# High Fidelity Photopatterning of Three-Dimensional OPF Hydrogels for Co-Culture Applications

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**Introduction:** Hydrogels provide a unique *in vitro* environment to study cellular behavior in 3D. Photolithography forms versatile hydrogel structures with high spatial resolution, allowing for the controlled patterning of cells. However, current photopatterning techniques are limited in their ability to produce hydrogels with thicknesses greater than 500μm, and thus do not fully recreate a 3D environment with thicknesses similar to many tissues. Here, we developed reproducible methods to produce photopatterned poly(ethylene glycol) (PEG) based hydrogels, using cytocompatible materials[1], with comparably improved fidelity and thickness by performing the crosslinking in a controlled, low-oxygen environment. In addition, a validation of this technique for the serial photopatterning of different cell types is demonstrated for potential application in a novel 3D co-culture system.

**Methods:** The polymer solution used was 75% w/v $H_2O$ and consisted of a 1:1 w/w mixture of oligo(poly(ethylene glycol) fumarate) (OPF) ($M_n$ = 10,000kDa) and poly(ethylene glycol) diacrylate ($M_n$ = 3,400kDa) (PEG-DA, Laysan) and 0.05% w/v D2959 photoinitiator (Ciba). OPF was synthesized through the dropwise addition of triethylamine (TEA) and fumaryl chloride (FuCl) to a solution of PEG dissolved in methylene chloride (MeCl) (0.9:1 and 2:1 molar ratios of FuCl to PEG and TEA to FuCl, respectively) under $N_2$, over the course of 5h at 4°C and 2 days further at room temperature[2]. MeCl was then removed through rotovaporation and TEA hydrochloride through filtration, and the OPF recrystallized in ethyl acetate, washed in ethyl ether, and vacuum dried at 25°C.

Photopatterning experiments were performed in a microfluidic device fabricated from polydimethylsiloxane (PDMS) (Sylgard 184, Corning). The 10,000dpi photomasks used consisted of a series of 800μm to 3000μm squares (CAD Art Srv). Devices were equilibrated with $N_2$ or room air prior to loading the polymer. The photomask was aligned and the polymer solution injected and allowed to crosslink under 365nm light for 12 min. The dimensions of the hydrogels immediately after crosslinking and after reaching equilibrium swelling were measured using a dissecting microscope (Leica) and ImageJ software (NIH). Measurements were compared using ANOVA and Tukey's post-hoc test ($n = 3$, $p \leq 0.05$). Linear regression was performed to determine the correlation between mask size and the size of the resulting hydrogel.

To employ these techniques in the serial photopatterning of different cell types, the initial polymer/cell population would need to be washed away completely to prevent mixing with the second population in between crosslinking steps. To ensure that existing photopatterned gels would not obstruct the complete removal of the first cell population, 10μm polystyrene beads (Sigma) used in place of cells were homogeneously dispersed in the polymer solution at $10\times10^6$ beads/mL. Hydrogels were photopatterned under $N_2$ in an array of alternating square blocks, with block spacing varying from 100μm to 750μm. An isotonic PEG solution was used to wash out the remaining polymer solution after photopatterning, and the washing process recorded using dark field microscopy.

**Results and Discussion:** We can easily form complex hydrogel shapes by altering the geometry of the photomask (Figure 1). Hydrogel thickness and fidelity are enhanced under a $N_2$ environment, resulting in sharper, well-defined corner and arc features. Gel width conformed nearly exactly to size of the photomask and strongly correlated with mask feature size ($r$ = 0.999). Crosslinking under $N_2$ also produced gels with a significant increase in thickness, regardless of mask size (Figure 2). This can be attributed to the possibility that oxygen, due to its ability to quench free radicals, serves to inhibit the gel formation. By removing oxygen from the crosslinking environment, we have successfully produced hydrogels of sufficient thickness to be used in a true 3D cell culture system.

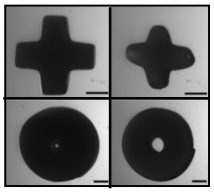

**Figure 1:** Hydrogels photopatterned with (left) or without (right) $N_2$, using photo-masks with cross and con-centric circle designs. Scale bar = 1mm.

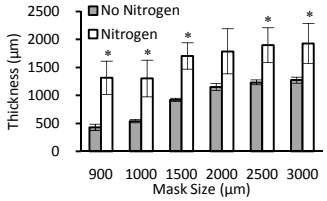


**Figure 2:** Thickness of hydrogels patterned with and without $N_2$ (after swelling, mean ± standard deviation). * Significant difference from the corresponding gel without $N_2$ ($p \leq 0.05$).

Video microscopy of the bead washing process revealed that nearly all of the polystyrene beads were removed from the main chamber (Figure 3). Hydrogel blocks could be spaced as close as 100μm without preventing the removal of beads during washing, suggesting an optimal spacing for the first series of gels so that the second set of gels can be patterned between these with no overlap in cell populations.

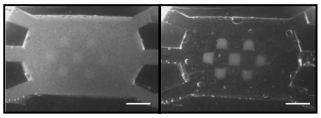

**Figure 3:** Microfluidic device before (left) and after (right) washing. Scale bar = 1mm.

Through these experiments, we have optimized methods for photopatterning cytocompatible, PEG-based hydrogels in a controlled environment with thicknesses greater than 1mm, sufficient for use in a 3D cell culture system. These novel techniques are particularly exciting as they will enable patterning of a wide variety of cell types for future co-culture experiments.

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**References:** [1]Shin H et al. Biomacromolecules. 2003;4:552-60. [2]Jo S et al. Macromolecules. 2001;34:2839-44.