MULTI-MATERIAL STEREOLITHOGRAPHY: SPATIALLY-CONTROLLED BIOACTIVE POLY(ETHYLENE GLYCOL) SCAFFOLDS FOR TISSUE ENGINEERING

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Abstract **Reviewed, accepted August 27, 2007**

Challenges remain in tissue engineering to control the spatial and temporal mechanical and biochemical architectures of scaffolds. Unique capabilities of stereolithography (SL) for fabricating multi-material spatially-controlled bioactive scaffolds were explored in this work. To accomplish multi-material builds with implantable materials, a new mini-vat setup was designed, constructed and placed on top of the existing build platform to allow for accurate and self-aligning X-Y registration during fabrication. Precise quantities of photocrosslinkable solution were added to and removed from the mini-vat using micro-pipettes. The mini-vat setup allowed the part to be easily removed and rinsed and different photocrosslinkable solutions could be easily removed and added to the vat to aid in multi-material fabrication. Two photocrosslinkable hydrogel biopolymers, poly(ethylene glycol dimethacrylate) (PEG-dma, molecular wt 1,000) and poly(ethylene glycol)-diacrylate (PEG-da, molecular wt 3,400), were used as the primary scaffold materials, and controlled concentrations of fluorescently labeled dextran or bioactive PEG were prescribed and fabricated in different regions of the scaffold using SL. The equilibrium swelling behavior of the two biopolymers after SL fabrication was determined and used to design constructs with the specified dimensions at the swollen state. Two methods were used to measure the spatial gradients enabled by this process with multi-material spatial control successfully demonstrated down to 500-µm. First, the presence of the fluorescent component in specific regions of the scaffold was analyzed with fluorescent microscopy. Second, human dermal fibroblast cells were seeded on top of the fabricated scaffolds with selective bioactivity, and phase contrast microscopy images were used to show specific localization of cells in the regions patterned with bioactive PEG. The use of multi-material SL and the relative ease of conjugating different bioactive ligands or growth factors to PEG allows for the fabrication of tailored three-dimensional constructs with specified spatially-controlled bioactivity.

Introduction

The use of solid freeform fabrication (SFF) or rapid prototyping (RP) technologies in the field of tissue engineering (TE) for fabricating biocompatible three-dimensional (3D) structures has grown rapidly in the last five years (Ang *et al.*, 2002; Arcaute *et al.*, 2005a, b, c, and 2006; Landers *et al.*, 2002; Liu and Bhatia, 2002; Cooke *et al.*, 2002; Dhariwala *et al.*, 2004; Vozzi *et al.*, 2003 and 2004). Different RP technologies and their modified versions have been used in combination with the available biomaterials for the creation of 3D scaffolds. Deposition technologies, such as 3D plotting, were early SFF technologies used in TE. Viscous solutions or pastes of agarose, chitosan, hydroxyapatite, and poly(lactic-co-glycolic acid) (PLGA) are some of the biomaterials used in 3D plotters (Ang *et al.*, 2002; Landers *et al.*, 2002; Vozzi *et al.*, 2003 and 2004). Biomaterials in a powder form such as microspheres of poly(caprolactone) have been

used with selective laser sintering (SLS) RP technology (Vozzi *et al.*, 2003 and 2004). Photolithographic methods involving the use of masks and a source of ultraviolet (UV) radiation have been used along with photoreactive hydrogel materials to produce micro-patterned scaffolds (Hahn *et al.*, 2006; Liu and Bathia, 2002). Although less than 50-μm feature sizes have been achieved using the developed photolithographic methods, the technique is challenging to employ for 3D complex scaffold primarily because of the masking requirements. Advancements in the chemistry of biomaterials have developed photocrosslinkable biopolymers with use in stereolithography (SL). Photocrosslinkable poly(propylene fumarate) is being used in SL to create complex scaffolds for bone regeneration (Cooke *et al.*, 2002), and poly(ethylene glycol) (PEG) with photoreactive end groups such as acrylates or methacrylates is being used in SL for soft TE applications (Dhariwala *et al.*, 2004; Arcaute *et al.*, 2005a, b, c, and 2006).

The use of RP technologies and biomaterials has allowed the creation of biocompatible 3D structures with specific architectural features, but these structures have primarily been limited to the use of single materials. The design of a functional implantable scaffold requires control over the scaffold's macro-scale design, as well as the use of different materials to tailor the micro-scale characteristics of the scaffold. The specific macro-scale design of the scaffold is driven by the overall structure of the tissue that it will eventually regenerate. Spatial control of the scaffold at smaller scales is required to provide a cellular micro-environment with specified bioactivity that promotes regeneration. In this work, we explore the use of SL to create 3D multi-material PEG scaffolds with specified spatially controlled characteristics.

