Tip Based Printing of Functionalized Hydrogels Microscale Patterns

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Introduction: Hydrogels are cross linked hydrophilic polymers that swell in water and can be synthesized from a variety of natural and synthetic polymers. Hydrogel scaffolds can be tuned to closely mimic the chemical and mechanical properties of extra cellular matrix and therefore could play a crucial role in tissue engineering research. However, in bulk hydrogels, it is difficult to control the 3D architecture and may cause cell necrosis due to diffusion limitation. A solution to this problem is the construction of microscale hydrogel patterns. Currently, microscale hydrogel patterns are generally constructed using techniques like photolithography and micromolding. But each of these methods has significant drawbacks.
In this report we present a novel method for the construction of chemically functional hydrogel microscale patterns. We use a tip based lithography method to directly deposit the hydrogel precursors at defined location and polymerize them to form hydrogels. This method allows for rapid fabrication of high resolution patterns.

Methods: A 1:2 (wt/wt) solution of Poly(ethylene glycol) dimethacrylate (PEG-DMA) with a molecular weight of 0.55 kDa and 1 kDa was used for printing hydrogels. A photoinitiator (2,2-diethoxyacetophenone) was added into the PEG-DMA ink solution to aid in polymerization and formation of hydrogels. A NLP 2000™ desktop nanolithography platform (NanoInk Inc., Skokie, IL USA) equipped with a 12 pen array (M-expV1 type) was used to construct the patterns (Fig 1) on chemically functionalized glass substrates. The hydrogel precursor patterns were polymerized by exposing them to 10 mW UV irradiation for 20 mins. To construct the functionalized hydrogel patterns, a 1:2 (wt/wt) solution of PEG-DMA (1 kDa) and 4-arm PEG-thiol (2 kDa) was used. For functional study, the hydrogel pattern was incubated with thiol-reactive rhodamine red C2 maleimide (Invitrogen) for 2 hrs and characterized by fluorescence microscopy.

Results: We have demonstrated that microscale hydrogel patterns can be easily generated using tip based lithography process. Uniform and size controllable hydrogel patterning could be obtained by temperature and humidity control during the deposition process. At 37ºC and 20% RH, relatively larger droplets (~5 µm) were printed while at 25ºC and 20% RH, smaller droplets (~2 µm) were deposited (Fig 2). AFM imaging of the polymerized hydrogel patterns confirmed the size and homogeneity of the array.
We have also successfully demonstrated the precise deposition of thiol functionalized PEG hydrogel on glass substrates. In addition, we have shown specific immobilization of the thiol-reactive rhodamine red C2 maleimide molecules on the surface of our microscale hydrogel patterns (Fig 3). Red-fluorescent was observed exclusively at the patterned area. By simply adjusting the ratio between the two PEG components, we should be able to fine-tune the number of free thiol functional groups in the hydrogel, and hence the amount of conjugated biomolecules. These hydrogels with different composition can be also loaded simultaneously onto the writing tips by an inkwell to create concentration gradient patterns in a single array.

Summary: Functionalized hydrogels have been proven to be very useful in biomedical applications. The methodology we report herein is an effective way to produce well-defined size arrays of hydrogel with selective immobilized biomolecules printed on glass substrates which are ideal systems for addressing different biological relevant issues.

Figure 1. *The desktop nanolithography platform NLP 2000^{TM} was used for patterning hydrogels.*

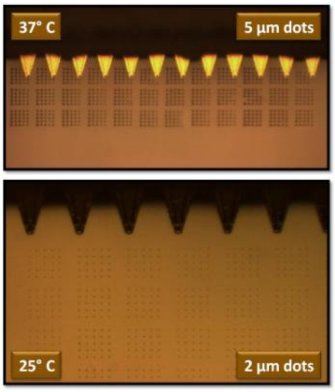

Figure 2. *Hydrogel spot size as a function of temperature. Environmental (temperature and humidity) conditions play a crucial role in the spot sizes that can be obtained by tip based lithography. At a constant relative humidity of 20%, larger spots can be printed at 37 ºC (top) compared to 25 ºC (bottom).*

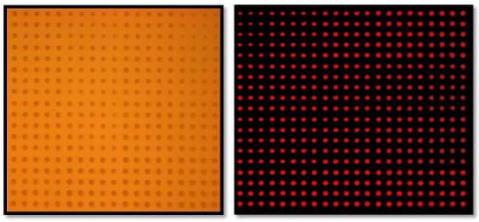

Figure 3. *Functionalized Hydrogel. (Left) Bright field image of functional hydrogel patterns that were constructed with excessive thiol groups. These patterns were incubated with thiol-reactive rhodamine red C2 maleimide molecules and interrogated by fluorescence microscopy (Right).*