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Three-Dimensional Photopatterning of Hydrogels Containing Living Cells

*Valerie A. Liu and Sangeeta N. Bhatia**
Departments of Bioengineering and Medicine, University of California, San Diego, La Jolla, CA 92093
E-mail: sbhatia@ucsd.edu

Abstract. **Recent advances in tissue engineering have leveraged progress in both polymer chemistry and cell biology. For example, photopolymerizable biomaterials have been developed that can be used to photoencapsulate cells in peptide-derivatized hydrogel networks. While these materials have been useful in bone, cartilage and vascular tissue engineering, they have limited applicability to more complex tissues that are characterized by precise cell and tissue organization (e.g., liver, kidney). Typically, the tissue shape has been defined solely by the container used for photopolymerization. In this paper, we describe the use of photolithographic techniques to broaden the capability of photopolymerizable PEG-based biomaterials by inclusion of structural features within the cell/hydrogel network. Specifically, we describe the development of a photopatterning technique that allows localized photoencapsulation of live mammalian cells to control the tissue architecture. In this study, we optimized the effect of ultraviolet (UV) exposure and photoinitiator concentration on both photopatterning resolution and cell viability. With regard to photopatterning resolution, we found that increased UV exposure broadens feature size, while photoinitiator concentration had no significant effect on patterning resolution. Cell viability was characterized using HepG2 cells, a human hepatoma cell line. We observed that UV exposure itself did not cause cell death over the doses and time scale studied, while the photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone was itself cytotoxic in a dose-dependent manner. Furthermore, the combination of UV and photoinitiator was the least biocompatible condition presumably due to formation of toxic free radicals. The utility of this method was demonstrated by photopatterning hydrogels containing live cells in various single layer structures, patterns of multiple cellular domains in a single ''hybrid'' hydrogel layer, and patterns of multiple cell types in multiple layers simulating use in a tissue engineering application. The combination of microfabrication approaches with photopolymerizable biomaterials will have implications in tissue engineering, elucidating fundamental structure–function relationships of tissues, and formation of immobilized cell arrays for biotechnological applications.**

Key Words. **photopolymerization, hydrogels, patterning, poly (ethylene glycol)**

Introduction

Tissue engineering seeks to repair, replace or restore tissue function, typically by combining biomaterials and living cells. The properties of the tissue engineered construct emerge from the local response of the cells to their 3-dimensional microenvironment (Bhatia and Chen, 1999). Therefore, it is of great importance to recreate biochemical and structural components of the *in vivo* cellular microenvironments when designing implantable tissue constructs. Traditional methods for controlling biomaterial scaffold architecture involve the production of porous polymer scaffolds or meshes as substrates for attachment of cells (Langer and Vacanti, 1993). Recent efforts to create 3-dimensional environments have moved from use of simple porogens to more intricate structures such as 3-dimensional printed scaffolds (Griffith et al., 1997), microtubular scaffolds (Ma and Zhang, 2001), and 3-dimensional micropatterned scaffolds using UV polymerization (Ward et al., 2001). However, the generation of more complex cellular tissue constructs in which cells can be placed in specified 3-dimensional configurations throughout a thick construct is limited by current scaffold-making techniques. For example, biomaterials scaffolds must be seeded with cells with the help of gravity, centrifugal forces, or convective flow (Yang et al., 2001). Alternatively, cells can be recruited to the graft by use of growth factors or chemokines (Lee et al., 2000; Badylak et al., 2001). Therefore, current techniques do not enable the formation of a thick tissue construct that is populated with living cells. Hydrogel biomaterials offer a potential solution to this dilemma.

Hydrogels are becoming an increasingly popular material for tissue engineering because their high water content and mechanical properties resemble those of tissues in the body. In addition, many of the hydrogels can be formed in the presence of cells by photocrosslinking, which allows homogeneous suspension of cells throughout the gels. Poly(ethylene glycol) (PEG)-based

*Corresponding author: Departments of Bioengineering and Medicine, 9500 Gilman Drive, Mail Code 0412, University of California at San Diego, La Jolla, CA 92093.

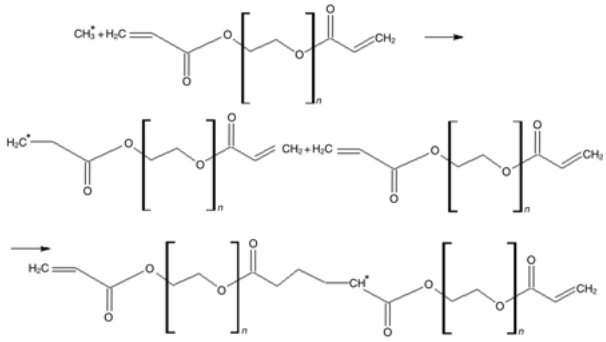
hydrogels are of particular interest because of their biocompatibility, hydrophilicity, and ability to be customized by changing the chain length or chemically adding biological molecules (Peppas et al., 2000). These types of hydrogels have been used to homogenously immobilize various cell types including chondrocytes (Bryant and Anseth, 2002; Elisseeff et al., 2000), vascular smooth muscle cells (Mann et al., 2001), and fibroblasts (Gobin and West, 2002; Hern and Hubbell, 1998) that can attach, grow, and produce matrix. One property of these hydrogel systems that has not yet been exploited is the use of the photocrosslinking step to form structural 3-dimensional hydrogel features containing cells. Elsewhere, in non-biological systems, the fundamentals of photolithography have indeed been applied to PEG-based hydrogel systems to create hydrogel valves within flow systems by controlling the regions of photocrosslinking using a mask (Beebe et al., 2000), though this process has not been adapted to the encapsulation of living cells.