PEG is a biocompatible material with numerous applications in medicine, biological sciences and tissue engineering. PEG is a material suitable to use in SL because it can be easily made photoreactive and crosslinkable by the incorporation of acrylate or methacrylate end groups into the PEG backbone (Zalispky and Harris, 1997). These photoreactive groups, in the presence of a photoinitiator and upon exposure to UV light, serve to crosslink the PEG into a hydrogel. Hydrogels are crosslinked polymeric structures that absorb large amounts of water; they are permeable to oxygen, nutrients, and other water-soluble metabolites and have a soft consistency that makes them similar to soft tissues (Nguyen and West, 2002).

Photopolymerizable PEG hydrogels have been investigated for a variety of TE applications including cell encapsulation (Bryant and Anseth, 2001; Bryant *et al.*, 2004; Burdick and Anseth, 2002; Mann *et al.*, 2001a; Williams *et al.*, 2003), creation of synthetic extracellular matrix (ECM) analogs (Gunn *et al.*, 2005; Mann *et al.*, 2001a; Mann *et al.*, 2001b), and for the formation of substrates with patterned arrays of immobilized proteins and/or cells (Hahn *et al.*, 2006; Liu and Bhatia, 2002). Research has been performed to understand the photocrosslinking of PEG hydrogels in SL and to successfully create complex 3D structures (Arcaute *et al.*, 2005a, b, c, and 2006), although further research is necessary to fabricate complex multi-material PEG structures with accurate dimensions. Since hydrogels are crosslinked polymeric structures that swell in water to an equilibrium state, it is important to characterize the physical properties of hydrogels at the equilibrium "swollen" state. Researchers use different parameters to define the equilibrium-swelling behavior of hydrogels. One widely used parameter is the swelling ratio (or weight degree of swelling), defined as the weight of the equilibrium swollen gel divided by the weight of the dry gel (Peppas, 2004). In this work, we introduce a new parameter to define the equilibrium swelling behavior of hydrogels, called the "dimensional swelling factor." The dimensional swelling factor (DSF) is defined as the ratio of a swollen dimension to that dimension as originally fabricated using SL (or as originally designed in CAD). Taking into

consideration the DSF in the design of the scaffold allows the fabrication of hydrogel scaffolds with the appropriate dimensions at the swollen state.

The present work describes the use of SL to fabricate multi-material 3D constructs with specified spatially-controlled characteristics. Controlled concentrations of fluorescently labeled dextran or bioactive PEG were prescribed and patterned in different regions of scaffolds to demonstrate the ability of SL to fabricate multi-material scaffolds over a range of scales down to 500-μm. Fluorescent images confirmed the presence of the fluorescent component in the specific regions of the scaffolds. Cell seeding experiments revealed specific localization of cells in the regions patterned with bioactive PEG containing the cell adhesion ligand RGDS. The following sections describe the experimentation and results from these studies in more detail.

Materials and Methods

Photopolymer solution

For the work described here, two photoreactive solutions were used as primary scaffold materials. These solutions were prepared using two biocompatible photopolymers: poly(ethylene glycol dimethacrylate) (PEG-dma, molecular wt 1,000; PEG-dma 1K) (Polysciences, Inc., Warrington, PA) and poly(ethylene glycol diacrylate) (PEG-da, molecular wt 3,400; PEG-da 3.4K) (Laysan Bio, Inc., Arab, AL). The photopolymers were dissolved in HEPES at a concentration of 20% (w/v). The photoinitiator Irgacure 2959 (I-2959) (2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone) (Ciba Speciality Chemicals Corp., Tarrytown, NY) was added to the photopolymer solution at a concentration of 0.5% (w/v).

To demonstrate the unique capabilities of SL for fabricating multi-material spatially-controlled scaffolds, fluorescently labeled (490/520 nm) dextran (FITC-dextran, Sigma-Aldrich, St. Louis, MO) or bioactive PEG were added to the photopolymer solution. The bioactive PEG contained the tetrapeptide Arg-Gly-Asp-Ser (RGDS) and was prepared by reacting the peptide with acryloyl–PEG–N-hydroxysuccinimide (acryloyl–PEG–NHS, 3400 Da; Nektar Therapeutics, Huntsville, AL) in 50 mM sodium bicarbonate buffer (pH 8.5) for 2 hours and subsequently freeze-dried (Mann *et al.*, 2001b). The photopolymer solutions with or without either FITC-dextran or bioactive PEG were used to fabricate multi-material scaffolds as described below.

Cell maintenance

Cell culture reagents were obtained from Sigma-Aldrich (Saint Louis, MO) unless otherwise specified. Human dermal fibroblasts (HDFs) were obtained from Cambrex BioScience (Walkersville, MD) and maintained on Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS; BioWhittaker, Walkersville, MD), 200 mM L-glutamine, 10,000 units/mL of penicillin and 10 mg/mL of streptomycin. Cells were maintained at 37 ºC in a 5% CO_2 environment. Cell seeding experiments were conducted using cultures at passage 12 or less.

