Nanostencil and InkJet Printing for Bionanotechnology Applications

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Abstract. In this contribution we describe the application of Ink-Jet printing and Stencil Lithography in bionanotechnology. Both techniques are alternative patterning methods that can be used for the fabrication of biocompatible micro- and nanostructures out of the costly and restricted clean room environment. The applications presented in this contribution are 1) the cell patterning using Au dot arrays deposited on PDMS,by stencil lithography, 2) the fabrication of biosensors based on localized surface plasmon resonance in Au nanodots deposited by stencil lithography and 3) the printing of cells and biomolecules by InkJet printing.

Keywords: Biosensor, Nanotechnology, Stencil, Inkjet, Plasmonics, Cell Patterning, Tissue Engineering, Cell Printing.

1 Introduction

Micro and nanotechnologies have been enthusiastically adopted by biologists because they offer them new tools for exploring cell behavior. For instance, micro-arrays have been adopted under various forms to study the behavior of single-cells and to improve experiment statistics and the effects of nanostructures on cells are being studied to better understand behavioral cues that cells receive from their microenvironments. Microfluidic systems have been adopted by many biologists as they permit the creation of miniaturized bioreactors and analysis platforms, among others.

Unfortunately, there is a limit to the extent that conventional cleanroom processes can be implemented to answer life-sciences questions due to biocompatibility issues. Furthermore, cleanroom processes are often costly and require a developed infrastructure which can place them out of the reach of more modest research labs and make them infeasible for the production of bio-devices and structures in bulk. Fortunately, two alternative patterning techniques – Stencil Lithography (SL) and InkJet printing – show very good compatibility with bio- and soft- materials and can be implemented at a lower cost than standard cleanroom processes. InkJet printing can be used for printing of cells and proteins. SL has the advantage of not requiring any resist processing, and has been used on soft materials like PDMS or bio-functionalized surfaces.

SL is essentially a shadow-mask patterning technique that allows the fabrication of structures at both μm and nm length-scales. [1-3] SL can be used for patterning by deposition, etching and particle implantation. [4-6] A stencil is comprised of a thin

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nitride membrane supported on a micromachined silicon support structure allowing it to be handled (Fig. 1a). The membrane contains patterned apertures with µm and nm dimensions. In SL micro and nanopatterning, a stencil is placed in contact with the substrate to be patterned, the patterning step is carried out, and then the stencil is removed and preserved for re-use (Fig.1b). As there are no organic solvents, UV light, or acids involved, SL can be applied to pattern any substrate compatible with the deposition or etching condition, making it excellent for patterning on biomaterials. Also, SL can be easily implemented outside of a cleanroom environment, making micro and nanopatterning accessible to a wider range of users. The use of SL for Bionanotechnology applications is discussed in Section 2.

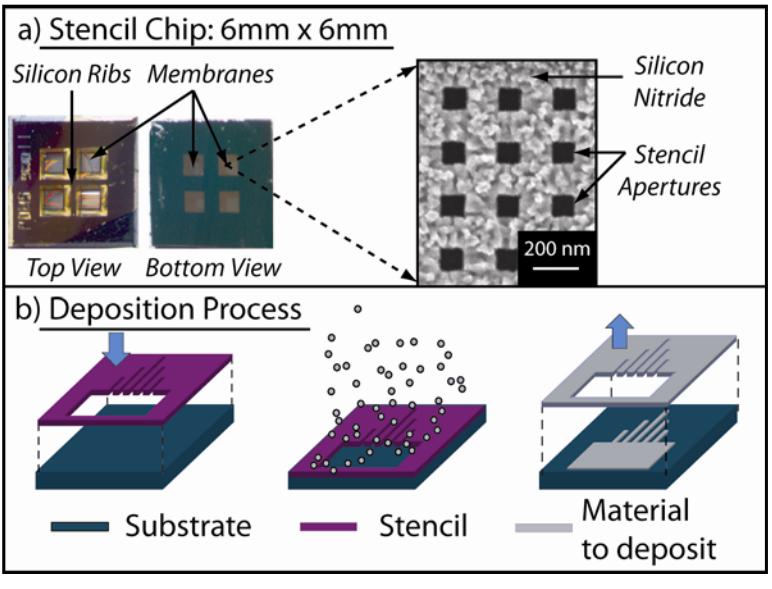

Fig. 1. Stencil overview. (a) Left: Photograph of top and bottom of 6 mm stencil chip with membranes and silicon ribs indicated. Right: SEM image of 100 nm square stencil apertures in membrane of structure at left. (b) Schematic of stencil deposition process. A stencil is mechanically fixed to a substrate, a material is deposited, and then the stencil is removed leaving deposited material in the same patterns as the stencil apertures.