In this paper, we present and characterize a method to combine hydrogel tissue engineering with photolithographic methods of hydrogel patterning to form microstructures of hydrogels that contain living cells. We also extend this technique to demonstrate the photopatterning of multiple cell types as well as the creation of multilayer structures. The hydrogel structures and arrays that are formed are covalently bound to glass wafers for ease of cell culture. Using this approach, we envision that intricate 3-dimensional tissues can be fabricated. The combination of microfabrication approaches with photopolymerizable biomaterials will have implications in tissue engineering, elucidating fundamental structure–function relationships of tissues, and formation of immobilized cell arrays for biotechnological applications.

Materials and Methods

Hydrogel chemistry

PEGDA hydrogel chemistry was based on a protocol previously described by West and co-workers (Mann et al., 2001). Poly(ethylene glycol) diacrylate (PEGDA) (3.4 kDa; Shearwater Polymers, Huntsville, AL) was dissolved in HEPES buffered saline (pH 7.4) to form a 20% w/v solution. The photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone (Sigma, St. Louis, MO) dissolved in 1-vinyl-2-pyrrolidinone (300 mg/mL) (Sigma) was added to the prepolymer solution immediately prior to UV exposure. The solution was then exposed to a UV light source (VWR, cat. no. 36595-020) at 365 nm and 10 mW/cm^2 to crosslink the polymer and form the hydrogel. The photocrosslinking reaction involves the formation of a reactive methyl radical from the photoinitiator, which then attacks double bonds in the PEGDA and initiates a chain reaction (Mellott et al., 2001).



Pretreatment of glass slides

Clean 2″ circular borosilicate glass wafers (Erie Scientific, Portsmouth, NH) were treated with a 2% v/v solution of 3-(trimethoxysilyl) propyl methacrylate (Aldrich, Milwaukee, WI) in 95% ethanol (pH 5 with acetic acid) for 2 minutes, rinsed with 100% ethanol, and then baked at 110 °C, leaving free methacrylate groups on the glass to react with the PEGDA during UV exposure.

Photopatterning of hydrogel

An apparatus was designed for photopatterning of the PEGDA hydrogels (Figure 1). Prepolymer solution was injected into a chamber with a Teflon base and a pretreated 2″ borosilicate glass wafer on top to allow penetration of the UV light. The height of the chamber was determined by the thickness of the silicone spacer (100 μm) separating the glass and Teflon.

Light patterning was made possible by creating an emulsion mask that allows UV light to pass through only desired regions. Masks were drawn using Corel Draw 9.0 and printed using a commercial Linotronic-Hercules 3300 dpi high-resolution line printer. The mask was placed on top of the glass wafer of the polymer chamber, and was pressed flat to the wafer by a glass slide. All layers were held together by caliper screws that also controlled the exact height of the chamber. Upon hydrogel crosslinking (365 nm light, 10 mW/cm^2), the remaining uncrosslinked prepolymer solution and cells were washed away with HEPES buffered saline solution. To add another cell type, the mask was changed and the next prepolymer/cell solution was injected and exposed to UV light. For additional hydrogel layers, a thicker spacer was also used.

Cell culture

HepG2 cells (American Type Culture Collection, Manassas, VA) were cultured in 175 cm^2 flasks (Fisher, Springfield, NJ) and were passaged preconfluency no more than 13 times. The cells were maintained in