Material characterization

The equilibrium-swelling behavior of hydrogels was determined as weight degree of swelling (q) and dimensional swelling factor (DSF). Hydrogel samples of the two different photopolymer solutions were crosslinked in SL. A simple ring pattern (ID = 1.72 mm, OD = 2.94 mm) was drawn by the laser to crosslink the samples. The dry (W_d) and swollen (W_s) weights of the samples were measured in order to calculate the weight degree of swelling (q). Also, digital images of the samples were taken at the swollen state using a stereomicroscope (MZ16, Leica

Microsystems, Germany) equipped with a CCD camera (Retiga 2000R Fast 1394, QImaging Corp., Canada). The outside diameter of the scaffolds was measured to estimate the DSF by comparing the swollen diameter with the diameter in the CAD drawing. Different samples of each photopolymer solution were measured to determine these equilibrium-swelling parameters according to the following equations:

$$q = \frac{W_s}{W_d}$$

$$DSF = \frac{swollen\ dimension}{design\ dimension}$$

Multi-material fabrication

A modified 3D Systems (Model 250/50) SL machine equipped with a He-Cd laser (325 nm wavelength) was utilized for layered multi-material manufacturing. The machine modifications involved the removal of the original vat and replacement with a self-aligning mini-vat setup. Figure 1 shows a schematic of the mini-vat setup. The setup consisted of a fixture and a mini-vat. The fixture incorporated a keyway design to secure the mini-vat in a specific X-Y orientation, and the fixture was affixed to the original elevator platform. The mini-vat consisted of two pieces: base and cylinder. The base includes the same keyway design to provide a press-fit into the fixture and maintain X-Y registration of the scaffold during fabrication. This feature provides an easy method for using multiple hydrogel materials for scaffold fabrication. The cylinder of the mini-vat can be interchanged (from small to large) during the build as needed to hold more photopolymer solution and thus manufacture longer scaffolds. The original elevator platform was used to hold the fixture and the mini-vat, and the elevator platform was set at a height at which the laser beam was circular with a diameter of ~250 μm. In addition, the elevator was lowered a distance equal to the elevation change of the liquid solution in the cylinder as new photopolymer solution was added to construct a new layer, maintaining the laser beam diameter at ~250 μm.

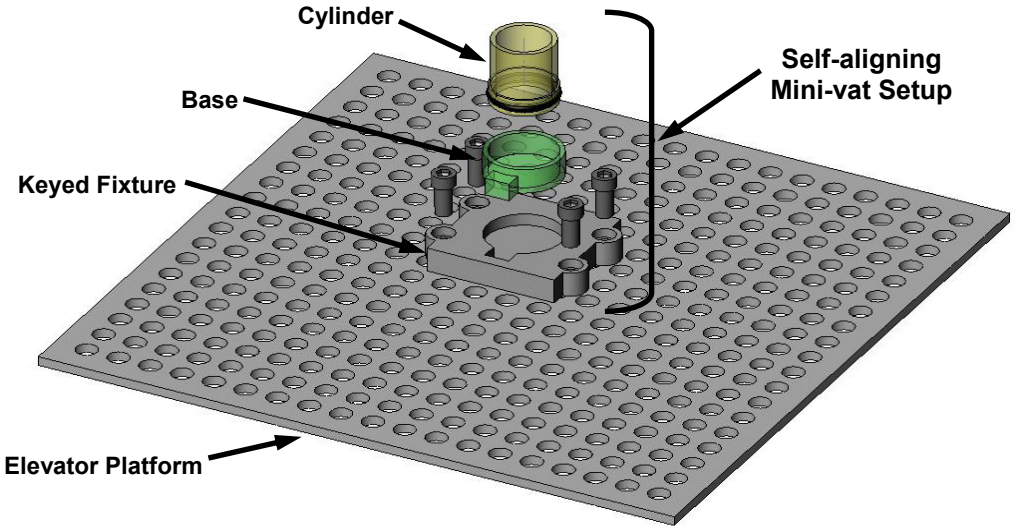

Figure 1. Schematic of the self-aligning mini-vat setup.

The procedure to fabricate a multi-material scaffold started with the CAD drawing of the scaffold in stl format. Build files were obtained using 3DLightyear^{TM}. The build files did not have an extra border, and the border and hatch vectors were commanded to have equivalent speeds so improved geometric accuracy could be achieved as described in Arcaute *et al*., 2006. Figure 2 shows the CAD drawings for the designs of the multi-material scaffolds. In the fabricated scaffolds, the dark regions in the CAD drawings (Figures 2A and 2B) were crosslinked using the photopolymer solution with either FITC-dextran or PEG-RGDS.

