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White-Paper: Acrylate-modified hydrophobicized methylcellulose (PolyVivo AI147) crosslinking and micro-patterning

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Introduction

Photolithography and related photo-crosslinking techniques have come to the forefront lately for use in creating scaffolds or patterns for cell growth and biological interactions [1-3]. Hydrophobicized methylcellulose has already proven itself a viable option for the growth of cells in three-dimensional shapes [4] [5]. A recently generated reagent-version of this material, activated with cross-linkable acrylate groups, has been created and is available from PolySciTech as PolyVivo cat# AI147. This white-paper relates methods for crosslinking this material and steps towards micro-patterning the material using conventional photolithography-based techniques.

Equipment

UVP Blak-Ray™ B-100AP High-Intensity UV Lamp
Olympus BX51 Microscope
AMscope digital camera

Reagents

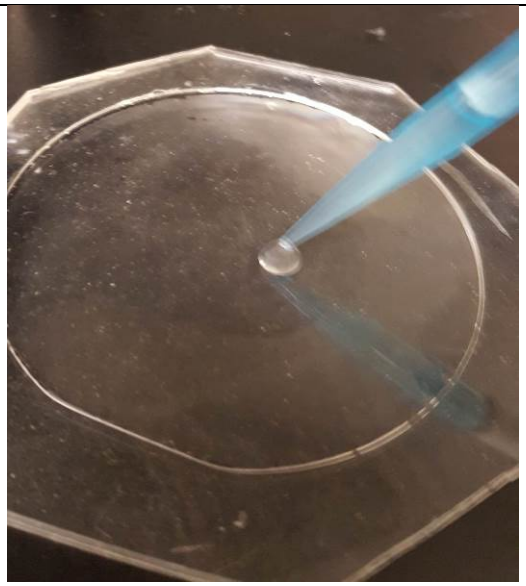
Acrylate modified hydrophobicized methylcellulose (PolyVivo Cat# AI147, lot# 60901BPR-A)
Deionized water (Sourced from Barnstead™ Easypure™ II)
Ethanol 200 proof (Decon Laboratories, Inc.)
Phosphate buffered saline (Aldrich cat # P4417)
2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) (Aldrich cat# 410896)

Soft-patterning method

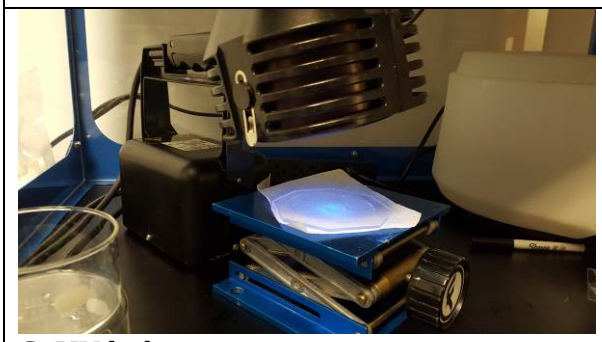
Acrylate modified hydrophobicized methylcellulose (PolyVivo Cat# AI147) was dissolved in deionized water at 2.5% w/v at 4°C overnight and stored at this temperature prior to use. A 70% v/v solution of ethanol in water was prepared and this solution was used to prepare a 10% w/v solution of photoinitiator (Irgacure 2959) in 70% ethanol. One milliliter of the prepared PolyVivo AI147 2.5% w/v solution was mixed with 0.1 ml of 10% Irgacure 2959 in ethanol and shook gently by hand to mix for a few minutes. This solution was then pipetted

onto a PDMS micro-template bearing columns 50 um in diameter x 50 um tall (prepared as described previously [6-10]) and exposed to UV light at a distance of ~10 cm for 20 minutes to cure. Subsequently, the cured hydrogel was gently pried from the template using flat-bladed tweezers and transferred, upside down, into a small petri dish. The dish was filled to cover over the hydrogel with phosphate buffered saline solution (prepared according to manufacturer instructions) and imaged under a light microscope at 4X and 10X magnification.

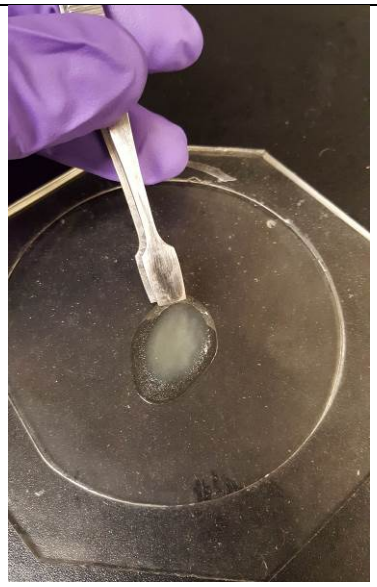
A. Mixing of gel-acrylate and photoinitiator



