APPLICATION NOTE

INNOPSYS

InnoStamp 40™ and InnoScan 1100AL™: a complete automated platform for microstructured cell arrays

Microstructured cell arrays are obtained by patterning of extracellular matrix (ECM) components on a substrate to control cell spread. Because such arrays are of interest to different fields related to cell biology, automatic production and parallelized assays are needed. With complete automation of ECM 2D patterning and whole-slide image acquisition, the InnoStamp 40™ and the InnoScan 1100AL™ are ideal tools for high-throughput assays for the study of cell fate decisions in the presence of external stimuli such as drugs, toxins or mechanical stress.

Cell culturing on micropatterned substrates has been shown to be a very powerful tool for studying cell behavior in response to well-controlled biochemical surface cues. Micropatterns of ECM components can be used to control cell attachment in a substrate, either to confine cell shape to a given geometry or to impose cell spread on adhesive and non-adhesive surfaces[1]. In the same way, the ECM and other environmental factors can be modulated to create structures with signaling microenvironments that can influence cell differentiation[2]. ECM composition can influence cell motility, and some studies with ECM gradients have also been used to study neuronal growth.

ECM microarrays are thus promising tools in cell biology. Nevertheless, two main issues have to be addressed before this technology can be translated into a high-throughput approach that is highly reproducible and easy to use in cell biology laboratories. The first one is the precise control of the size and position of the patterns on the surface (alignment) together with the transfer of reproducible and homogeneous quantities of different proteins of the ECM. Microcontact printing (μCP) is a cost-effective solution for creating micropatterned ECM arrays. In μCP, a polydimethylsiloxane (PDMS) stamp is used to transfer ECM molecules with the desired microfeatures (**Fig. 1**). However, there are limitations to the quality of protein transfer in manual μCP resulting from uncontrolled inking techniques and contact forces, making manual μCP difficult to use as a routine technique.

The second limitation concerns image acquisition. ECM arrays, like all cell arrays, need to undergo whole-slide imaging in order for all of the conditions displayed on the slide to be compared under the same imaging conditions. Also, high-throughput approaches need automated processes with reduced human intervention. Although motorized microscopes and other digital microscopes can be used to obtain whole-slide images, most of them use a tiling system. To obtain a whole-slide digital image, tiling systems acquire multiple individual images that are then stitched together. This process can be tedious and long, and very often it requires the supervision of the user, which makes the process a poor candidate for automation.

To overcome the challenges associated with both ECM patterning and cell-array image acquisition, Innopsys has developed a complete platform consisting of an automated μCP printer, the InnoStamp 40™, and a three-color fluorescence scanner, the InnoScan 1100AL™, capable of whole-slide high-resolution imaging.

Magnetic assisted μCP for automated ECM patterning

We previously reported the use of magnetostatics in μCP[3]. The InnoStamp 40™ implements this technology and uses magnetized PDMS stamps and permanent magnets to automate the μCP process while controlling the contact force. This magnetic assisted μCP allows for homogeneous transfer and alignment of molecules such as ECM components and other signaling proteins without altering protein properties.

The automated platform is outlined in **Figure 1**. In a typical patterned cell assay, magnetic PDMS stamps with the desired patterns are inked with different ECM proteins (fibronectin, collagen or laminins) at 100 μg ml^{-1} for 1 min (**Fig. 1b**). After drying, a magnetic stamp is automatically carried to the printing area, aligned and brought into contact with a chemically activated microscope slide for 1 min with a controlled contact force. **Figure 1h** shows some examples of patterns transferred onto glass slides.

Once the ECM proteins have been transferred to the slide, the slide is treated with polylysine-grafted polyethyleneglycol (PLL-g-PEG) to