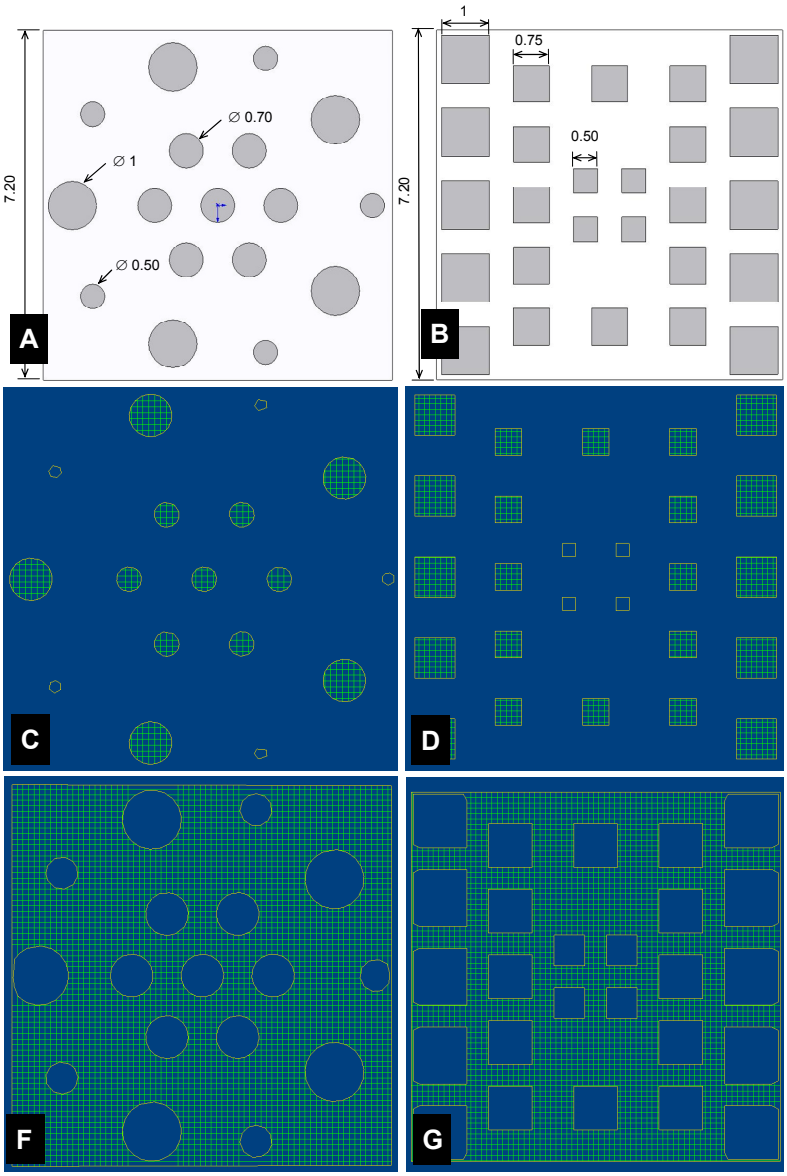

Figure 2. Multi-material scaffold design. A and B: computer-aid design. C and D: vector file of the regions patterned using the photopolymer solution containing fluorescently-labeled dextran or bioactive PEG. E and F: vector file of the regions patterned using the primary photopolymer solution. Units are in mm.

Process planning plays an important role in multi-material fabrication. For the designs in Figure 2, the regions patterned with the photopolymer solution containing FITC-dextran or bioactive PEG were crosslinked first (dark circles or squares with different dimensions) using the build files shown in Figures 2C and 2D. A small amount of photopolymer solution (for a layer thickness of ~ 1mm) containing FITC-dextran or PEG-RGDS was added to the mini-vat, and the individual build file to command the laser to crosslink the specific pattern was used. Once the pattern was crosslinked, the photopolymer solution was removed from the mini-vat to be reused. The mini-vat was removed from the fixture to rinse the patterned scaffold with copious amounts of distilled water. Excess water in the crosslinked pattern was removed using a Kimwipe. The mini-vat was then placed in the fixture, and a precise amount of photopolymer solution (without FITC-dextran or PEG-RGDS) was added using a micro-pipette to have a ~1-mm thick layer. Then, the corresponding build file was used to crosslink the new desired pattern (see Figure 2F and 2G). Once the pattern was

crosslinked, the photopolymer solution was removed to be reused and the scaffold was rinsed to remove any unreacted photopolymer. The completed scaffold was then removed from the mini-vat.

The multi-material scaffolds fabricated with the photopolymer solution containing FITC-dextran were placed in water, and fluorescent and brightfield images were taken to examine the fluorescently patterned regions. The concentration of the fluorescently labeled dextran in the photopolymer solution was 20 mg/mL. The scaffolds fabricated with the photopolymer solution containing PEG-RGDS were allowed to swell in media overnight. Cells were then seeded on top of the scaffolds and observed using phase contrast microscopy.