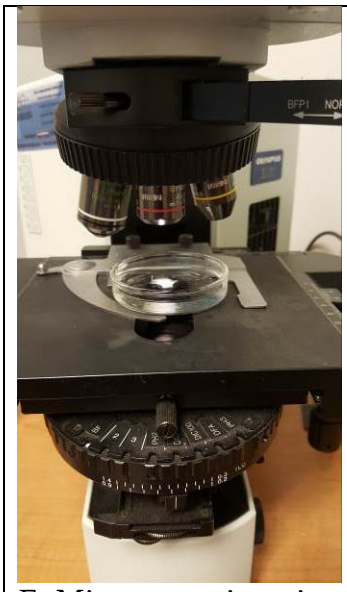
B. Addition to PDMS micro-template



C. UV light exposure.



D. Removing cured piece from template



E. Microscope imaging crosslinked gel in saline.

Figure 1. Image series from soft-patterning hydrogel preparation.

Results

The formed hydrogel was observed to not re-dissolve in the room-temperature phosphate buffered saline indicating that it was cross-linked.

Under the microscope, several regions of the hydrogel were observed to have successfully received an imprint of the micro-patterned template (Fig 2) though the pattern form was not completely uniform across the entire surface.

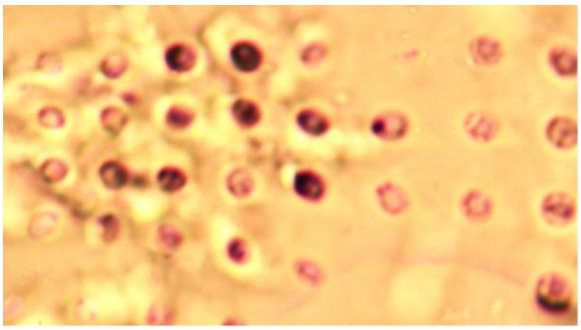


Figure 2. Partial template transfer onto crosslinked hydrogel (10X microscope magnification).

Full-patterning method

Acrylate modified hydrophobicized methylcellulose (PolyVivo Cat# AI147) was dissolved in deionized water at 5% w/v at 4°C overnight and stored at this temperature prior to use. A 20 mg amount of photoinitiator (Irgacure 2959) was dissolved in 100 µL 100% ethanol with the use of vortexing . Each sample solution was made by pipetting 1 mL of the previously made 5% w/v AI147 into

the scintillation vial containing the dissolved Irgacure 2959. This solution was then stirred using the pipette tip before being pipetted onto a PDMS micro-template bearing duct-pattern (kindly provided by Dr. Sophie Lelievre's lab after preparation as previously described [11-13]) and exposed to UV light at a distance of ~5-10 cm for about 90 minutes to cure. Subsequently, the cured hydrogel was gently pried from the template using flat-bladed tweezers and transferred in between two microscope slides and imaged under a light microscope at 4X and 10X magnification.

<table>
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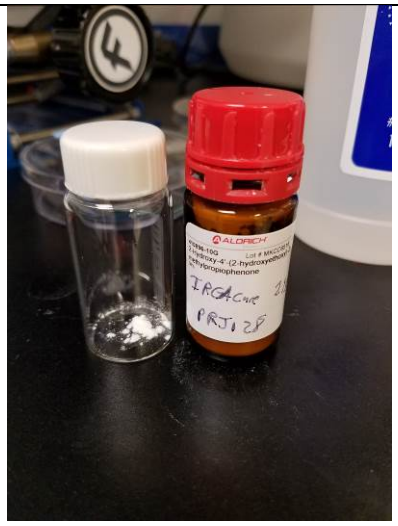
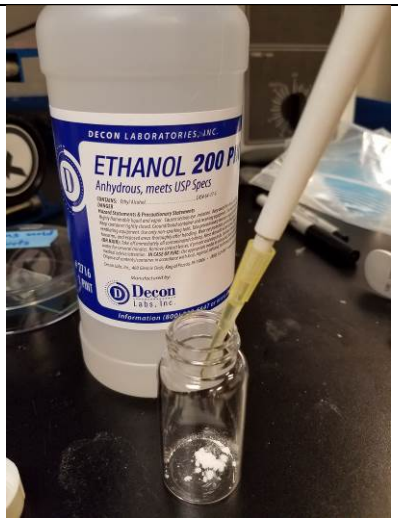
<td> 