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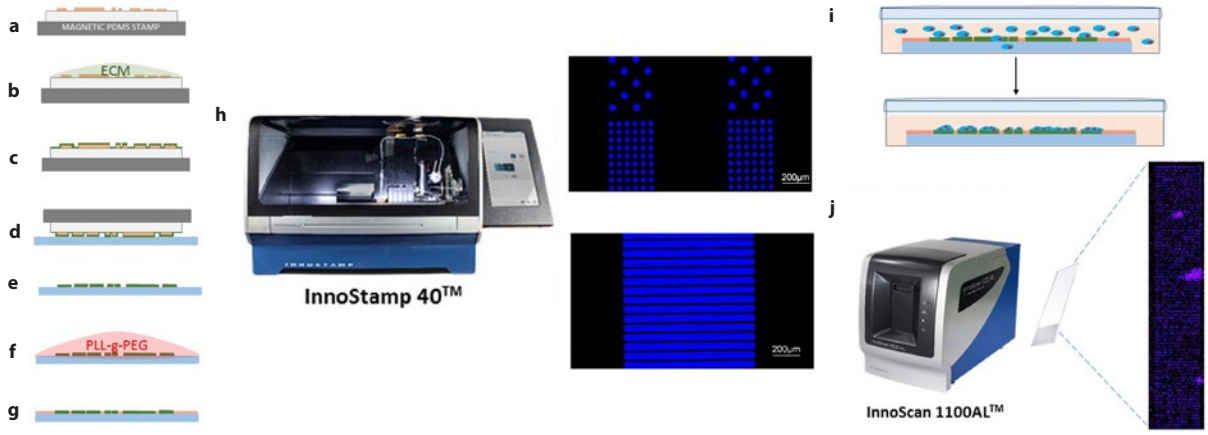

Figure 1 | Automatic microstructured cell array process used in InnoStamp 40™. (**a**) A magnetic PDMS stamp containing the features is used as a transfer stamp. (**b**) The magnetic PDMS stamp is inked with ECM components. (**c**) The stamp is air dried. (d) The magnetic stamp is put in contact with the substrate. (**e**) ECM components are transferred to the substrate according to the stamp pattern. (**f**) The slide is treated with a non-adhesive molecule such as PLL-g-PEG. (**g**) The antifouling molecule covers the regions around the ECM pattern. (**h**) The InnoStamp 40 automated microcontact printer together with some examples of ECM patterned slides. The spot diameter and line width range from 50 to 150 µm. (**i**) Cells cultured in micropatterned slides; after 24 h cells selectively attach to sites containing ECM molecules. (**j**) After fluorescence staining, cells are detected with the InnoScan 1100AL scanner. A whole-slide image at a resolution of 0.5 µm per pixel is shown.

prevent cell adhesion outside the printed ECM patterns. The slides are then incubated with prostate cancer cells expressing green fluorescent protein (PC3-GFP cells) for 24 h. After incubation, cells are dyed with DRAQ5 for nuclei detection, fixed with 10% paraformaldehyde for 30 min and air dried.

Whole-slide imaging

After cell fixation, the slides are scanned using the InnoScan 1100AL™ fluorescence scanner. We carried out whole-slide scanning at a resolution of 0.5 µm per pixel with an auto-focus system (the equivalent of a 20× microscope objective). Two-color acquisition was done with DRAQ5 dye to detect nuclei in the 635-nm channel, and a 488-nm excitation wavelength was used to detect the expression of GFP.

As shown in **Figure 2**, cells selectively attached to the patterned sites containing ECM protein, in this case collagen. PC3-GFP cells that attached to printed collagen patterns showed very good spread and a good shape. In contrast, the few cells that attached to PLL-g-PEG–coated regions exhibited a typical round shape, indicating poor viability. This demonstrates that micropatterning was successfully achieved.

Conclusion

Combining magnetic assisted µCP with whole-slide imaging, the InnoStamp 40™ and the InnoScan 1100AL™ form a full automated platform for microstructured cell array assays. The InnoStamp 40 provides homogeneous deposition of various ECM proteins and precise alignment of patterns. With the magnetic technology, PDMS

