REVERSIBLY SEALABLE STEEL STENCILS FOR CELL AND PROTEIN PATTERNING

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Introduction:
Polydimethylsiloxane (PDMS) stencils have been extensively used to micropattern biomolecules and to create small cellular incubation chambers as tools for high-throughput screening, tissue engineering and *in vitro* diagnosis [1,2,3]. PDMS stencils are easily fabricated but suffer from lack of rigidity which leads to distortions when sealing them onto surfaces. This makes the alignment and position registration of the microwells difficult. Precise alignment is required to interface PDMS stencils with automated liquid handling systems, to deliver distinct chemicals, protein solutions or drugs in each compartment.
Here, we introduce reversibly sealable and reusable stencils composed of a rigid stainless steel stencil support patterned with self-sealing rubber ring gaskets underneath (Fig 1a). The developed stencils, when sealed onto flat surfaces (Figure 1b), create a perfectly aligned nL-capacity microwell array (Nanowell plate), overcoming the limitations of PDMS stencils. The regular distribution of the compartments allows each one to be addressed using pre-encoded positions with an automated liquid handling robot, making it possible to use each microwell as an incubation microchamber.

Experimental Methods:
We used UV curable elastomers [4,5] in combination with photolithography to define self-sealable rubber ring gaskets on chemically etched steel stencils (Figure 2). Photopatterning of the ring gaskets is not a trivial process (Figure 3). The direct spin coating of the UV curable pre-polymer on the steel stencil is not possible due to the presence of the trough holes. In order to overcome this issue the pre-polymer was spin coated on a cover slip (Figure 3a) and transferred uncured onto the steel stencil (Figure 3b). During the alignment procedure between the stencil and the photomask, in order to achieve the correct positioning of the ring gaskets on the steel stencil, the cover slip has to remain in place in order to protect the photomask from the tacky pre-polymer. The parameters used to choose the best polymer were feature resolution, softness (Shore A30 and below - the softness improve stencil sealing properties), development quality, and adhesion to steel surfaces.

Results:
The developed stencils can create, when sealed, 3 nL-capacity microwells on a wide range of surfaces, such as PS petri dishes, coated microscope slides or cover slips. These properties make it feasible to use an ink-jet microarrayer to fill, with different protein solutions, up to 3000 microwells created on microscope slides (Figure 4).

Conclusion:
We used sealable stainless steel stencils to produce miniaturized microwell arrays on a wide range of surfaces (Nanowell plates). The position accuracy of the wells, emulating commercial microtiter plates, allows the controlled delivery of nL amounts of solutions in distinct compartments. Future work will focus on real miniaturized immunoassays and multiplexed cell culture studies.

References:

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[2] Folch, A. *et al*. "Microfabricated Elastomeric Stencils for Micropatterning Cell Cultures", *J. Biomed. Mater. Res.* **2000**, 52, 346-353.
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[4] Desai, Salil P. *et al*. "A Photopatternable Silicone for Biological Applications". *Langmuir*, **2008**, 24(2), 575-581.
[5] B. Torbiéro M. *et al*. "Mass Patterning of Polysiloxane Layers Using Spin Coating and Photolithography Techniques", *Microelectron. J.*, **2006**, 37(2), 133-136.

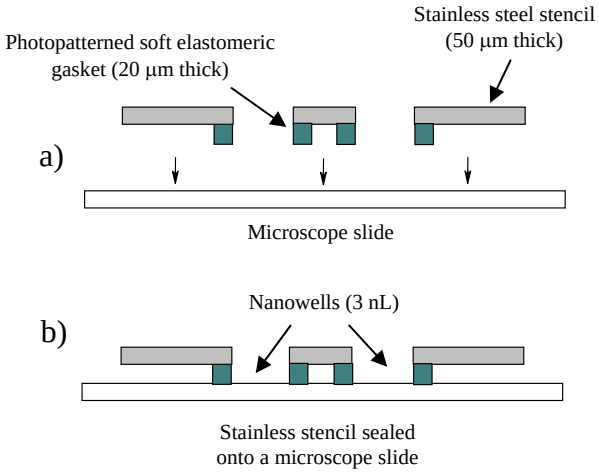

Figure 1. a) Stainless steel stencils and b) Nanowell plate (NP) formation after sealing composite steel stencils onto flat surfaces.

