Magnetic-microcontact printing based ECM nano-patterning allows homogeneous controlling of cell growth and behavior

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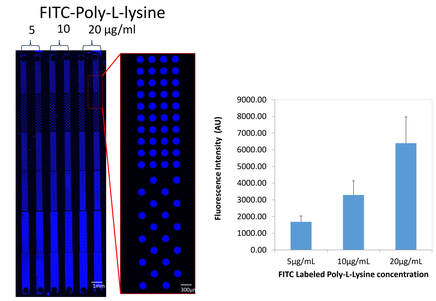
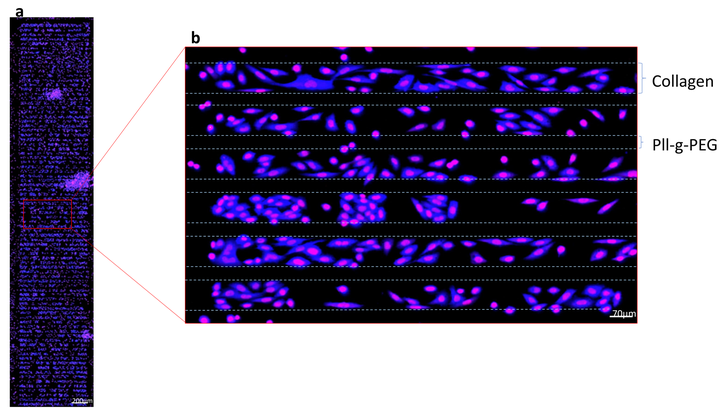
Abstract

Surface nano-patterning with biochemical cues has been shown to be a very powerful tool to control cell growth and attachment. However, manual patterning methods are unable to assure pattern homogeneity in large surface, introducing biases in the analysis of cell response face to external stimuli such as drugs, toxins or others. Here we present a new patterning method that allows to automatically pattern large surfaces such as whole microscope slides or 4in wafers. Using magnetic-microcontact printing, microscope slides were printed with different 50-150 µm patterns of extracellular matrix proteins (ECM) with a pitch of 40-220 µm. PC3-GFP cells were cultured on patterned slides. Fluorescence detection was used to evaluate cell spread homogeneity on nano-patterns. Results show homogeneous cell growth and attachment on defined patterns all along the patterned surface. Using this new methodology, the study of cell behavior in response to well-controlled biochemical surface cues can be studied. In the context of multiplexed assays for high-throughput screening, automated magnetic-microcontact printing is a method of choice providing a high level of reproducibility and homogeneity over large surface of analysis.

Results

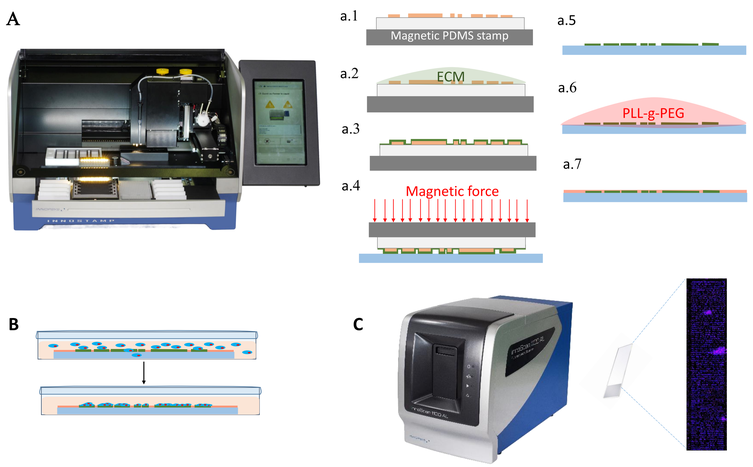


ECM protein nanopaterning: In order to test the uniformity of protein patterns, microscope slides were patterned with different concentrations of fluorescent-labeled ECM proteins using the InnoStamp 40 automate. A representative image for FITC-labeled poly-L-Lysine patterning is shown. Different 50-150 µm patterns such as horizontal or vertical lines and dots were used as nanopaterns (pitch 40-220µm).
Fluorescence intensity was quantified for each of the 87 independent slides, a linear correlation between fluorescence intensity and protein concentration is observed.



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 with the InnoScan 1100 AL imager. (**b**) Zoomed view of the image in **a**. Cell attachment and growth is confined in the collagen patterns.

Methods



Microstructured cell array process. **Panel A. Automatic ECM patterning using the InnoStamp 40 magnetic-µCP automate: a.1** A magnetic PDMS stamp containing the features is used as a transfer stamp. **a.2** The magnetic PDMS stamp is inked with ECM components. **a.3** The stamp is air dried. **a.4** The magnetic stamp is put in contact with the substrate. Controlled magnetic force is applied. **a.5** ECM components are transferred to the substrate according to the stamp pattern. **a.6** The slide is treated with an antifouling molecule such as PLL-g-PEG. **a.7** The antifouling molecule covers the regions around the ECM pattern. **Panel B.** Cells cultured in micropatterned slides; after 24 h cells selectively attach to sites containing ECM molecules. **Panel C.** After fluorescence staining, cells are detected with the InnoScan 1100AL scanner which allows to image the whole slide at 20x magnification in a single step.

Conclusions

- Magnetic assisted µCP is a useful tool to nanopatern substrates such as ECM proteins. This methodology has been proved to be simple and highly reproducible.
- 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 stamps can be manipulated automatically through all the steps of a full µCP process without user intervention.
- The InnoScan 1100AL™ allows for whole-slide imaging in a single step, by using content-based autofocus the scan is focalized 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: Cell migration studies, Cell – microenvironment interaction studies, Extracellular Matrix composition assays, Cell microarrays and microfluidic devices manufacturing.

References

- *Magnetic µCP:* 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
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- *To learn about magnetic µCP automation and automatic whole slide fluorescence detection:* www.innopsys.com

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