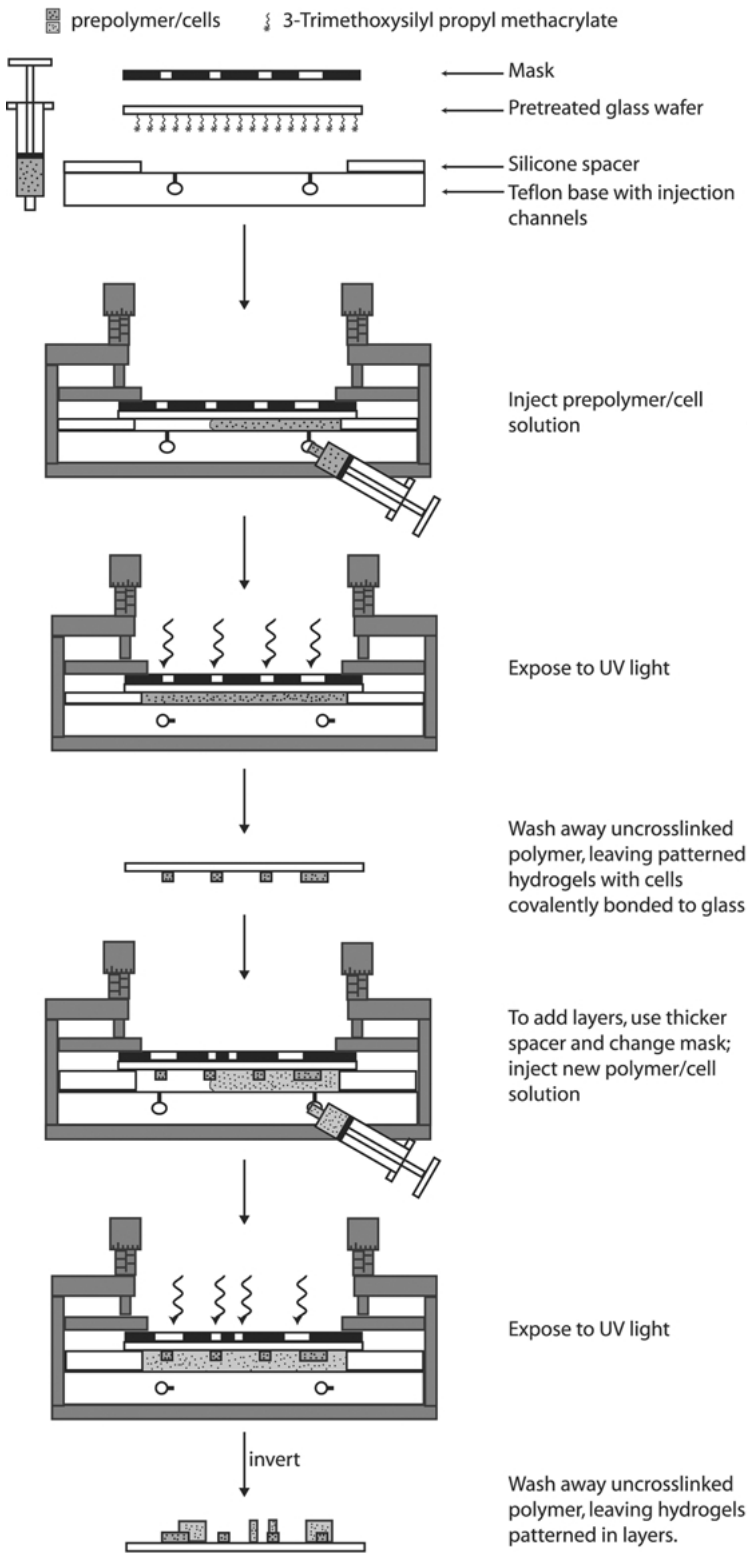


Fig. 1. *Process for formation of hydrogels microstructures containing living cells. The apparatus is assembled, including a pretreated glass wafer with reactive methacrylate groups on its surface, and a Teflon base with an inlet and outlet. Once the cells and prepolymer solution are injected, the inlet and outlet are closed, and the unit is exposed to UV light. The resulting patterned hydrogels containing cells are covalently bound to the glass wafer. At this time, a thicker spacer can be used in conjunction with a new mask to add another layer of cells. This process can be repeated several times.*

minimal essential medium (MEM; Gibco, Grand Island, NY) supplemented with 5% fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin and incubated at 5% CO_2 and 37 °C. The cells to be incorporated into hydrogels were added to the prepolymer solution containing initiator and were mixed gently prior to UV exposure.

Cell viability

In order to explore the effects of photoinitiator and UV exposure on cell death, individual components were tested in HepG2 cultures. 3.0×10^5 cells were seeded in six-well culture dishes and allowed to proliferate until near confluency. A solution of 300 mg/mL of 2,2-dimethyl-2-phenyl-acetophenone (Aldrich) in 1-vinyl-2-pyrrolidinone (Sigma) was added to MEM in concentrations of 0, 3, 5, and 10 uL/mL. The six-well plates were then rinsed with fresh MEM media to remove any dead or non-adherent cells, and 1 mL of the media containing initiator were added to each well. Cells were then exposed to 0, 30, or 60 seconds of UV light (365 nm, 10 mW/cm^2), and allowed to incubate at 37 °C for 2 hours. The effects of the initiator solvent 1-vinyl-2-pyrrolidinone alone were also tested in the same manner. This incubation time was considered to be an overestimate of exposure time during photopolymerization protocols. Cell viability was measured by an MTT viability assay, which forms a purple precipitate by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma, St. Louis, MO) was added to cells in medium without phenol red at a concentration of 0.5 mg/mL. After an incubation time of 3 hours, live cells had formed a purple precipitate, which was dissolved in a DMSO/Isopropanol solution. The absorbance was measured at 570 nm with a SpectraMax Plus spectrophotometer (Molecular Devices, Sunnyvale, CA). Viability was also viewed qualitatively using the Calcein AM and ethidium homodimer live/dead fluorescent stains (Molecular Probes, Eugene, OR).

Microscopy

Hydrogels and cells were observed and recorded using a Nikon Diaphot microscope equipped with a SPOT digital camera (SPOT Diagnostic Equipment, Sterling Heights, MI), and MetaMorph Image Analysis System (Universal Imaging, Westchester, PA) for digital image acquisition. Cells labeled with chloromethylfluorescein diacetate (CMFDA, C-2925, Molecular Probes) and chloromethylbenzoylaminotetramethyl rhodamine (CMTMR, C-2927) were observed by fluorescence microscopy with ex/em: 492/517 and 541/565 nm. In some cases, Hoechst nuclear stain was used (Molecular Probes) and viewed using fluorescent microscopy. Hydrogel resolution was determined by measuring line width using phase contrast microscopy and comparing to the actual size of the mask features. Three measurements were made for each condition, using MetaMorph software.

Statistical analysis

Error bars represent standard deviation, with $n = 3$ for resolution experiments and $n = 6$ for viability studies. Statistical significance was determined using one-way ANOVA (analysis of variance) followed by Tukey's Multiple Comparison Test. Bar graphs in Figure 2 show data for selected feature sizes with trends representative of all feature sizes tested (30, 50, 80, 100, 200, 300, 500 μm line widths).