Cell seeding

The multi-material scaffolds used for the cell seeding experiments contained regions patterned using the photopolymer solution containing 20mg/mL of bioactive PEG. The bioactive PEG contained the cell adhesion ligand RGD found in many extracellular matrix proteins, including fibronectin and laminin (Yamada, 1991). Upon photopolymerization, the ligand is attached to the scaffold through a PEG spacer chain, and the ligand can interact freely with cells, since plain PEG has been shown to be cell non-adhesive, by providing attachment sites for the cells in specific regions of the scaffold (Zalipsky and Harris, 1997).

The solutions used to prepare the multi-material scaffolds for the cell seeding experiments were filter sterilized (0.22 μm filter; Cole-Parmer, Vernon Hills, IL) prior to crosslinking. The scaffolds fabricated for the cell seeding experiments were made using the PEG-da 3.4K photopolymer solution. Once the scaffolds were fabricated, they were immediately rinsed with Dulbecco's phosphate buffered saline (D-PBS) solution, and then placed in individual wells of a 24-well plate with media. The plate was kept at 37ºC and 5% CO_2 overnight for the scaffolds to swell to equilibrium and for the unreacted material trapped within the gel to leach out. The media was removed, and sterile silicone rings (Pharmed® BPT, Saint-Gobain Performance Plastics, Akron, OH) were used to secure the scaffolds at the bottom of the well, preventing cells from going underneath the scaffold and attaching to the well's surface. A cell suspension containing 10,000 cells was added to each scaffold (two scaffolds for each design), and then the scaffolds were incubated at 37ºC and 5% CO_2. Cells plated on wells without scaffolds served as controls. After 24 hours, the media was replaced to remove any non-adherent cells. Cell adhesion was examined using phase contrast microscopy (Leica DMIRB, Leica Microsystems, Germany) at 24 and 48 hours.

Results

Material characterization

The experimental results for the equilibrium swelling behavior are shown in Figure 3. The swollen and dry weights of the scaffolds made with the two different solutions of photoreactive PEG are shown in Figure 3A. The calculated weight degree of swelling (q) of each solution is also included in the graph. The graph in Figure 3B compares the dimensions of the scaffold (outside diameter) at the equilibrium swollen state with the specified dimension (from the CAD file). The graph in Figure 3B shows a significant change (DSF of 1.25) in dimensions for the gels made out of PEG-da 3.4K. As a result, the scaffolds made using a 20% (w/v) PEG-da 3.4K solution swell to a final dimension 25% larger than the specified dimension. This dimensional change can be taken into account in the CAD file so that the final dimensions for the PEG-da 3.4K scaffolds can be prescribed in the equilibrium swollen state. The calculated DSF for the

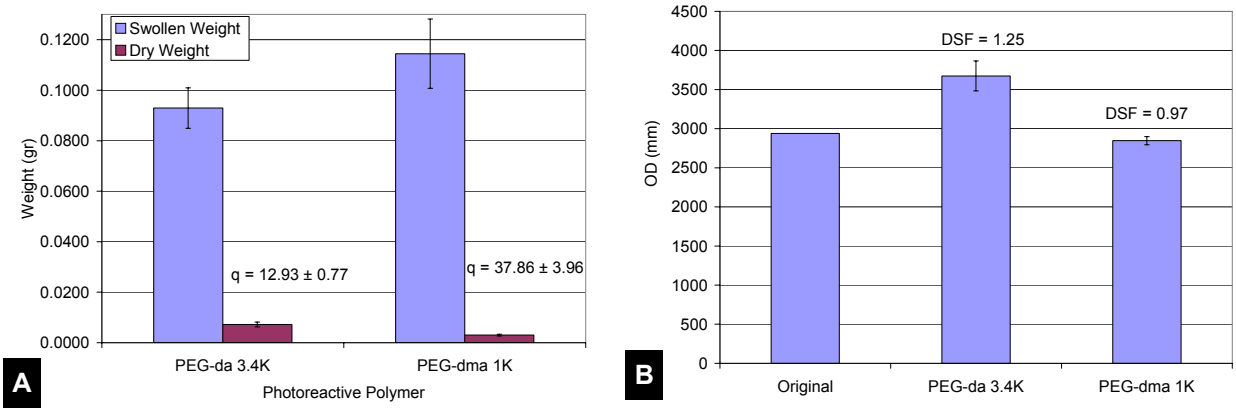

Figure 3. Equilibrium swelling behavior parameters. A: Weight degree of swelling for the different photopolymers used. B: Dimensional swelling factor calculated comparing the outside diameter of the original scaffold design and the measured outside diameter of the fabricated scaffolds at the equilibrium swollen state for the different photopolymers used. The photopolymer concentration was 20% (w/v).

PEG-dma 1K gels is 0.97, indicating a slight shrinkage in dimensions. This shrinkage can also be taken into account in the CAD file, although the original CAD file for the PEG-dma 1K gels was not modified for this case. Statistical analysis could be used to more precisely specify the DSFs for these solutions (and this will be performed as we explore the limits on micro-scale fabrication using these techniques), but the preliminary analysis performed here was sufficient for this study.

