HYDROGELS IN STEREOLITHOGRAPHY

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Abstract **Reviewed, accepted August 23, 2005**

The use of stereolithography (SL) for fabricating complex three-dimensional (3D) tissue engineered scaffolds of aqueous poly(ethylene glycol) (PEG) hydrogel solutions is described. The primary polymer used in the study was PEG-dimethacrylate (PEG-dma) with an average molecular weight (M_W) of 1000 in distilled water with the photoinitiator Irgacure 2959 (I-2959). Successful layered manufacturing (LM) with embedded channel architecture required investigation of the photopolymerization characteristics of the PEG solution (measured as hydrogel thickness or cure depth) as a function of photoinitiator concentration and laser energy dosage for a specific photoinitiator type and polymer concentration in solution. Hydrogel thickness was a strong function of PI concentration and energy dosage. Curves of hydrogel thickness were utilized to successfully plan, perform, and demonstrate layered manufacturing of highly complex hydrogel scaffold structures, including structures with internal channels of various orientations. Successful fabrication of 3D, multi-layered bioactive PEG scaffolds containing cells was accomplished using a slightly modified commercial SL system (with 325 nm wavelength laser) and procedure. Human dermal fibroblast (HDF) cells were encapsulated in PEG hydrogels using small concentrations (~ 5 mg/ml) of acryloyl-PEG-RGDS (M_W 3400) added to the photopolymerizable PEG solution to promote cell attachment. HDF cells were combined with the PEG solution, photocrosslinked using SL, and successfully shown to survive the fabrication process. The combined use of SL and photocrosslinkable biomaterials such as PEG makes it possible to fabricate complex 3D scaffolds that provide site-specific and tailored mechanical properties (i.e., multiple polymer materials) with a polymer matrix that allows transport of nutrients and waste at the macroscale and facilitates cellular processes at the microscale through precisely placed bioactive agents.

1. Introduction

When stereolithography (SL), a rapid prototyping (RP) or layered manufacturing (LM) process that could automatically build detailed three-dimensional (3D) parts, became commercially available in the mid-1980s, the manufacturing industry was profoundly impacted by significantly improving the way in which prototypes were fabricated (Wohlers, 2004). Commercial line-scan SL solidifies thin layers of photo-reactive liquid polymer using an ultraviolet (UV) laser that selectively crosslinks the liquid photopolymer by rastering across the surface of the polymer contained in a vat. 3D shapes are built one layer at a time by attaching the desired part to a build platform that begins at the surface of the liquid photopolymer and traverses deeper into the liquid after each successive layer. Since the introduction of SL, a number of new RP technologies have been developed and commercialized with widely varying applications in engineering design, manufacturing, medicine, and more.

During the last few years, RP technologies have been employed quite extensively in the field of tissue engineering (TE) for manufacturing 3D scaffolds that provide the mechanical support and structure for cells to grow and regenerate damaged tissue. Researchers have investigated a wide variety of strategies for engineering implantable scaffolds for tissue regeneration. The

manufacturing processes used for engineering implantable scaffolds depend on the material used to fabricate the scaffold and the intended application. Porous polymer scaffolds that act as substrates for cell attachment are produced using techniques such as fiber bonding, membrane lamination, and solvent casting-particulate leaching (Lu and Mikos, 1996; Ma, 2004). However, complex scaffolds that mimic tissue structures have been difficult to produce, or the fabrication method has been difficult to scale up to a manufacturing level. Also, using traditional manufacturing processes has not allowed the fabrication of scaffolds with predetermined internal structures that provide the cells with an optimum local microenvironment. Precise control of the scaffold microenvironment allows the incorporation of the necessary biochemical stimulus for the cells to proliferate, differentiate, migrate, and undergo apoptosis. RP technologies are increasingly demonstrating the potential for fabricating 3D structures with precise control of the micro- and macro-scale characteristics (Liu and Bhatia, 2004). Some researchers have developed new RP technologies for the fabrication of tissue scaffolds based on the 3D dispensing of aqueous solutions and pastes (Landers *et al*., 2002; Ang *et al*., 2002). Other researchers (Cooke *et al*., 2002; Dhariwala *et al*.,2004; Comeau *et al*. 2005) have explored the capabilities of SL for manufacturing biodegradable polymeric scaffolds. Using SL, Cooke *et al*. (2002) were able to fabricate acellular TE scaffolds using poly(propylene fumarate) (PPF) with applications in bone regeneration where the scaffold contained 3D macroscale features and interconnecting microscale porous networks. In addition to the PPF, a class of hydrophilic polymer materials, called hydrogels, exists that are widely used in tissue engineering and can be photocrosslinked.

Poly(ethylene glycol) (PEG) is a synthetic hydrogel polymer used in tissue regeneration because of its characteristics as being considered non-toxic, non-immunogenic and easily cleared from the body. PEG is water soluble and can be modified with photoreactive and crosslinkable groups like acrylates or methacrylates (Harris and Zalipsky, 1997). Further, PEG can be made bioactive by incorporating adhesion ligands, growth factors, and cytokines to the polymer solution during the photocrosslinking process. Bioactive factors can be attached to the scaffold through functional groups or may simply be trapped within the hydrogel during crosslinking (Mann *et al.*, 2001a and b). These characteristics make PEG a strong candidate for creating photocrosslinkable hydrogel tissue engineered scaffolds (Arcaute *et al.*, 2005b).