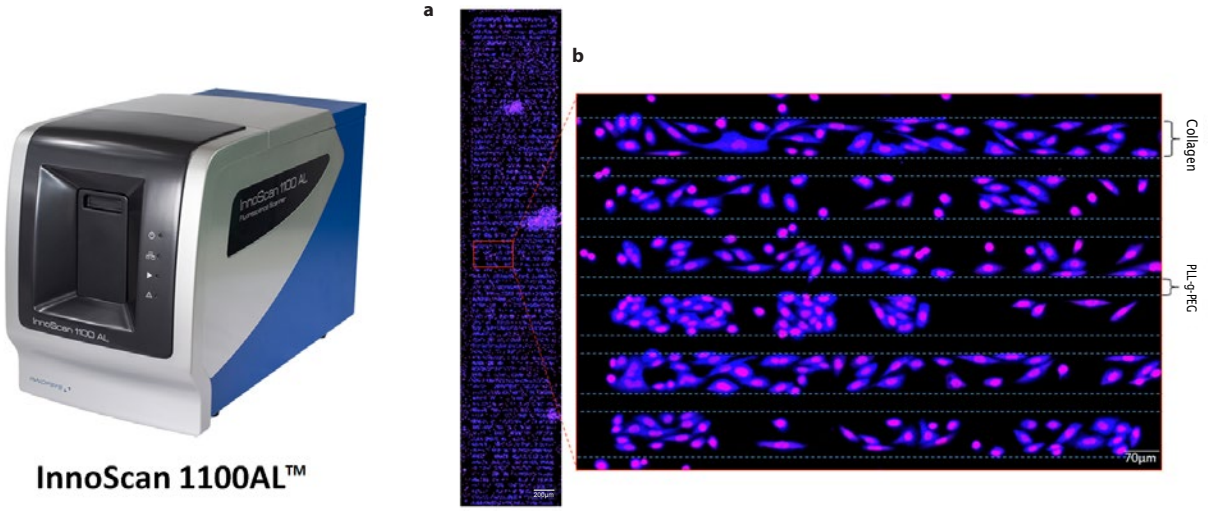

Figure 2 | ECM cell-spread patterning. PC3-GFP cells were cultured on an array of printed lines of collagen surrounded by antifouling PLL-g-PEG zones. Nuclei were labeled with DRAQ5 dye detected in the 635-nm channel (magenta). GFP (excitation/emission wavelength: 488/509 nm) was detected in the 488-nm channel (blue). (**a**) A whole-slide image scanned at a resolution of 0.5 µm per pixel. (**b**) Zoomed view of the image in **a**.

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stamps can be manipulated automatically through all the steps of a full μCP process without user intervention (**Figure 3**), and the contact between the PDMS stamp and the substrate can be controlled over a whole slide or a 4-inch wafer, leading to a large-surface, homogeneous transfer of ECM protein patterns.

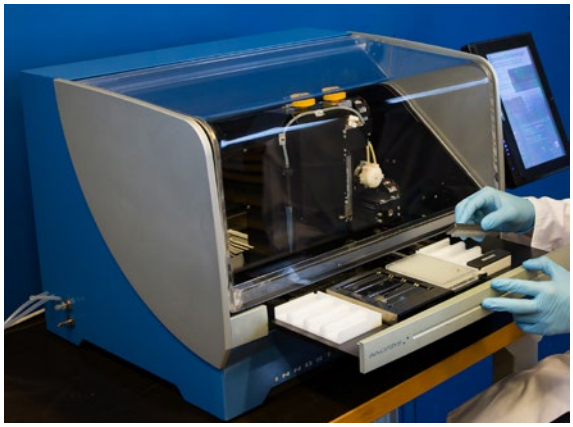

Figure 3 | The Innopsys InnoStamp 40™ uses permanent magnets to automate the full microcontact printing process.

Whole-slide scanning is crucial to obtain cell images with the same scan conditions over a whole slide. Automatic scans are necessary to standardize both imaging and interpretation methods. Tiling methods are not satisfactory because they need user validation for image stitching and smoothing, processes that can be long and tedious. The InnoScan 1100AL™ allows for whole-slide imaging either at a fixed focus or with a content-based autofocus allowing the user to focus on cell-containing sites. With three independent lasers and associated photomultipliers, the InnoScan 1100AL™ is able to scan signals of three different dyes simultaneously without any user intervention.

This platform allowed us to create structured culture systems in which cells were selectively attached to ECM proteins. Moreover, with the automatic alignment provided by the InnoStamp 40 device, repeated microcontact printing steps are easily performed, making it possible to achieve multipatterning through the deposition of several distinct proteins at specific locations. Applications in cell biology and medical fields, microfluidics and cell chips can be considered.

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3. Cau, J.C., Lafforgue, L., Nogues, M., Lagraulet, A. & Paveau, V. Magnetic field assisted microcontact printing: a new concept of fully automated and calibrated process. *Microelec. Eng.* **110**, 207–214 (2013).