A. ~20 mg of Irgacure 2959 in scintillation vial</td>
<td> 

B. Addition of 100 µL 100% Ethanol to dissolve Irgacure 2959</td>
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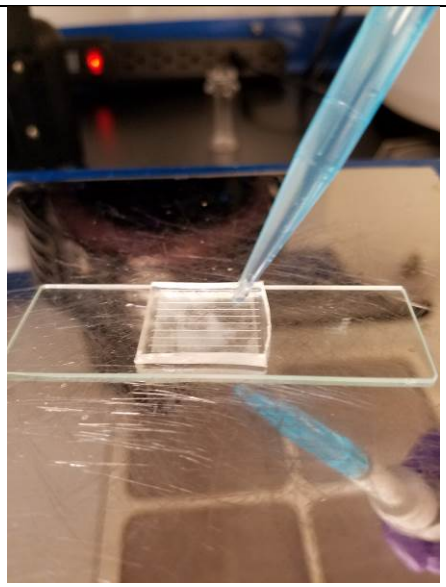
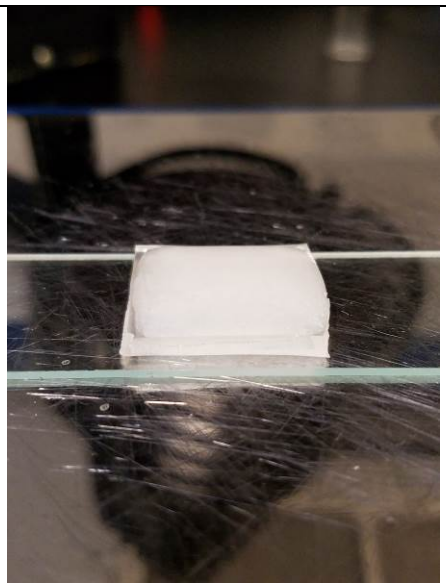
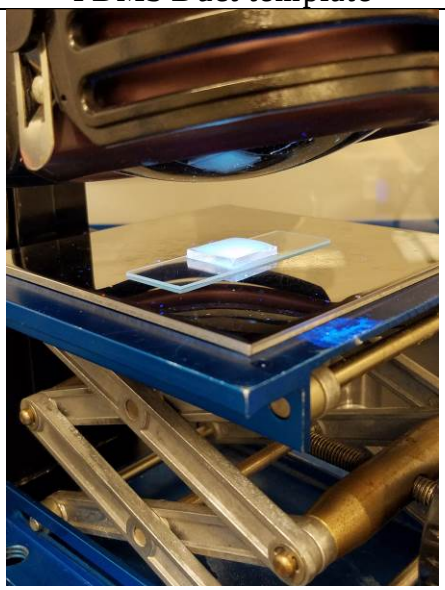
<tr>
<td> 
C. Left, 5% AI147. Right, Irgacure 2959 dissolved in Ethanol</td>
<td> 
D. Transfer of 1 mL 5% AI147 into vial containing dissolved Irgacure 2959</td>
</tr>
</table>



E. Prior to transfer of solution onto PDMS Duct template



F. Post transfer of solution onto template



G. Curing under Blak Ray B100 AP

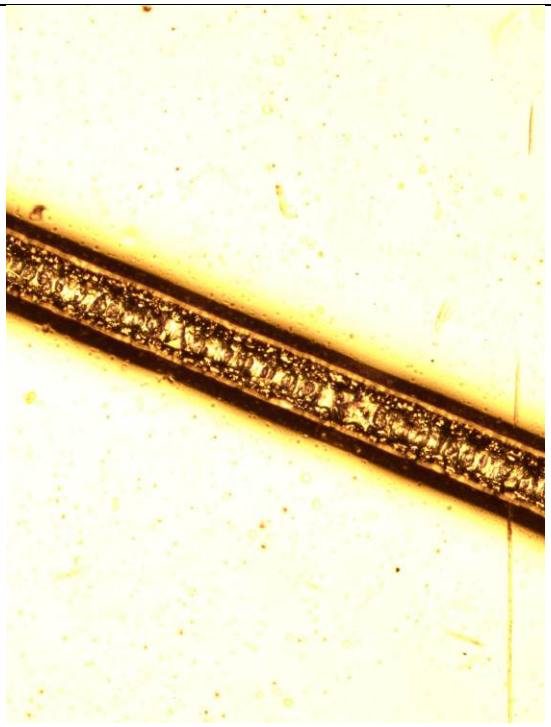
Figure 3. Image series from Full-patterning hydrogel preparation.