InkJet printing has been widely used in computer peripherals for many years. Recently, it has attracted significant attention as a means of fabricating polymer electronics devices such as OLEDs. In most InkJet printers, a pressure wave is generated and travels to a small aperture where it forces the ejection of a microscopic droplet. The pressure wave is typically generated by a piezoelectric or localized boiling of the ink by heating. InkJet apertures typically range from 10 – 100 µm in diameter, resulting in ejected droplets of the same. The system is well suited to the patterning of biomaterials because it is inherently 'wet', and the volumes of liquid involved are minuscule so there is little waste of expensive biomaterials. Furthermore, InkJet printed droplets are large enough to hold cells making it an ideal cell-delivery system. Similarly to SL, InkJet printing can easily be implemented outside of a cleanroom

environment, and printers can be controlled by widely-available software. The use of InkJet printing for Bionanotechnology applications is discussed in Section 3.

2 Stencil Lithography

2.1 Cell Patterning by Stencil Lithography

As mentioned earlier, one of the key advantages of SL is that it permits patterns to be made on biocompatible substrates which would not be compatible with existing patterning techniques. We have created metallic patterns on PDMS (Fig. 2a), tissue-culture polystyrene, glass, and polytetrafluoroethylene (PTFE) among others. To our knowledge, this is the only technique capable of creating metal nanopatterns directly on a PDMS surface rather than transferring them from another surface.

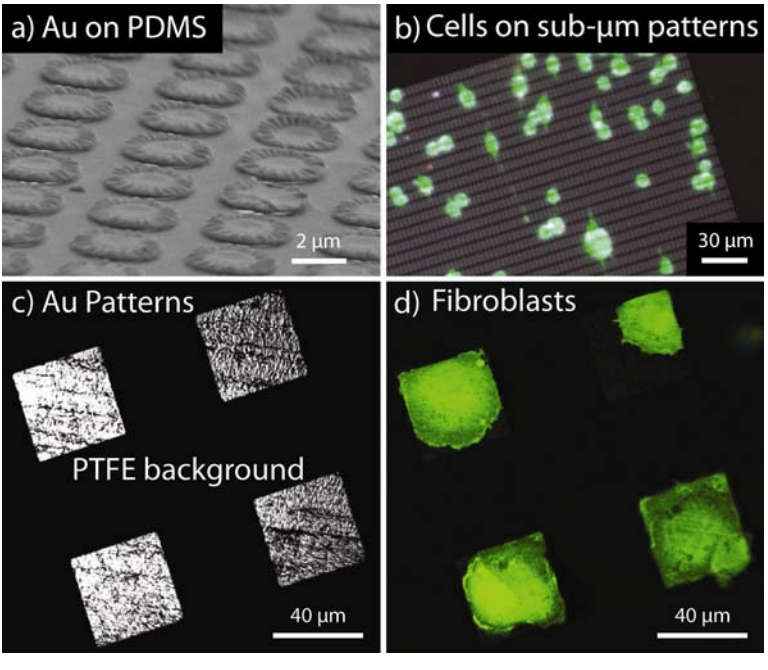

Fig. 2. Cell Patterning. (a) Stenciled array of 2 µm Au circles on PDMS. (b) MDA cells align along 5 µm x 0.5 µm rectangles spaced 2.5 µm longitudinally and 2 µm laterally. (c) Reflected light image of 40 µm Au squares on PTFE. (d) Green fluorescent Human Dermal Fibroblasts are effectively confined and only spread on the Au patterns.

Biologists have been using cell patterning strategies such as micro-contact printing and photolithography to study the response of cells to geometrical cues. Théry et al. showed that when cells are cultured on elongated shapes such as rectangles, they tend to divide along their short axis [7]. Similarly, Dalby et al. showed that nanoscale disorder is a sufficient condition to cause bone-precursor cells to differentiate and secrete bone mineral [8].