Results

Photocrosslinking method

A method was developed to create hydrogel microstructures containing living cells in one layer or in multiple patterned layers. An apparatus was constructed that allows photocrosslinking of hydrogels of various heights that can be patterned using emulsion masks (Figure 1). Because the photocrosslinking free radical reaction is quenched by oxygen, it was necessary to contain the prepolymer solution within a closed chamber while exposing to UV light. The Teflon base provides a surface on which the polymer sits, but prevents adhesion so that the hydrogel can be easily removed after crosslinking. The height of the hydrogel is easily varied by using silicone spacers of different thicknesses. A glass wafer placed on top of the spacer both allows transmission of UV light and also acts as a surface to which the hydrogel adheres. In preliminary experiments, it was found that hydrogel features would lift off the glass wafer when submerged in buffer solution. This problem was solved by pretreating the glass with 3-(trimethoxysilyl) propyl methacrylate, thereby leaving methacrylate groups on the surface that would covalently bind to the hydrogel during photocrosslinking. This apparatus provides a way to photopattern hydrogels containing living cells, and also allows for the addition of cell types and layers of patterned hydrogels.

Patterning resolution

The effects of UV exposure time and photoinitiator concentration on resolution of hydrogel patterning were studied to characterize the limitations of the technique. Masks with line features of various widths were used to pattern hydrogel under different UV and initiator conditions. Images of features were recorded within minutes after UV exposure. Indistinct edges were seen in some cases, in particular for larger feature sizes (Figure 2A). In

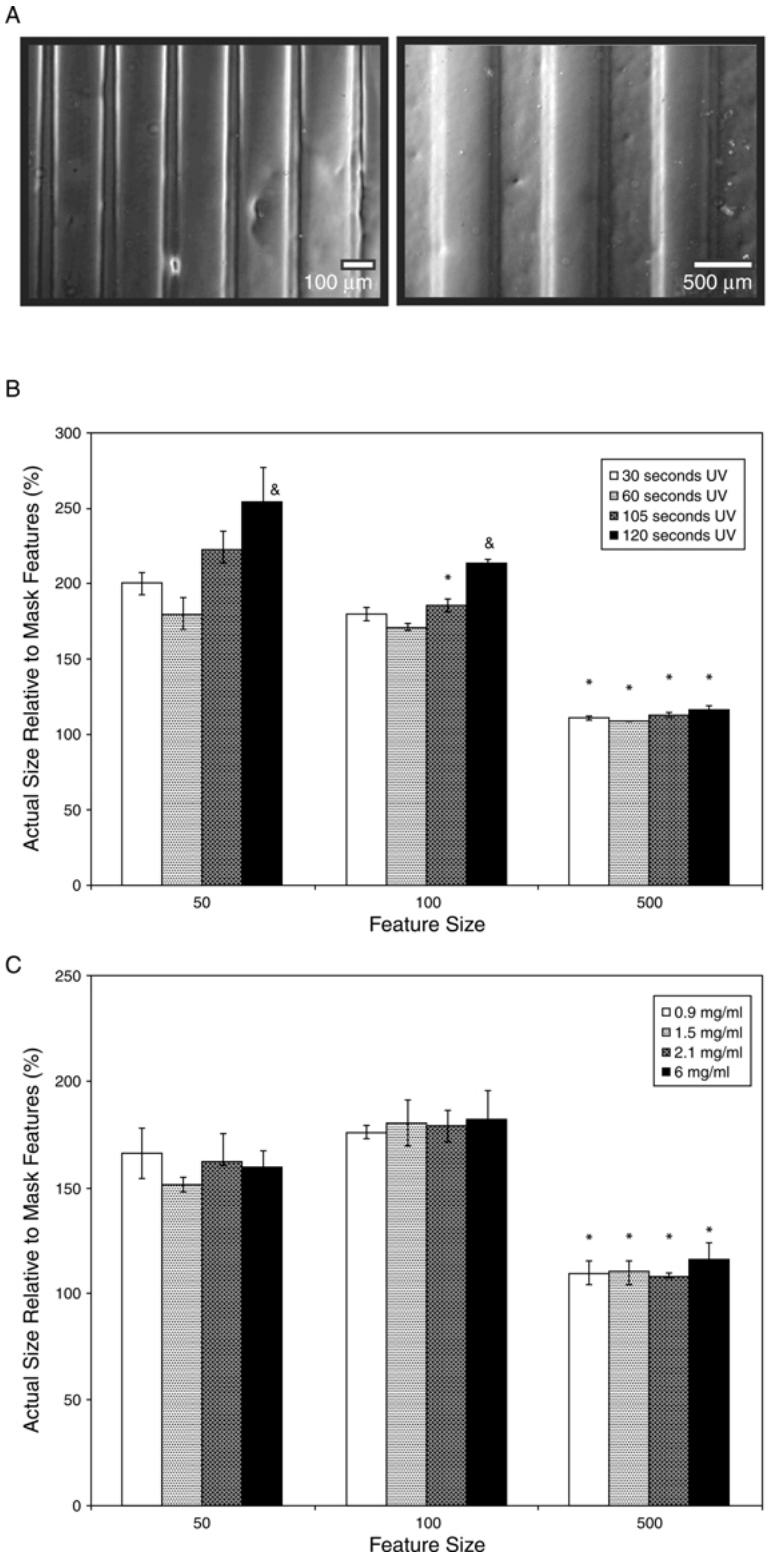


Fig. 2. *Resolution of photopatterning. (A) Hydrogel lines used to measure photopatterning resolution. (B) Feature fidelity as a function of feature size and UV exposure.* & $= p < 0.05$ *compared to all other UV exposures for the same feature size.* $^{*} = p < 0.001$ *compared to all other feature sizes for the same UV exposure time. (C) Feature fidelity as a function of feature size and photoinitiator concentration.* $^{*} = p < 0.05$ *compared to all other feature sizes of the same photoinitiator concentration. The data indicate that UV exposure has an effect on patterning resolution for smaller feature sizes, while photoinitiator concentration does not affect resolution. In addition, patterning of large feature sizes* ($> 200\,\mu m$) *results in better feature fidelity, regardless of UV or photoinitiator amounts.*