Results

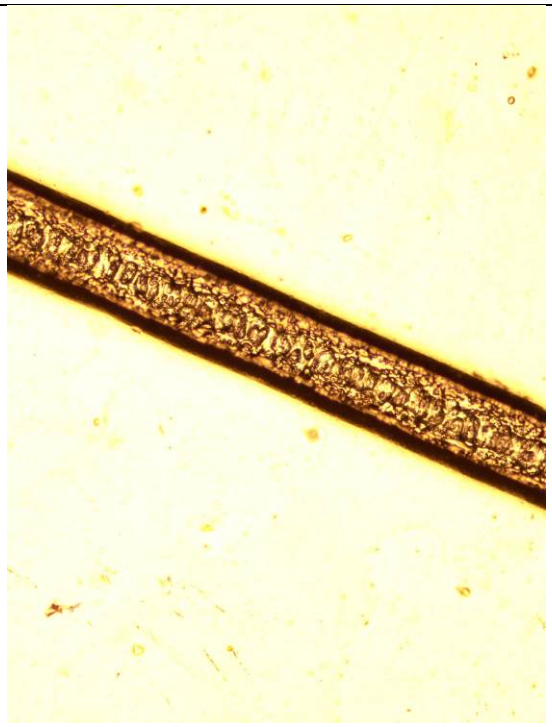
The formed hydrogel was observed to not re-dissolve in 37°C deionized water indicating that it was cross-linked. Expected hydrogel swelling behavior was observed after allowing the sample to soak in the deionized water for approximately 8 hours.

Under the microscope, the channels were seen to be successfully imprinted. However, there were a few areas which had noticeable narrowing of the channel. This was not seen all throughout and was not to be found present in every

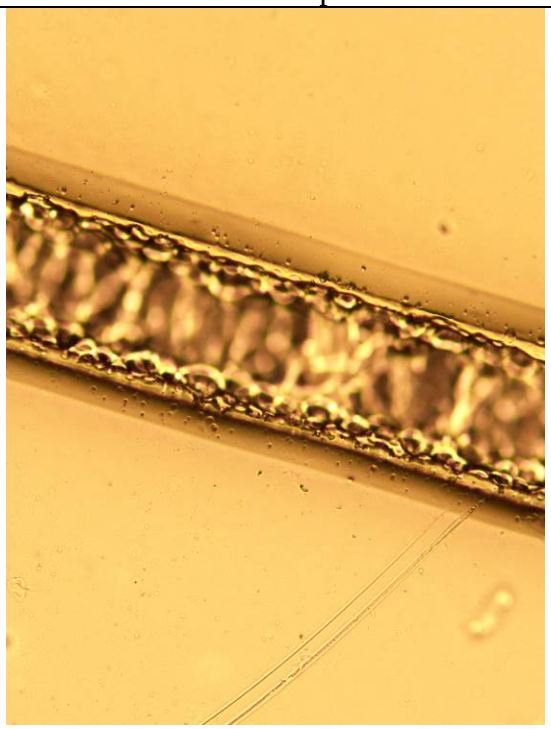
replicate. On the other hand, it was seen that the texture inside all of the channels was not smooth, rather it was fibrous.



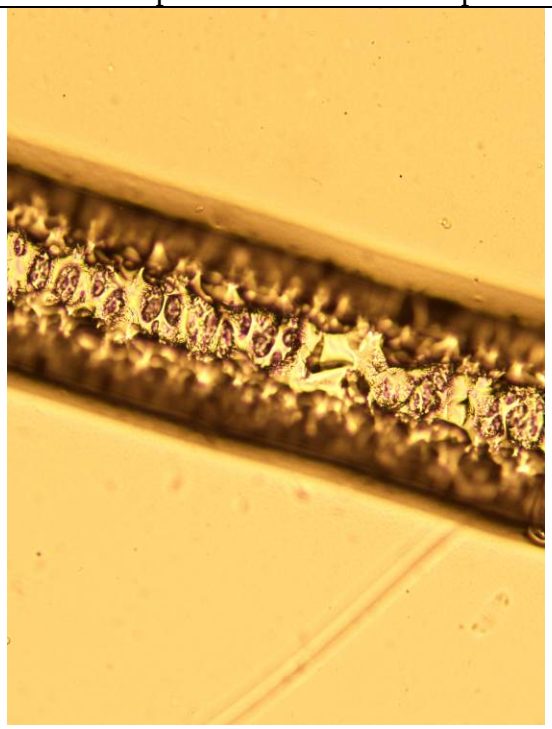
A. 4X microscope magnification of channel imprint



B. 4X microscope magnification of channel imprint on a different replicate



C. 10X microscope magnification with the outside of channel in-focus



D. 10X microscope magnification with the inside of channel in-focus

Figure 4. Images from crosslinked hydrogel (4X & 10X microscope magnification).

Conclusion

The acrylate-modified HMMC material (PolyVivo AI147) can be used to form photo-crosslinkable gels using conventional photo-reagents, such as Irgacure 2959, and these techniques can be potentially applied to lithography based micro-manufacturing methods. To achieve good template transfer for lithography approaches, a 5% w/v concentration of AI147 and 2% w/v concentration of Irgacure 2959 photoinitiator is necessary.

References

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