To create cell adhesive micro or nanopatterns, a metal is patterned through a stencil by e-beam evaporation. Following this, the metal is rendered cell-adhesive by physical adsorption of proteins or using a specific surface modification chemistry such as thiol chemistry in the case of Au and Pt, or phosphate in the case of titanium oxides. In the work we describe here, the peptide chain Ac-GCGRGDSPG-NH2 is grafted to stenciled Au patterns by means of the thiol on the cystein – rendering the Au patterns cell-adhesive.

To test the efficacy of sub-micron patterning, MDA cells were cultured on an array of 0.5 µm x 5 µm Au rectangles spaced 2.5 µm longitudinally and 2 µm laterally. The substrate was polystyrene. Interestingly, MDA cells tended to align lengthwise along the rectangles in spite of having to bridge a 2.5 µm gap between rectangles as opposed to a 2 µm gap laterally (Fig. 2b).

To test the efficiency of confining cells to specific geometry, GFP+ Human Dermal Fibroblasts were cultured on 40 µm Au squares on a PTFE substrate. The cells spread on the square patterns and were clearly confined at the edges of the square, demonstrating the capacity of this technique for controlling cell geometry with µm-scale patterns (Fig. 2c & 2d).

2.2 Nanodot Arrays Fabricated by SL for Biosensing Applications

The use of metallic nanodots for biosensing applications using localized surface plasmon resonance (LSPR) has been broadly studied recently [9 ,10]. Using stencil lithography, we have deposited Au nanodot arrays using stencils containing 100 nm thick silicon nitride membranes with nanoapertures defined by electron beam lithography. [11] The membranes of the stencil contain arrays of 100 nm diameter holes, with spacing between them of 100, 200 and 300 nm. The lateral size of the arrays varies from 10 to 30 µm. Fig. 3a shows a stencil membrane with 100 nm holes. The stencils were fixed on the substrate (silicon or glass) followed by the deposition of 80 nm thick Au by e-beam evaporation at 4 Å/s. Fig. 3b shows the deposited structures consisting of nanodots 120 nm in diameter.

The nanodot arrays have been used for biomolecular detection based on the wavelength shift of LSPR in nanostructures when biomolecules bind to the nanodots [9]. The wavelength resonance λ_R depends on several factors, one of them being refractive index n of the media surrounding the nanostructures. Fig. 3c shows the localized surface plasmon spectra of arrays of 100 nm nanodots with different spacing of ~100, 200 and 300 nm. The peak of resonance is at λ_R ~800 nm. Fig. 3d shows the change in resonance wavelength of the dots (100 nm diameter, spaced 300 nm) when PEL-PEG-biotin and streptavidin are added in a buffer solution (Buffer: HEPES pH 7.4, PLL-PEG-Biotin: 100 µg/ml, Streptavidin: 20 µg/ml). The substrate containing the dots was put into a flow cell that allows the controlled dispensing of liquids while monitoring the extinction spectra from the dot array. First a buffer is introduced, followed by the dispensing PLL-PEG-Biotin that adheres to the substrate and the gold dots, shifting the resonance wavelength of the dot array. To remove any PLL-PEG-Biotin non-attached to Au, another flow of buffer is applied. Then, the streptavidin is added, generating another shift in resonance wavelength. Finally, there is a last flow of buffer to remove any streptavidin left in solution. This demonstrates the application of nanostructures fabricated by SL for biosensing applications.