such cases, the feature was measured from the outermost part of the hydrogel. Results showed that large features ($> 200\,\mu m$) had greater fidelity than smaller features ($< 80\,\mu m$), which tended to result in hydrogel lines wider than the intended line width (Figure 2). This was the case for all UV exposure times and initiator concentrations tested. For the smaller features, increased UV exposure was shown to increase the width of hydrogel features and reduce patterning resolution (Figure 2A). Unexpectedly, varying the amount of initiator did not affect the resolution of patterning (Figure 2B). Thus, the smallest amount of initiator needed to achieve photocrosslinking can be used to reduce cell toxicity, without concern that resolution will be lost. Hydrogel feature sizes were compared to mask features both in relative percentage compared to the mask as well as by looking at the absolute change in line width (data not shown). The absolute line width change was approximately 30 μm per edge for large feature sizes (300–500 μm) and 50 μm for smaller feature sizes (50–200 μm).

Cell viability

Free radicals generated during the photocrosslinking process can cause cell death. Thus, a toxicity study was conducted on HepG2 cells in culture. Cells grown on 2-dimensional culture wells were subjected to photoinitiator and UV exposure, and viability was assessed after 2 hours of incubation. Results from the MTT viability assay can be seen in Figure 3C. UV exposure did

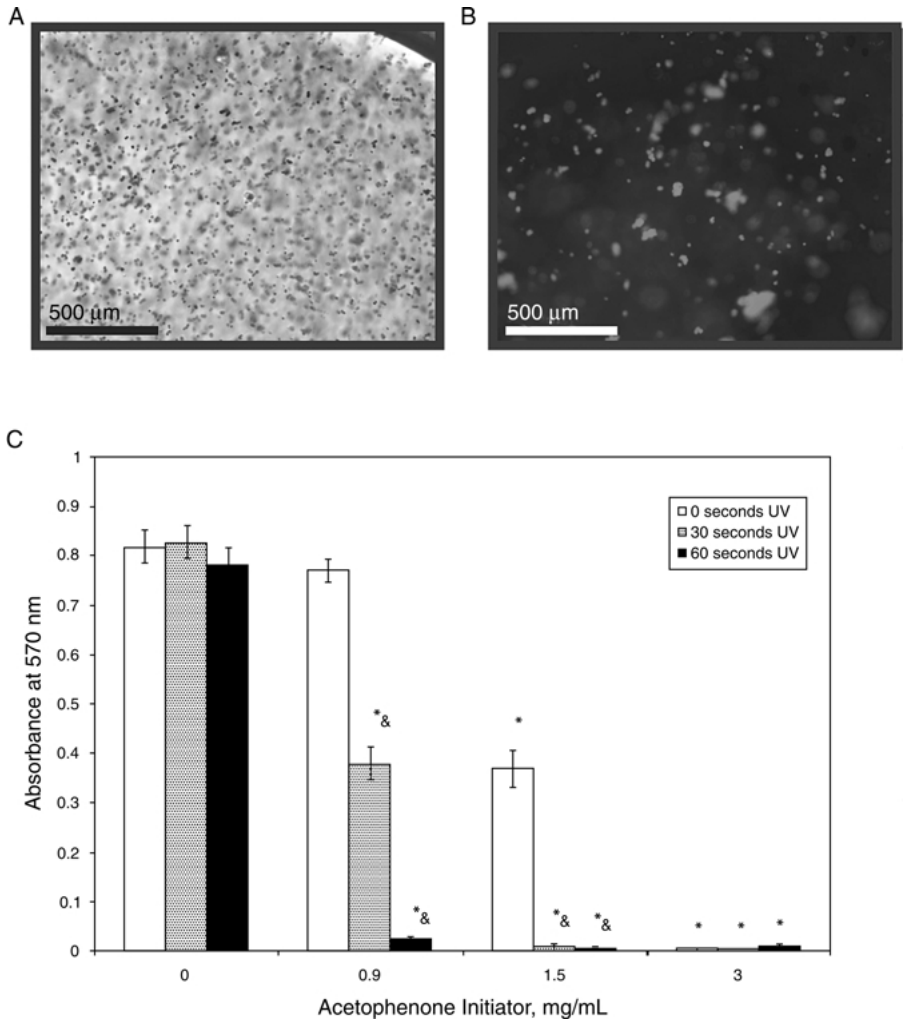


Fig. 3. *HepG2 viability. (A) MTT viability stain (live cells stain dark purple) of cells in bulk hydrogel. (B) Live (green) /dead (red) stain of HepG2 cells in PEGDA unpatterned hydrogel. A, B demonstrate relative spatial uniformity of viable cells. (C) Toxicity study of effects of photoinitiator and UV exposure on HepG2 cells.* & $= p < 0.001$ *compared to 0 initiator for the same UV exposure time.* $^{*} = p < 0.001$ *compared to 0 seconds UV exposure for the same photoinitiator concentration. Viability was measured using the MTT assay, in which the absorbance measurement increases with increased metabolic function. The data indicate that UV exposure alone is not toxic, but the photoinitiator solution results in cell death in a dose-dependent manner. The combination of UV exposure and photoinitiator has the most deleterious effects on cells.*

not significantly reduce cell viability in the absence of photoinitiator over the range of doses we studied. In contrast, the acetophenone (photoinitiator) solution did prove to have toxic effects for HepG2 cells at amounts greater than 0.9 mg/mL. In addition, the combination of acetophenone and UV exposure further increased the toxicity of the initiator, presumably by producing harmful free radicals. It should be noted that these experiments were conducted in the absence of the polymer PEGDA, which would serve as an additional sink for free radicals during photocrosslinking. Therefore, the conditions induced in our viability study were an overestimation of the toxic effect seen by live cells photoencapsulated in this hydrogel system. In addition, the initiator solvent 1-vinyl-2-pyrrolidinone alone was not found to be toxic to cells in amounts used for photocrosslinking. The distribution of cell viability within the hydrogel network was assessed microscopically by both an MTT stain (Figure 3A) and a fluorescent live/dead stain (Figure 3B). No significant spatial variation in cell viability was observed.