Scaffold fabrication

Simple scaffolds made with different materials were fabricated using stereolithography. Brightfield images of the multi-material scaffolds fabricated with both photopolymer solutions containing FITC-dextran are shown in Figure 4. The pictures on top of Figure 4 (A and B) correspond to the scaffolds fabricated using PEG-dma 1K while pictures C and D correspond to the scaffolds fabricated using PEG-da 3.4K. Figure 5 shows the corresponding fluorescent images of the scaffolds in Figure 4. The fabrication of the multi-material scaffolds shown in Figures 4 and 5 took approximately 10 minutes. During fabrication, the rinsing (using a wash bottle with distilled water) and removal of excess water (using a Kimwipe) were the most time consuming steps since care was required in order to avoid damaging the scaffold. The scan times for the scaffolds were less than 5s for the fluorescently labeled (or bioactive) regions and approximately 20s for the plain PEG regions.

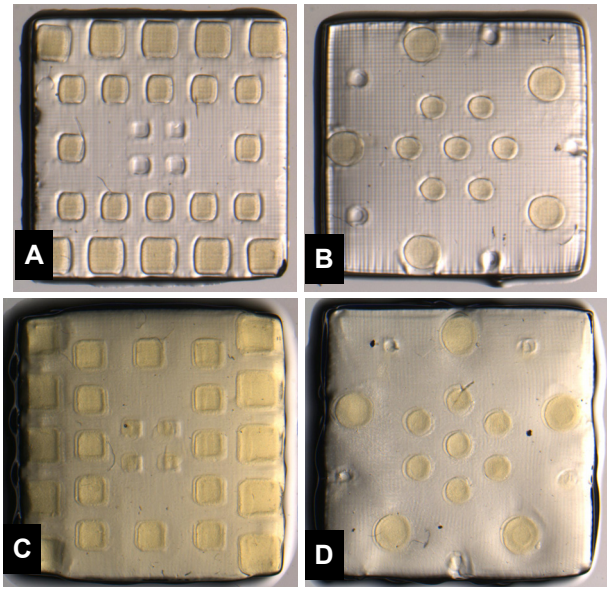

Figure 4. Brightfield images of the multi-material scaffolds fabricated using fluorescently-labeled dextran. A and B(10X): scaffolds fabricated with PEG-dma 1K as the main material. C and D (7.1X): scaffolds fabricated with PEG-da 3.4K as the main material.

Figure 4 shows that the smallest features (∅ = 500 µm, L = 500 µm) in the fluorescent pattern were not crosslinked when using the PEG-dma 1K photopolymer solution. This can be seen more clearly in the corresponding fluorescent images in Figure 5 as there is no fluorescence in the regions where the smallest features should be located (see Figure 2 for the original CAD files and build files). The absence of these features is due to the SL machine building only border vectors (without hatching) and so the solution is not receiving sufficient crosslinking energy. This can be accounted for by adding additional border vectors for the smallest features (which can be done by including an additional stl file of the small features only, for example). As the scales get even smaller (below 500 µm), micro-fabrication issues such as this will need to be explored (see Wicker *et al.*, 2005).

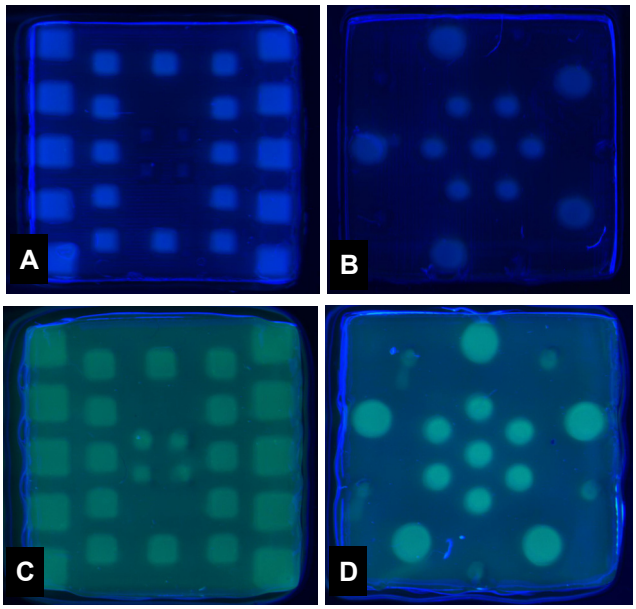

Figure 5. Fluorescent images of the multi-material scaffolds fabricated using fluorescently-labeled dextran. A and B (10X): scaffolds fabricated with PEG-dma 1K as the main material. C and D (7.1X): scaffolds fabricated with PEG-da 3.4K as the main material

