Humidified Microcontact Printing of Proteins: Universal Patterning of Proteins on Both Low and High Free Energy Surfaces

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Figure S1. In HμCP, a 1 minute incubation of water in the gap formed between the stamp and the hydrophobic surface suffices to transfer the protein from the stamp to the surface as demonstrated by the fluorescence which was 4 times stronger after 1 min. Stamps with posts 125 µm in diameter were used in HμCP to transfer a fluorescently labeled IgG to a hydrophobic surface. The required incubation time of water in the gap was assessed by allowing the stamps to remain in contact with water for a determined length of time. The protein transfer was shown to depend on the presence of water as shown by the lack of protein transfer at t=0 min, whereas after a water incubation in the gap for as short as 1 min, protein was transferred to the surface throughout the circular region of the stamp in contact with the surface. The scale bars corresponds to 50 µm.
Figure S2. A variety of biomolecules can be patterned with HμCP. HμCP was conducted with pillar stamps of 125 μm diameter to transfer fluorescently labeled (a) Immunoglobulins (IgG), (b) Bovine Serum Albumin (BSA) and (c) Arginylglycylaspartic acid (RGD) peptide to a PDMS surface. When proteins were printed by μCP, no transfer occurred (insets). This demonstrates that HμCP can be used to pattern a large variety of biomolecules on low energy surfaces. The scale bars correspond to 50 μm.
Figure S3. Time course of water saturation at the center line of the stripe in contact with the substrate for different stripe widths. The channel width was equal to the stripe (or ridge) width and the channel depth was 50 µm for all tested stamps with stripes of different widths. Water from the channel diffused into the stripe, and into the stamp, and while close to the channel saturation is 100% and quickly reached, as one moves away it takes longer for water to diffuse and the saturation reduced as water diffuses into the bulk PDMS above the stamp, while eventually reaching a steady state. For 150 µm and 300 µm stripes, the equilibrium state at the center of the stripe is reached rapidly as it is only 75 µm and 150 µm from the edge, respectively, while exceeding the 88% saturation threshold (dotted red line) for HµCP. For the 300 µm stripe, over 200 s are needed to surpass the threshold. For wider stripes the humidity at the center line increases much more slowly, and does not surpass the 88% threshold in the steady state, as shown here and in Fig. 3. 100% water saturation is not reached due to the diffusion of the water molecules in the stamp.
Figure S4. Cells adhere and spread on biologically active multi-protein patterns. HμCP was employed to pattern alternating stripes of fibronectin (red) and netrin-1 (green). Both proteins were immunostained and C2C12 myoblasts were grown on the patterns and subsequently fixed and stained for actin (blue) and nuclear components (white). Positive staining of the cells for fibronectin can be attributed to the secretion of fibronectin by myoblasts. The morphology of C2C12 myoblasts grown on patterns obtained through HμCP is comparable to the morphology observed on incubated fibronectin patterns (Ricoult et al., 2014). The adhesion of the cells to the pattern confirms that the printed proteins remained biologically active. Scale bar corresponds to 20 μm.
**Figure S5. HμCP can be employed to pattern 3 or more solutions.** (a) A multi-patterned PDMS substrate that may be created by HμCP and microfluidic patterning as described previously in the manuscript is (b) contacted with a plasma activated stamp. (c) Upon lift-off, the ridges of the stamp detach an alternating stripe pattern composed of two proteins. (d) The stamp is then contacted with the target surface and (e) a third solution is flown into the channels to transfer the other two proteins and simultaneously coat the surface in the channels. (f) Detachment of the stamp yields a self-aligned 3-protein pattern. The cycle could be repeated to produce a substrate composed of more than 3 different molecules while geometries that differ from straight lines may be used as well.