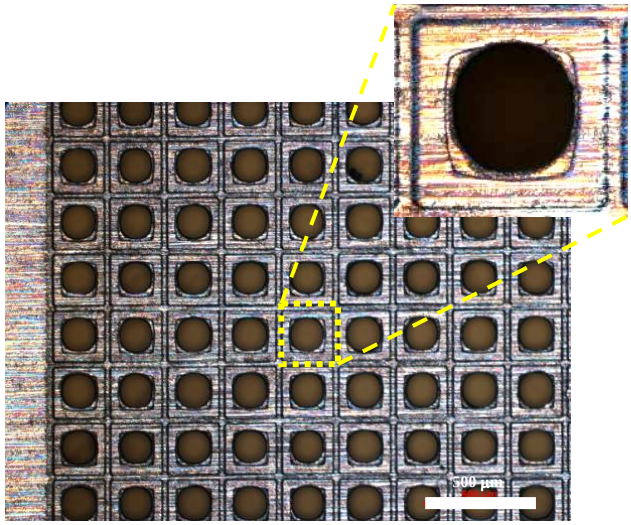

Figure 2. Stainless stencil with sealed elastomer rings on a cover slip.

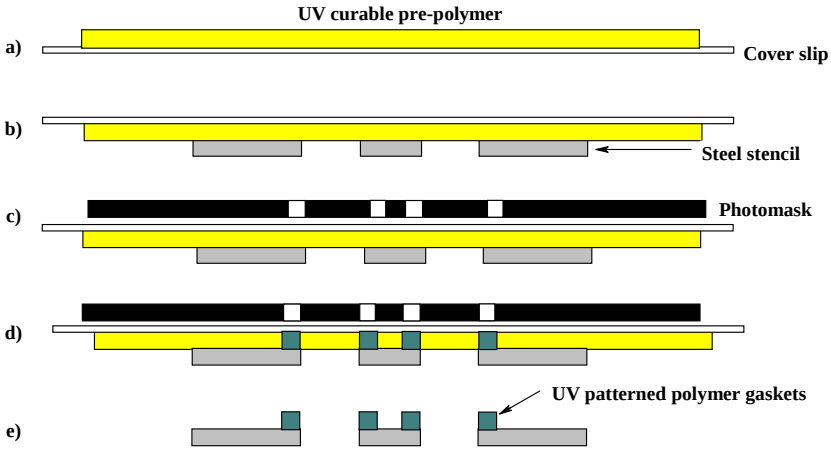

Figure 3. Reversibly sealable stainless stencils fabrication procedure. a) Spin coating of UV curable pre-polymer onto a cover slip, b) pre-polymer transfer onto stainless stencil (cover slip remains in place), c) alignment of photomask, d) UV exposure, e) removal of cover slip and development of uncured pre-polymer.

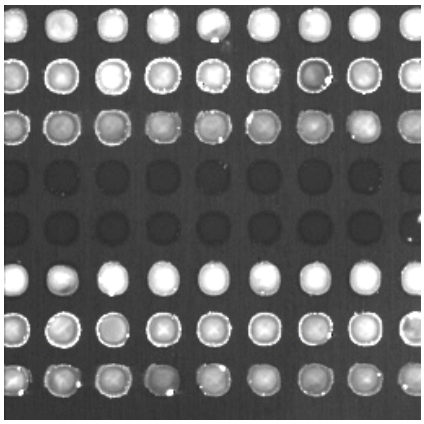

Figure 4. Image showing fluorescently labeled protein spotted into distinct microwells. No protein was delivered in the central microwell rows.