ImageJ. By piling up all the images of a same nominal feature over the whole array, the average representation of each motif can be computed (Fig 5). The coefficient of variation of the area of each motif can also be calculated either inside an array of a printed surface (intra-slide) or from a print to another print (inter-slides).

**Qualification of the reproducibility**

Over 40 printed glass slides, 99.8% of the existing patterns of the stamp were transferred to the substrate. The variation coefficient of areas printed as a function of the shape of the pattern is presented in the chart 1.

Concerning disks, which are the most common printed features, the variation of the printed area is 6% (intra slide) and reaches 8% from slide to slide. The contractility of immobilized cells over an adhesive micropattern is known to depend on their spreading area. Researchers interested in comparing the mechanical stiffnesses of various cells thus need to compare cells in adhesion over a well-defined area [1]. Our method of cell immobilization on protein micro-patterns obtained by µCP is thus capable of setting with a very good accuracy the adhesion of each cell. For example, printed disks of 20 µm in diameter will exhibit a reproducible area of 315 ± 19 µm² over a large array.

The shape of the patterns is also crucial for inducing or investigating a specific cell response. The developed ImageJ macro computes the average representation of all present motifs, over thousands of features. Examples are shown on figure 5. With this kind of statistical representation, we can reveal details of the printed patterns that cannot be observed on the original image of the scanner. In some conditions, the quantity of proteins deposited at the geometrical center of the pattern is higher. This phenomenon is spacing dependent (Fig 5. two right columns) but it is reduced when a rinsing step of the stamp prior to printing is performed.
Conclusion

The automatization of the microcontact printing process thanks to the Innostamp40 enables the production of large arrays of protein patterns exhibiting an excellent reproducibility in shape and dimensions. This quality of patterning is essential for the production of useful cell micro-arrays for advanced research or pharmacological projects.

References


