APPLICATION NOTE

Hydrogel Patterning

NanoInk, Inc. NanoFabrication Systems

Introduction

Hydrogels are three-dimensional cross-linked polymer networks that have physical characteristics similar to those of natural tissue. The versatility of poly(ethylene glycol) (PEG) chemistry and the excellent biocompatibility of PEG-based hydrogels have been instrumental in hydrogel advances related to controlled material release, directed cellular function, and regenerative medicine applications. Sub-cellular scale patterned hydrogels with defined mechanical properties are valuable as scaffolds for tissue engineering work and for *in vitro* cell culture studies.

Dip Pen Nanolithography® (DPN®) is an established method of nanofabrication in which materials are deposited onto a surface using a sharp tip. DPN enables controlled deposition of a wide variety of materials onto various substrates with nanoscale registry, all under ambient conditions. Here we demonstrate consistent and reproducible direct deposition of hydrogel precursors at defined locations and subsequent polymerization of these precursors to form PEG-based hydrogels.

Principles of PEG-Based Hydrogel Formation

Since they exhibit high degrees of hydrophilicity and biocompatability, PEG-based hydrogels have been used extensively in tissue engineering and drug delivery applications. PEG is a versatile material; it is available in a wide range of molecular weights and with various functional end groups.[3] The mechanical and swelling properties of PEG hydrogels can be fine-tuned by altering the PEG molecule chain length or by varying the degree of cross-linking, achieved by modifying the number of acrylate groups or by adjusting the UV exposure time, respectively. Hydrogel patterns can be printed on a variety of surfaces including silicon, glass and gold.

A schematic of the PEG hydrogel deposition process is shown in Figure 1. PEG-dimethacrylate (PEG-DMA) is used as the precursor material. Prior to DPN precursor patterning, a small amount of photo-initiator (~ 1% of total volume) is added to the precursor to assist in the later polymerization reaction. NanoInk's desktop nanolithography platform, the NLP 2000 System, is used to pattern the precursors. Precursors are loaded into reservoirs on the NLP 2000 System's "Inkwells". These Inkwell reservoirs feed microfluidic channels specifically engineered to transport liquids and to match NanoInk's M-type cantilever "pen" tip geometry. Precursor material then moves from the Inkwells to load each cantilever "pen" tip. The loaded tips print the desired DPN pattern, and resulting PEG-DMA features are polymerized using UV-induced cross linking to from hydrogels.

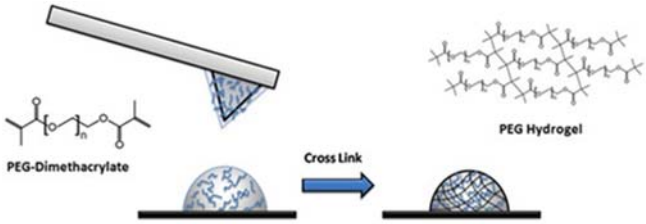

Figure 1. Schematic of the DPN PEG hydrogel printing process.

Uniform PEG Hydrogel Microarrays

To demonstrate the feasibility of using DPN to create large-area hydrogel microarrays, a one-dimensional 12-pen M-type cantilever "pen" array was used to print PEG hydrogel patterns as shown in Figure 2A. Within five minutes, the DPN system deposited 3000 hydrogel domains, covering a total area of 0.8 × 0.6 mm^2, on a glass substrate. Features within the PEG hydrogel array had an average diameter of 1.25 µm (Figure 2B) and a pitch of 13 µm. The patterned PEG hydrogel domains exhibited uniform size distribution. Average feature size diameter was calculated for four individual "pen" tips (Figure 2B, red) as well as for entire arrays (Figure 2B, blue). The coefficient of variation calculated over the entire pattern was 16%, while the CV for individual tips varied between 6 - 16%. These hydrogels patterns were generated at 25˚C and 20% RH. The diameter of the printed hydrogel domains increased to 6 µm by increasing the deposition temperature to 37˚C.

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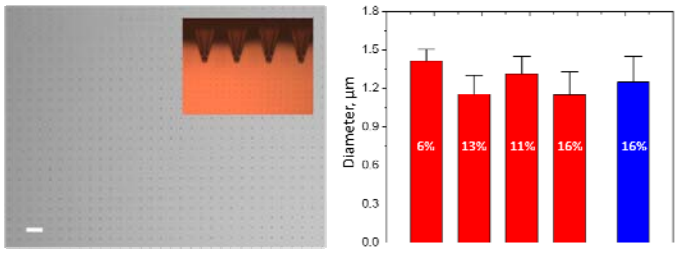

Figure 2. (Left) Optical image of PEG hydrogel pattern. Scale bar is 20 µm. (Right) Average diameter of PEG hydrogel features. The red bars represent individual "pen" tips and the blue bar represents the entire pattern. Intra-bar numbers indicate coefficient of variation.

PEG Hydrogel Nanostructures

DPN can also be used to fabricate PEG hydrogel nanostructures. Submicron-sized PEG hydrogel patterns are easily generated by lowering print molecule diffusion rate. This rate decrease is achieved by using larger-chain length PEG-DMA precursors and patterning at low humidity conditions with print times of less than 1 second per spot. As an example, Figure 3 shows an AFM micrograph of PEG hydrogel nanostructures on a gold substrate, where average nanostructure height and diameter is 37 nm and 150 nm, respectively.

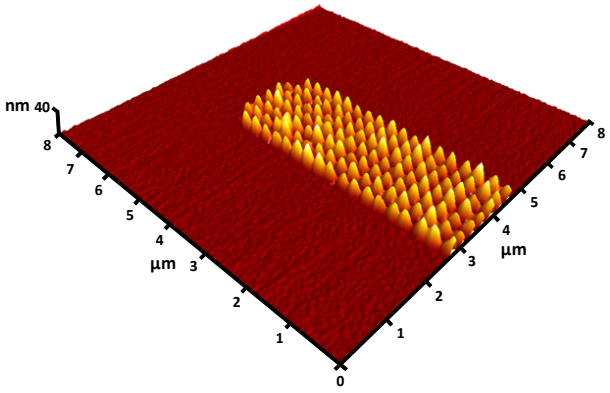

Figure 3. Three-dimensional topographic tapping mode AFM image of PEG hydrogel nanostructures.

Conclusion

Patterns of PEG hydrogels at micron and submicron scales are easily generated using NanoInk's DPN instrument systems. Using a 1D cantilever "pen" tip array to parallel print PEG precursors, an area of 0.8 × 0.6 mm^2 can be patterned with PEG hydrogels in a relatively short period of time. Resulting PEG hydrogel large-scale microarrays exhibit uniform spot size.

Reference

1. Lin, C.-C.; Anseth, K. S., PEG Hydrogels for the Controlled Release of Biomolecules in Regenerative Medicine, *Pharm. Res.* 26 (2009) 631-643.
2. Krsko, P.; Libera, M., Biointeractive hydrogels, *Mater. Today* 8 (2005) 36-44.
3. Nuttelman, C. R.; Rice, M. A.; Rydholm, A. E.; Salinas, C. N.; Shah, D. N.; Anseth, K. S., Macromolecular monomers for the synthesis of hydrogel niches and their applications in cell encapsulation and tissue engineering. *Prog. Polym. Sci.* 33 (2008) 167-179.

NanoInk Products Used

NLP 2000 Platform
DPN® Pen Arrays: Type M
DPN® Inkwell Arrays: Type M-12MW

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