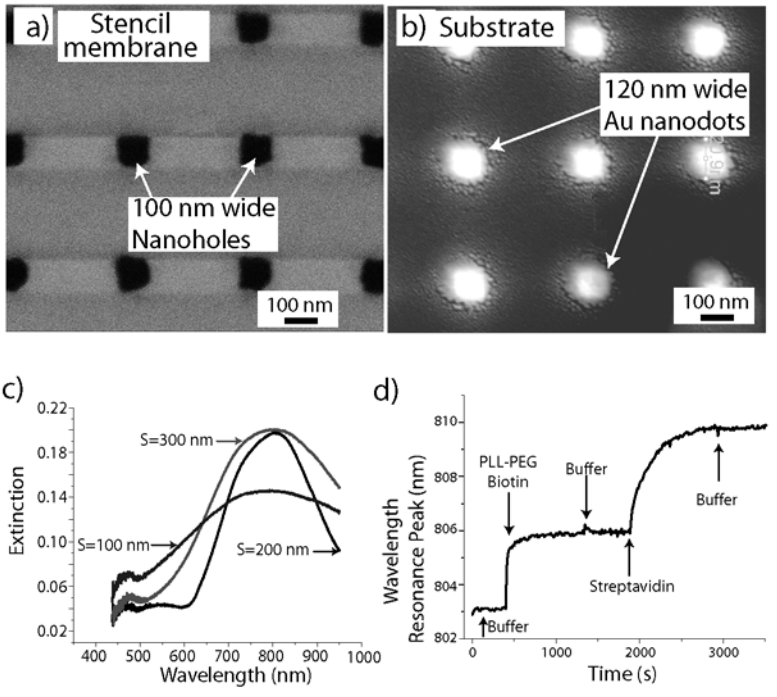

Fig. 3. Stencil. (a) Stencil containing 100 nm wide holes array. (b) 120 nm wide Au dots deposited through the stencil in (a). (c) LSPR extinction spectra of 120 nm wide Au dots with spacing of: 100,200 and 300 nm. (d) Response of the Au nanodots (120 nm wide, 300 nm spaced) when adding biotin and streptavidin. (Buffer: HEPES pH 7.4, PLL-PEG-Biotin: 100 µg/ml, Streptavidin: 20 µg/ml).

3 InkJet Printing for Bionanotechnology Applications – Cell and Biomaterials Printing

In recent years, InkJet printing has received more and more attention as a possible means of cell and biomaterial handling as a microscopic quantity of liquid is delivered to a surface. This is an ideal transport means for cells as they must remain in a liquid environment to remain viable. In fact, practice of cell handling in microscopic fluid droplets has already been accepted by biologists in a common tool – Fluoresence Activated Cell Sorting (FACS). Beyond simple cell handling, InkJet printing has been proposed as a tissue engineering tool as it would allow one to print a tissue mimetic with different cells arranged at different sites as proposed by Boland [12]. InkJet printing is also suited for cell and biomaterial printing as it is low cost. In fact, most of Boland's work is carried out with a modified HP Bubble-Jet printer [12]. InkJet printers can be controlled with widely available computer software. Thus, this widely-available technology can be used to create complex arrays of DNA, organic molecules, and proteins [13].

The InkJet printing research in our lab is carried out on a 50 µm diameter piezoelectric InkJet head from Microdrop Technologies GmbH (Germany) as piezoelectric InkJet heads offer a high-degree of cell viability after printing [14]. We have InkJet printed both B16F10 murine melanoma and HEK293T cells and found that both attached to fibronectin coated slides and migrated after printing (Fig. 4a).

Additionaly, we have carried out studies on the population statistics in printed droplets. To successfully count the number of cells per droplet, cells were printed on a glass microscope slide and then placed under another glass microscope slide. The spacing between slides was dictated by the frosting and measured to be approximately 20 µm using a micro-gauge sheet. Cells were imaged by bright and darkfield microscope (Fig. 4b). The population distribution of cells in the microdrops appeared to be Poissonian, with the deviations occurring at higher cell number likely caused by cell aggregation (Fig. 4c).