Hydrogel microstructures containing living cells

The fabrication method we developed was used to create various complex 3-dimensional hydrogel microstructures (Figures 4 and 5). First, cells in hydrogels were photopatterned in various shapes, demonstrating the basic utility of our method to incorporate living mammalian cells in hydrogel microstructures of arbitrary form (Figure 4A). Typical microstructures were on the order of 200 μm. We extended this technique to form a composite hydrogel structure that had two distinct cellular constituents in well-defined domains (Figure 4B). This was achieved by photopatterning one cell type

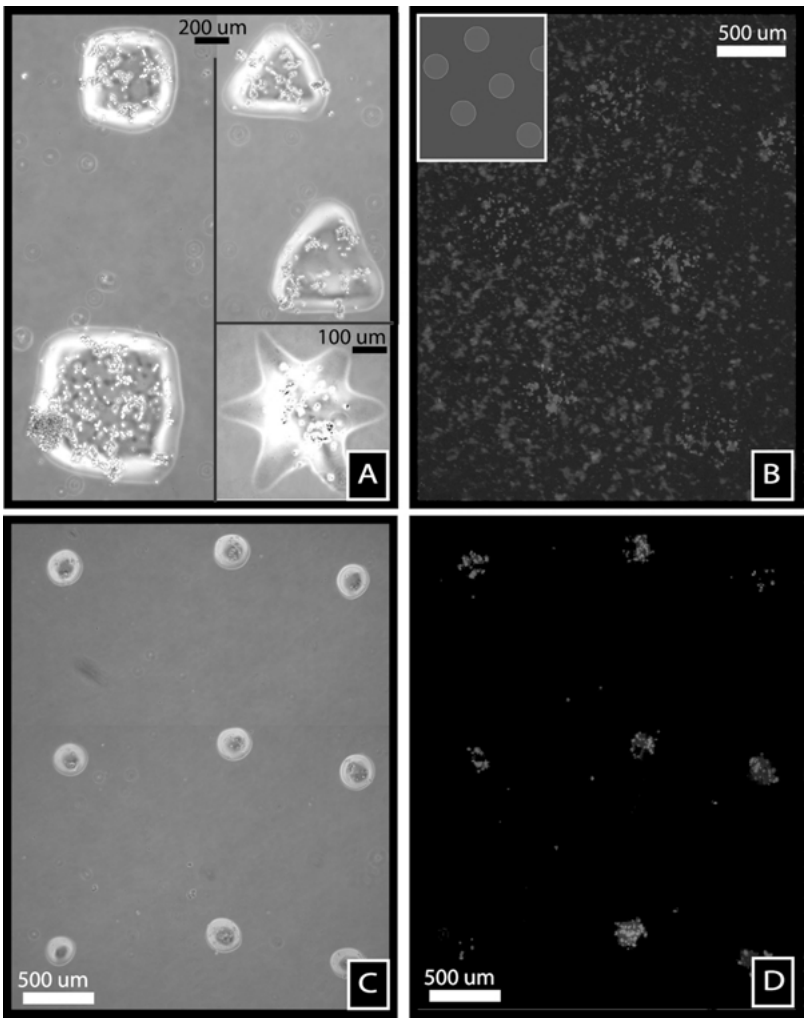


Fig. 4. *Examples of hydrogel microstructures containing living cells. (A) Cells entrapped in PEGDA hydrogels patterned in various shapes. (B) Red and green labeled cells patterned within distinct domains of a single hydrogel layer. (C) Phase microscopy of cellular array covalently linked to glass substrate. (D) Fluorescent image of the cellular array, showing two different cell types (green, red).*