The overall fluorescence of the scaffolds made with PEG-da 3.4K is also higher than the PEG-dma 1K (Figure 5). This can be explained by our simple fabrication strategy. Since the fluorescent dextran was physically trapped within the crosslinked gel, it leaches (diffuses) out of the matrix once it is crosslinked. Since the molecular weight of the PEG 3.4K is 3.4 times that of

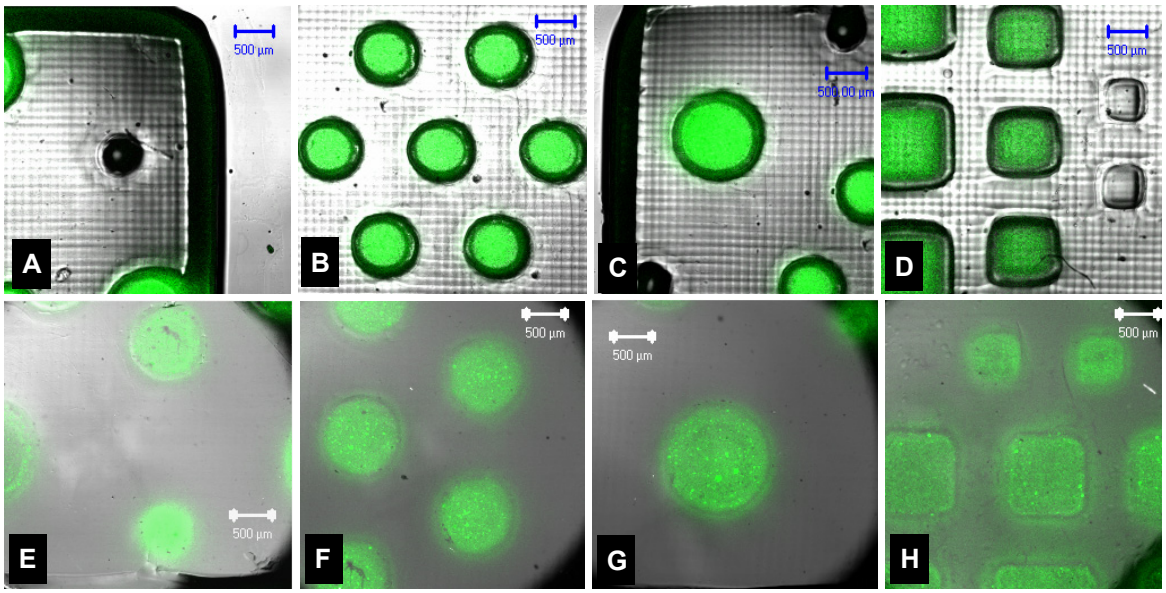

Figure 6. Confocal microscope images (25X) of the multi-material scaffolds fabricated using fluorescently-labeled dextran. A-D: scaffolds fabricated with PEG-dma 1K as the main material. F-I: scaffolds fabricated with PEG-da 3.4K as the main material.

PEG 1K, the FITC-dextran molecules leach out faster in the higher molecular weight PEGs. Fluorescent confocal microscopy images of the scaffolds taken at higher magnification corroborate these results. Brightfield and fluorescent images were acquired using a confocal laser scanning microscope (Zeiss LSM-5 Pascal, Carl Zeiss MicroImaging, Germany). The images in Figure 6 are the combination of both brightfield and fluorescent images. Figures 6A, C, and D show the empty spaces where the smallest features should be located. Also, it can be seen in Figure 6H how quickly the fluorescently labeled dextran leaches out from the crosslinked PEG-da 3.4K gel. The images in Figures 4, 5, and 6 were taken quickly after fabrication (and not at equilibrium) in order to avoid the leaching effect. We are presently covalently attaching FITC to PEG so that this effect can be eliminated.

Figure 7. Complex 3D structure manufactured in multi-material SL. PEG-dma 1K at a 20% (w/v) concentration was used as the main material. The solution used to create the staircase contained fluorescent microspheres. Scale bar represents 5mm.

A complex, multi-material 3D structure was fabricated using different solutions of PEG hydrogel as a demonstration of our fabrication technique. The rook shown in Figure 7 was made using a photopolymer solution of PEG-dma 1K at a 20% (w/v) concentration. The solution used to create the staircase contained fluorescent (580/605 nm) 10-μm microspheres (FluoSpheres®, Molecular Probes, Inc., Eugene, OR). The multi-material rook was manufactured in 0.010-in layers semi-automatically in three stages. In the first stage, almost the entire piece was fabricated except for the staircase and the top part that includes the ceiling and the parapet. Then, the staircase was built using a different photopolymer solution containing fluorescent spheres. Finally, the top of the rook and the parapet were fabricated using the same photopolymer solution used in the first stage. The fabrication of the multi-material rook took approximately 3 hours using the multi-material fabrication procedure described here. The same rook fabricated out of plain PEG using this procedure requires less than 2 hours, and a single rook fabricated out of commercial SL resin and using the automated commercial system requires 2.5 hours. Thus, the build time for the multi-material fabrication process shown here is comparable to the automated commercial system primarily because sweeping is not required between layers (the viscosity of the solution is essentially that of water). The multi-material