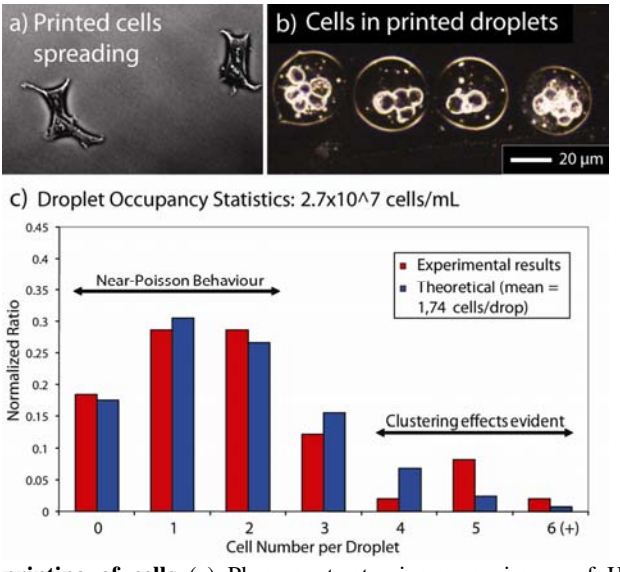

Fig. 4. InkJet printing of cells (a) Phase-contrast microscope image of HEK293T cells attached to a fibronectin-coated cover slide after printing. (b) Darkfield image of cells visible in individual droplets – as used for the statistics in c). (c) Graph showing nearly Poissonian droplet occupancy statistics for 27 million cells / mL. Deviation at higher cell number is due to cell clustering as seen in b). Data: N = 49 droplets.

References

1. Brugger, J., Berenschot, J.W., Kuiper, S., Nijdam, W., Otter, B., Elwenspoek, M.: Resistless patterning of sub-micron structures by evaporation through nanostencils. Microelectron. Eng. 53, 403–405 (2000)
2. Deshmukh, M.M., Ralph, D.C., Thomas, M., Silcox, J.: Nanofabrication using a stencil mask. Appl. Phys. Lett. 75, 1631–1633 (1999)

3. Vazquez-Mena, O., Villanueva, G., Savu, V., Sidler, K., van den Boogaart, M.A.F., Brugger, J.: Metallic Nanowires by Full Wafer Stencil Lithography. Nano Lett. 8, 3675–3682 (2008)
4. Viallet, B., Grisolia, J., Ressier, L., Van Den Boogaart, M.A.F., Brugger, J., Lebraud, T.: Stencil-assisted reactive ion etching for micro and nano patterning. Microelectro. Eng. 85, 1705–1708 (2008)
5. Villanueva, G., Martin, C., Vazquez-Mena, O., Montserrat, J., Langlet, P., Bausells, J., Brugger, J.: In: MNE 2008, Athens, Greece (2008)
6. Villanueva, G., Vazquez-Mena, O., van den Boogaart, M.A.F., Sidler, K., Pataky, K., Savu, V., Brugger, J.: Etching of sub-micrometer structures through Stencil. Microelectron. Eng. 85, 1010–1014 (2008)
7. Théry, M., Racine, V., Pépin, A., Piel, M., Chen, Y., Sibarita, J.B., Bornens, M.: The extracellular matrix guides the orientation of the cell division axis. Nat. Cell Biol. 7, 947–953 (2005)
8. Dalby, M.J., Gadegaard, N., Tare, R., Andar, A., Riehle, M.O., Herzyk, P., Wilkinson, C.D.W., Oreffo, R.O.C.: The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nat. Mater. 6, 997–1003 (2007)
9. Anker, J.N., Hall, W.P., Lyandres, O., Shah, N.C., Zhao, J., Van Duyne, R.P.: Biosensing with plasmonic nanosensors. Nat. Mater. 7, 442–453 (2008)
10. Homola, J.: Surface Plasmon Resonance Sensors for Detection of Chemical and Biological Species. Chem. Rev. 108, 462–493 (2008)
11. Vazquez-Mena, O., Sannomiya, T., Tosun, M., Villanueva, G., Vörös, J., Brugger, J.: Analysis and Applications of Nanostructures created by Stencil Lithography. In: Transducers, Denver, Co. U.S.A. (2009)
12. Boland, T., Tao, X., Damon, B.J., Manley, B., Kesari, P., Jalota, S., Bhaduri, S.: Drop-on-demand printing of cells and materials for designer tissue constructs. Mat. Sci. Eng. C 27, 372–376 (2007)
13. Wilson Jr., W.C., Boland, T.: Cell and organ printing 1: Protein and cell printers. Anatomical Record - Part A Discoveries in Molecular, Cellular, and Evolutionary Biology 272, 491–496 (2003)
14. Saunders, R.E., Gough, J.E., Derby, B.: Delivery of human fibroblast cells by piezoelectric drop-on-demand inkjet printing. Biomaterials 29, 193–203 (2008)