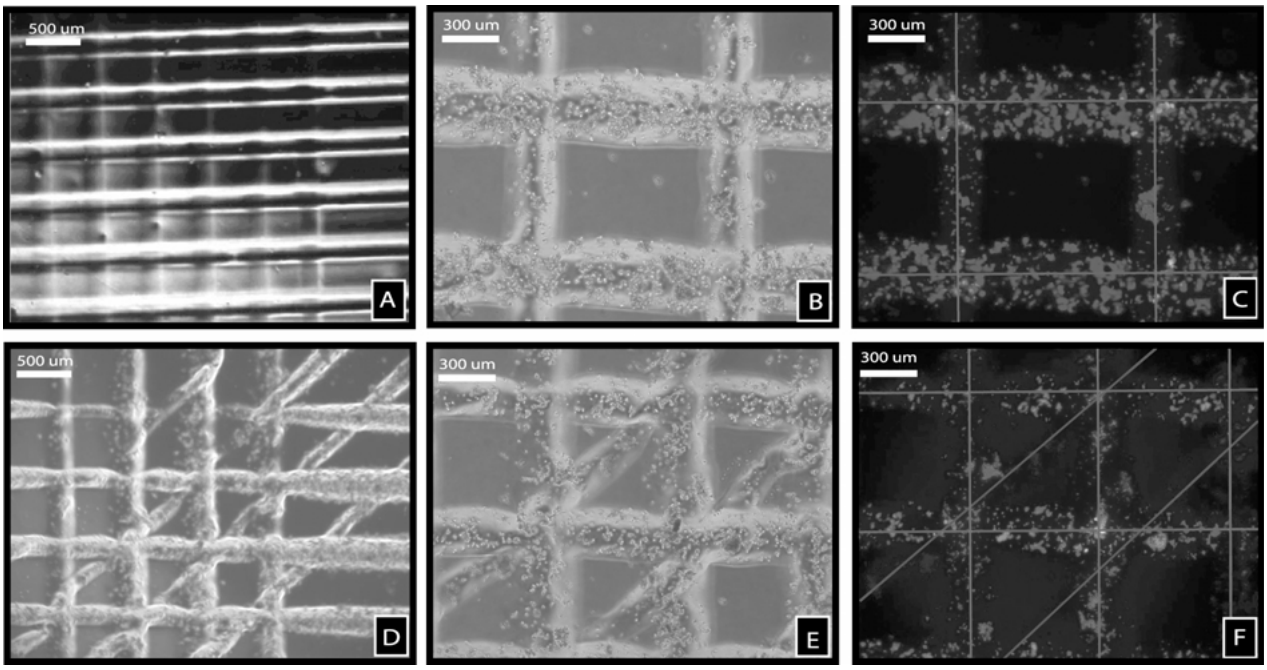


Fig. 5. *Examples of multilayer hydrogel microstructures containing living cells. (A) Two layers of patterned PEGDA lines. (B) Two layers of patterned PEGDA lines containing cells. (C) Fluorescent image of patterned hydrogel lines containing red and green tracked cells, demonstrating different cells in each layer. (D) Three layers of patterned PEGDA lines containing cells at low magnification. (E) Three layers of patterned PEGDA lines containing cells at higher magnification. (F) Fluorescent image of three layers, with one layer containing red labeled cells, one layer containing green labeled cells, and all layers counter-stained blue.*

(green), rinsing away the uncrosslinked polymer and cells, addition of a second cell type (red) in the bare regions followed by uniform exposure to UV light. This type of tissue structure cannot be achieved using other existing techniques.

A similar approach was utilized to photoimmobilize cells in hydrogel microstructures in an array format for applications in cell-based screening. Specifically, a first cell type was photopatterned (red), the remaining polymer and cells were washed away, a second cell type (green) was introduced and exposure to UV light was conducted through a second mask (Figures 4C–D).

By increasing the thickness of the spacer in the apparatus, multilayer hydrogel structures were formed. Sequential fabrication techniques of this genre are referred to as solid free form fabrication and are typically utilized for rapid prototyping. Here, we extend this approach to the fabrication of living tissues. In our example, a mask of parallel lines was used for the first layer, then rotated and used in conjunction with a thicker spacer to create a second layer of hydrogel that is taller than the first (Figure 5A, no cells). When different cell populations were incorporated into each layer, the result can be seen in Figures 5B and 5C, in which green cells are entrapped within horizontal lines in one layer, and red cells are contained within the layer of vertical lines. Addition of a third layer resulted in an even more complex 3-dimensional structure (Figures 5D–F). Figure 5F displays the three separate layers, where cells in one layer are labeled with red, cells within another layer are labeled green, and all three layers are counter-stained with Hoechst dye (blue). This type of tissue structure has not been achieved using other techniques.

Discussion

In order to improve upon current tissue engineering efforts to control tissue architecture, we have developed a method for 3-dimensional patterning of hydrogel microstructures containing living mammalian cells. The principals of photolithography were applied to PEG-based hydrogel chemistry to localize the UV light and thus photocrosslinking, as seen in conventional negative photoresists. We studied the effects of ultraviolet light exposure and photoinitiator concentration on both patterning resolution and cell viability. In developing our system, several design considerations were incorporated: oxygen quenching of the radical polymerization reaction was reduced by enclosing the prepolymer solution within a sealed chamber, hydrogels were covalently bound to the glass wafers to enable subsequent cell culture, and the capability to form multilayer structures was incorporated by use of a

variable height chamber. The utility of this method was demonstrated by photopatterning hydrogels containing live cells in various single layer structures, patterns of multiple cellular domains in a single ''hybrid'' hydrogel layer, and patterns of multiple cell types in multiple layers simulating use in a tissue engineering application.

We found that the pattern fidelity is fairly high for features on the order of hundreds of microns, while we saw feature magnification for smaller feature sizes. This may be a limitation for creating very small features on the order of a few cells, but the achievable resolution is sufficient for producing complex 3-dimensional structures that vary on the same length scale of most tissues ($\sim 100\,\mu m$) (Bhatia and Chen, 1999). We examined the effects of two parameters—UV exposure and photoinitiator concentration—on pattern fidelity. We found that while UV exposure must be limited to preserve patterning resolution, the initiator concentration did not affect the resolution of hydrogel patterning. Thus, the lowest possible initiator concentration should be used that can still initiate crosslinking in order to minimize toxic effects to the cells. The absolute width increase of the lines was not uniform for all feature sizes. The precise mechanism for increased feature widening at small dimensions is not clear. We speculate that hydrogel swelling may be playing a role as the surface area/volume ratio is higher for smaller features. However, since images were taken immediately after photocrosslinking in hopes of minimizing swelling effects, there may be a true non-linearity in the photocrosslinking process. In the future, we plan to develop a simple chemical kinetic model of the photocrosslinking process that may help explain our findings. Using the polymer system in this study, we were able to generate hydrogel features greater than 200 μm with resolution within 10%, while very small feature sizes (30–50 μm) resulted in feature magnification of up to 200%. Previous methods of patterning hydrogels with higher resolution ($< 5\,\mu m$) have patterned a very thin dehydrated polymer and subsequently hydrated the structure to form a swollen hydrogel (Chen et al., 1998; Yu et al., 2000). This approach is not appropriate for fabrication of hydrogel microstructures containing living cells. Others have used very small polymer chains and high polymer densities or very high UV intensities (Ward et al., 2001; Beebe et al., 2000) that are not amenable to cell survival. Because we use longer polymer chains in our system, the hydrogel absorbs a larger quantity of water, which is preferable for living cells, but results in large amounts of swelling that can distort the intended patterns.

