Biomolecules Immobilization on Microfabricated Functional Hydrogels

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Statement of Purpose: In this report we present a novel method for the construction of chemically functional hydrogel microscale patterns. We use Dip Pen Nanolithography® to directly deposit the hydrogel precursors at defined locations and polymerize them to form hydrogels. This method allows for rapid fabrication of high resolution patterns.

Methods: A range of multi-functionalized polyethylene glycol (PEG) was used as precursor ink solution for deposition, which would polymerize and form hydrogels with excessive and unreacted functional groups for further reactions. All patterns were fabricated on glass substrates by NLP 2000 desktop nanolithography platform (NanoInk Inc., Skokie, IL USA) equipped with a 12 pen array (M-type) and a NanoInk inkwell. A solution with equal molarity of polyethylene glycol dimethacrylate (PEG-DMA, 1 kDa) and 4-arm PEG-thiol (2 kDa) was prepared for thiol-consisting hydrogel printing. Different ratios were also prepared for the multiplexing experiment. Patterned hydrogels were polymerized by exposing them to 10 mW UV radiation for 20 mins. A 2:1 mixture of 4-arm PEG epoxide (2 kDa) and 4-arm PEG amine (2 kDa) was used to produce the epoxy functionalized hydrogel. Polymerization occurred at 55°C in 2 hrs. For functional studies, thiol hydrogel patterns were incubated with thiol-reactive rhodamine red maleimide (Invitrogen) for 2 hrs. Epoxy hydrogels were exposed to normal mouse and rabbit IgG respectively for 3 hrs, followed by a mixture of the corresponding Alexa Fluro dye tapped anti-IgGs for another 1 hr. All the samples were rinsed by PBS and water before characterizing by fluorescence microscopy.

Results: We have demonstrated that microscale hydrogel patterns can be easily achieved by using Dip Pen Nanolithography techniques. Highly uniform feature sizes were obtained in all the printings with coefficient of variation less than 10%. We have also successfully shown the possibility of simultaneously depositing different PEG compositions to create concentration gradient patterns in a single array (Figure 1) by using an inkwell for parallel ink loading. AFM imaging of the polymerized hydrogel patterns confirmed the size and homogeneity of the arrays. In addition, we have demonstrated specific biomolecule immobilizations on both thiol and epoxy functional hydrogel surfaces.

Figure 2 illustrates the conjugation of rhodamine red C2 molecules on the hydrogel surfaces through the reaction between thiol and maleimide groups. Red-fluorescence was observed exclusively at the patterned area. By applying the epoxy-amine reaction, specific conjugations of mouse and rabbit IgG on the epoxy hydrogel surfaces were also observed.

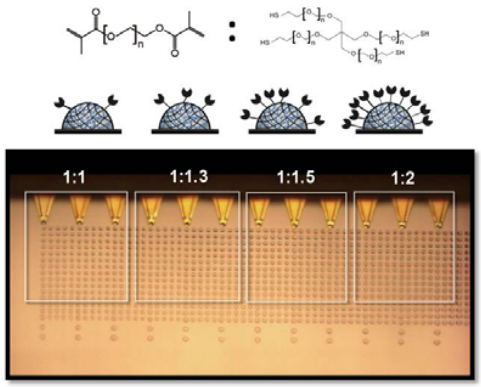

Figure 1. A bright field image of thiol hydrogels shows the parallel deposition of mixed polymer ratios.

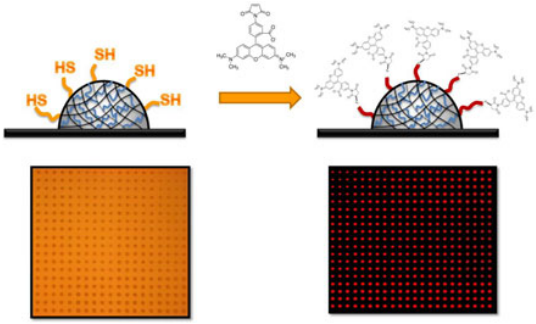

Figure 2. (Left) A bright field image of thiol functional hydrogel patterns, and (Right) a fluorescence image of the same pattern with rhodamine red C2 molecules conjugated on the surfaces.

Conclusions: 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.

References: (Reddy SK. Polymer, 2005;46:4212-4222.) (Hamid ZA. Biomaterials, 2010;31:6454-6467.)