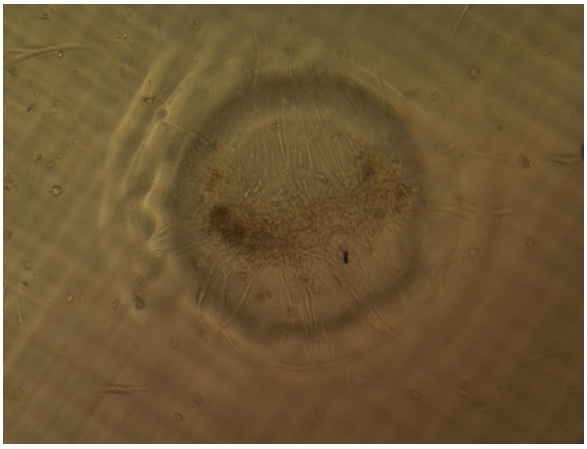

Figure 8. Representative phase contrast image (50X) of HDFs attached to the regions patterned with the photopolymer solution containing bioactive PEG-RGDS.

build times could be reduced by automating the material fill and removal procedure, including developing an intermediate scaffold cleaning procedure.

Cell seeding

Cell seeding experiments revealed specific localization of cells in the regions patterned with bioactive PEG containing the cell adhesion ligand RGDS. Procedures identical to the ones described above were used to fabricate bioactive scaffolds, where in this experiment, bioactive PEG replaced the fluorescently labeled PEG described above. As expected, the bioactive PEG provided a preferential attachment location for the cells. Phase contrast microscopy images were taken at 24 and 48 hours after cell seeding. Figure 8 shows a representative image of the HDF cells attaching to a circular region where the PEG-RGDS is located.

Conclusions

A commercial SL system was successfully modified to allow the fabrication of 3D PEG-based multi-material structures for tissue engineering applications. The SL system modifications involved the removal of the original vat and replacement with a self-aligning mini-vat setup. The design of the mini-vat allowed for repeatable and precise X-Y registration of the construct during fabrication so complex 3D multi-material structures could be created. Photocrosslinkable solution was added to and removed from the mini-vat using micro-pipettes in order to construct the part in a layer-by-layer fashion. During fabrication, the fixture was removed so the construct could be separately rinsed. Using the self-aligning feature, the fixture was replaced and a multiple material build continued by adding a different photocrosslinkable material to the build chamber. This strategy enabled multiple material fabrication within and across layers with achievable features down to 500-μm. Extending this to smaller scales is currently underway with realistic expectations of features on the order of 100-μm in the near term (using the commercially available 75-μm beam diameter) and smaller features in the longer term. We also believe the fabrication techniques described here could be automated fairly easily for specific applications, although we are not presently working on automating these techniques.

Furthermore, this work characterized the equilibrium swelling behavior by measuring the swelling ratio and the dimensional swelling factor for the two photocrosslinkable hydrogel biopolymers: PEG-dma 1K and PEG-da 3.4K. By measuring the dimensional swelling factor, hydrogel constructs could be designed and constructed with specified dimensions at the swollen state. Most importantly, the use of multi-material SL for the fabrication of tailored 3D constructs with specified spatially-controlled characteristics was demonstrated. Controlled concentrations of fluorescently labeled dextran or bioactive PEG were prescribed and fabricated in different regions of the scaffold. Fluorescent images confirmed the presence of the fluorescent component in the specific regions of the scaffolds. Cell seeding experiments revealed specific localization of cells in the regions patterned with bioactive PEG containing the cell adhesion ligand RGDS. We believe the use of multi-material SL and the relative ease of conjugating different bioactive ligands or growth factors to PEG provide for the fabrication of 3D multi-material constructs with specified spatially-controlled bioactivity. This technology and the spatial control over bioactivity that it provides can be applied to a number of current tissue engineering challenges.

Acknowledgements

The facilities within the W.M. Keck Center for 3D Innovation (Keck Center) used here contain equipment purchased through Grant Number 11804 from the W.M. Keck Foundation and a faculty STARS Award from the University of Texas System. This work was funded, in part,

through the Chihuahua Government (scholarship to KA) and the Mr. and Mrs. MacIntosh Murchison Chair I in Engineering (RW). The authors are grateful for the assistance of Frank Medina, Luis Ochoa, and Julieta Ventura of the Keck Center on various aspects of the project. The authors would also like to thank Marian Viveros from the UTEP Department of Biological Sciences for her microscopy technical assistance. Equipment and facilities in the Analytical Cytology Core Facility of the Biological Sciences Department used here are maintained through NCRR Grant Number 5G12 RR008124.

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