Photo nanoimprint lithography of biological samples using microfabricated PDMS stencils

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Bioimprint is a nanoimprint lithography technique capable of permanently replicating cellular features into rigid methacrylate copolymer [1,2]. While previous work has investigated the *Bioimprint* for high-resolution imaging and cell analysis applications, recent investigations center on using the *Bioimprint* as an independent cell culture substrate. We theorize increased and preferential adhesion to the *Bioimprinted* regions. Verification of this hypothesis, however, requires an accurate before-and-after rendering of the substrate surface.

Micropatterning cell cultures by physically restricting growth area has been well documented [3,4]. Master patterns are defined and constructed in SU-8 via traditional photolithography methods. Elastomeric stencils are then fabricated by exclusion-molding[5]. This ensures through-holes in the stencil to allow cells access to the desired substrate. Lithographically defining and physically limiting the growth regions available to seeded cells essentially creates a map of cell-feature locations imprinted into the methacrylate substrate (Fig 1).

Ishikawa cancer cells are seeded and grown within PDMS stencils (Fig. 2a) on glass microscope slides for 24 hours before imprinting. The stencils are removed and the liquid methacrylate copolymer is poured and UV-cured to create a *bioimprinted* surface (Fig. 2b). The resulting imprint contains both the nanoscale cell features typical of the *bioimprint* technique and the micropattern defined by the PDMS stencils (Fig. 2c). After intensive cleaning and sterilization, another passage of cells is cultured on the *bioimprinted* substrates. Following secondary cell culture, staining elucidates cell location and density. Results of the interactions of cells with the *bioimprinted* patterns will be presented and discussed.

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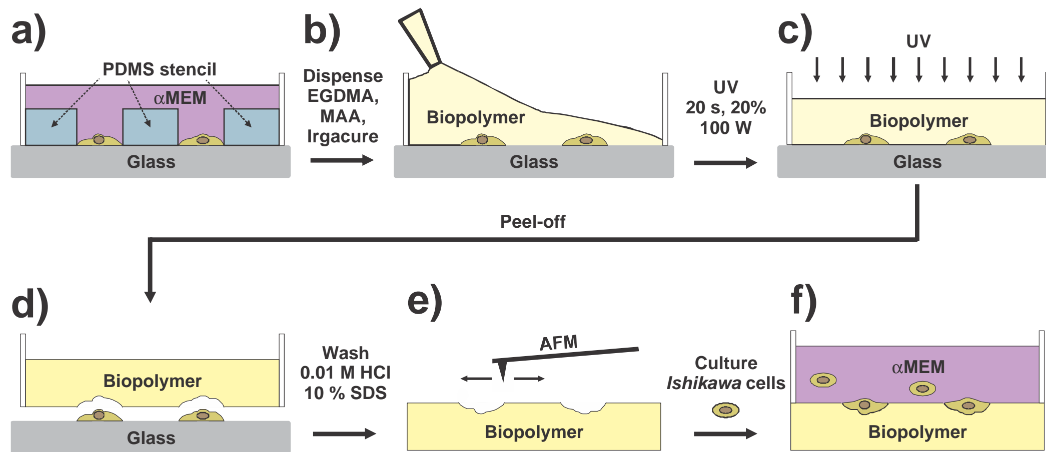

Fig. 1: Schematic of the substrate patterning process using Bioimprint. (a) Cells are cultured to shape in a PDMS stencil placed on glass. (b) The stencil is removed and the media replaced with UV-cureable biopolymer. (c) The biopolymer is UV-cured and peeled off (d) from the cell culture. (e) AFM and optical microscopy are used to characterize the patterned polymer substrate. (f) Primary cells are cultured on the polymer substrate and selectively attached on the patterned areas.

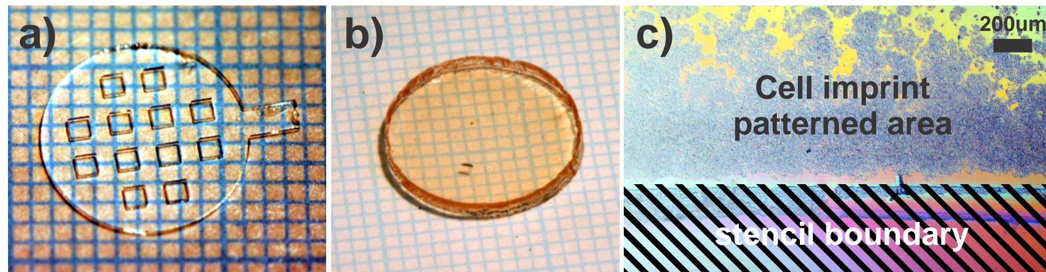

Fig. 2: (a) Photograph of a micro-fabricated PDMS stencil containing an array of square holes used to pattern cells on a glass substrate. (b) Photograph of the cured *Bioimprint* after removal from the pre-patterned cells. (c) DIC micrograph showing the boundary between the cell imprinted and the stencil covered areas. Artifacts appearing at the boundary are due topoor sealing of the PDMS stencil.