There are several parameters that can affect the resolution in our system. The resolution limitations may be caused by the thickness of the polymer solution layer, the quality of the emulsion mask, the non-parallel light source, the scattering of light throughout the polymer solution, and the diffusion of radicals throughout the solution. In some configurations, especially larger feature sizes, interesting edge effects were observed (Figure 2A). This may have been caused by non-uniform transmission of light through the mask (Madou, 1997), or by beam divergence. A more uniform light source and/or focused or collimated light could improve the accuracy of the hydrogel photopatterning. Resolution can also be affected by quenching of the radicals by oxygen. While the polymerization steps were conducted in a sealed chamber, oxygen can be further removed from the system to increase the pattern resolution by bubbling nitrogen gas through the prepolymer solution (Ward et al., 2001) prior to addition of the cells. In addition to the light source and dosage, other aspects of our hydrogel photopatterning method can also degrade the resolution of the patterns. The use of an emulsion mask may contribute to some loss of feature fidelity. This may be improved upon by use of a conventional chrome mask; however, we believe that for the range of feature sizes reported here, the emulsion masks should be sufficient. Emulsion masks offer the advantage of rapid production time and minimal cost. The thickness of the hydrogel can also impact the feature fidelity. While the 100 μm thickness we used is thin as compared to other polymer scaffolds (on the order of millimeters), it is thick as compared to other photopatterned polymers and photoresists, which are typically in the range of 1–25 μm. As the thickness increases, the amount of light scattering also increases, which can significantly affect photopatterning resolution. Light scattering is particularly prevalent in our system as compared with other photolithographic techniques because of the presence of cells within the hydrogel. We believe that a majority of the feature widening may be due to the uncollimated light and the swelling of the hydrogel with water after photocrosslinking. Other factors such as the type of initiator, incorporation of polymerization accelerators, polymer concentration, and polymer chain length may be factors to explore in the future to improve the resolution of this technique.

The photocrosslinking process can be harmful for living cells. Thus, it was of great importance to determine the impact of key variables on cell viability. Due to our interest in tissue engineering of the liver, we conducted our preliminary experiments using a well-characterized human hepatoma cell line. We found that UV exposure itself did not immediately cause cell death; however, the photoinitiator that we used decreased cell viability in a dose dependent fashion. Furthermore, the combination of UV exposure and initiator proved to be the most toxic, presumably due to free radical generation (Figure 3C). Notably, the viability experiments presented

here overestimate the toxicity as compared to normal photocrosslinking conditions. This overestimation arises from a 2-hour incubation time in the viability study, as compared to approximately 10 minutes under photopatterning conditions and the lack of PEGDA to serve as a site for free radical termination.

To improve viability in the future, some of the other parameters can be optimized. For example, other photoinitiators may be less toxic to the cells (Bryant et al., 2000). Specifically, the use of more hydrophilic photoinitiators that are less likely to intercalate in the hydrophobic cell membrane may be beneficial. Also, higher molecular weight PEG polymers could be used to increase the pore size of the hydrogel, although the chain length of PEG has been shown not to be a particularly sensitive parameter for cell viability when tested for porcine islet cells (Cruise et al., 1998). Protective agents such as antioxidants or surfactants can also be added to the cells before addition to the prepolymer solution. Viability may also be increased by stabilization via preaggregation of cells, by coculture with stabilizing cell types (Bhatia et al., 1999), or by addition of biological molecules such as adhesive ligands for adhesion-dependent cells (Hern and Hubbell, 1998; Mann et al., 2001). Because similar PEG-based hydrogel systems have been successfully used with other cell types (Bryant and Anseth, 2002; Elisseeff et al., 2000; Mann et al., 2001; Gobin and West, 2002), we are confident that our method can be optimized to create hydrogel patterns containing a high fraction of cells that can survive, grow, and function.

Progress in tissue engineering is ongoing at a rapid rate; however, novel approaches to forming 3-dimensional tissues will be required to move forward in the future. To our knowledge, existing methods do not allow the spatial localization of multiple cell types in 3-dimensional space. The technique that is presented here lays the groundwork for progress towards solid free form fabrication of living tissues. This methodology will also be useful to study the structure–function relationships of cells and their 3-dimensional microenvironment. In the future, specific adhesion peptides can also be incorporated into the hydrogels to study model cell-matrix interactions in 3-dimensions (Mann et al., 2001; Gobin and West, 2002; Hern and Hubbell, 1998). In addition, the hydrogel chemistry can be tailored to alter and study the role of the mechanical and transport properties of the hydrogel (Cruise et al., 1998). In summary, the methods presented here demonstrate yet another step in the merging of biological sciences with existing engineering technologies. This fusion allowed us to create complex 3-dimensional cellular structures unobtainable using previous methods. In doing so, we hope to better recreate the microarchitecture of tissues, as we strive to develop functional implantable tissue engineered constructs. Finally, in addition to being a powerful tool for tissue engineering, immobilized cellular arrays can be created using this technique and may be useful for cell-based assays for pharmaceutical drug development.

Acknowledgments

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