Our research group has been developing new multiple material SL fabrication technologies for use in TE applications (Wicker *et al.*, 2004) and investigating the use of PEG in SL for TE applications (Arcaute *et al.*, 2005a; Arcaute *et al.*, 2005b; Pallante *et al.*, 2005). Recent attention in peripheral nerve regeneration has focused on developing multi-material and multi-lumen PEG-based nerve guidance conduits (NGCs) with precisely placed bioactive agents and cells to regenerate peripheral nerves faster and over longer distances. SL has demonstrated that it has the capability of accurately manufacturing these multi-material and multi-lumen NGCs with the additional advantage of being able to scale up for mass production (Arcaute *et al.*, 2005b).

As a result, the present work describes our experiences with using SL for fabricating complex 3D tissue engineered PEG-based scaffolds. The primary polymer used here was PEG-dimethacrylate (PEG-dma) with an average molecular weight (M_W) of 1000 in distilled water with the photoinitiator Irgacure 2959 (I-2959). The photopolymerization characteristics of the PEG solution were investigated where hydrogel thickness or cure depth was measured as a function of photoinitiator concentration and laser energy dosage. Curves of hydrogel thickness were utilized to successfully plan, perform, and demonstrate layered manufacturing of highly complex hydrogel scaffold structures, including structures with internal channels of various orientations. Successful fabrication of 3D, multi-layered bioactive PEG scaffolds containing

cells was accomplished using a slightly modified commercial SL system and procedure. Human dermal fibroblast (HDF) cells were encapsulated in the PEG matrix during SL and tested for cell viability. In the cell encapsulation experiments, small concentrations (~ 5 mg/ml) of acryloyl-PEG-RGDS were incorporated in the photopolymerizable PEG solution to fabricate bioactive scaffolds that promote cell attachment. The following sections describe the methods and results of these investigations.

2. Experimental Methods

2.1. Cure Depth Experiments

Investigation of the cure depth characteristics of a hydrogel material as a function of different factors was carried out previously (Arcaute *et al*., 2005a). In this earlier study, cure depth, hydrogel thickness, or simply gel thickness was found to be a strong function of photoinitiator (PI) type and concentration, energy dosage, and polymer concentration in the solution. For the two PIs considered in the previous study, Irgacure 2959 (I-2959) appeared to be a superior candidate to use in the fabrication of scaffolds in a layer-by-layer fashion. Additional cure depth experiments were carried out and are described in the following sections to better understand the photocrosslinking process of a hydrogel material.

2.1.1. Materials

The polymer used was PEG-dma with average M_W of 1000 (Cat. No. 15178, Polysciences, Inc., Warrington, PA). PEG was chosen because it is a synthetic material with important use in tissue regeneration. The PI used was I-2959 (2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone) (PN 1559163, Ciba Speciality Chemicals Corp., Tarrytown, NY). Cytocompatibiblity studies (Bryant *et al*., 2000; Leach *et al*., 2003) have shown that I-2959 is an advantageous radical-forming photoinitiator in cell encapsulation applications since I-2959 does not have a significant detrimental effect on cell survival. Also, I-2959 allows the photopolymerization of PEG-based solutions as thin polymer networks that permit the fabrication of 3D PEG scaffolds using SL (Arcaute *et al.*, 2005a). The polymer was dissolved in distilled water to prepare a 30% (w/v) solution. Aliquot portions were separated from this solution, and different concentrations of I-2959 were added. The PEG-dma solution with PI is the photoreactive solution used in the cure depth experiments.

2.1.2. Apparatus

A 3D Systems Model 250/50 SL machine equipped with a He-Cd laser (325 nm) was used in this study. The original setup of the SL machine was modified slightly to carry out the gel thickness experiments. The modifications consisted of removing the original vat of material from the machine and setting the elevator platform at a fixed height. A flat-top cylindrical container was placed on the center of the platform as depicted in Figure 1. At the test height of the elevator platform, the laser beam was circular with a diameter of ~250 μm.

2.1.3. Procedure

The PEG-dma solution with PI was pipetted inside the container and filled up to the rim. A glass slide was placed on top of the container and in contact with the PEG-dma solution. The glass slide acted as a substrate for gel attachment and facilitated measurement of gel thickness. Samples were cured by writing a vector pattern through the glass slide and into the PEG-dma container at different energy dosages. The vector pattern consisted of a series of nineteen parallel lines with each line ~250 µm wide, or one laser beam width, 7.62 mm long and spaced ~355 µm apart. This vector pattern formed a ~6.64 mm by 7.62 mm rectangle (in the x and y dimensions) from which the sample cure depths were measured (in z). The laser beam was commanded to draw the vector pattern, and the energy dosage was varied by changing the SL machine parameters that control the scan speed of the laser (Arcaute *et al.*, 2005a).

After polymerization, the glass slide was lifted off the container with the polymerized gel attached to the slide. The cured gels were flushed with distilled water to remove unreacted PEG-dma solution, and the thickness (in z) of the gels was then measured with a caliper. Five sample gels were cured for each of the PEG-dma solutions, and the results presented in the next section represent the average thickness measurements for the samples. It was determined by using a laser power meter that the glass slide filtered the laser power by ~18%. The measured power of the He-Cd laser (rated at 40 mW) at the fixed platform height was 29.5 to 30.6 mW and 35.8 to 37.1 mW with and without the glass slide, respectively. The measured laser powers with the glass slide along with the speed of the laser beam given by the SL machine were used to determine the laser energy dosages.

2.2. Scaffold Fabrication

The modified SL machine used in the cure depth experiments was used for the fabrication of PEG-based scaffolds. A flat-bottom container is used as the receptacle for the photoreactive solution instead of the original vat. In this modified setup, a thin (0.45 mm) glass slide was placed within the flat-bottom container to act as a platform for the scaffolds and facilitate their removal once the scaffolds were built. The elevator platform started at a height at which the laser beam was circular with a diameter of ~250 µm, and the receptacle contained sufficient photoreactive polymer to form a single layer. The elevator was lowered every time more

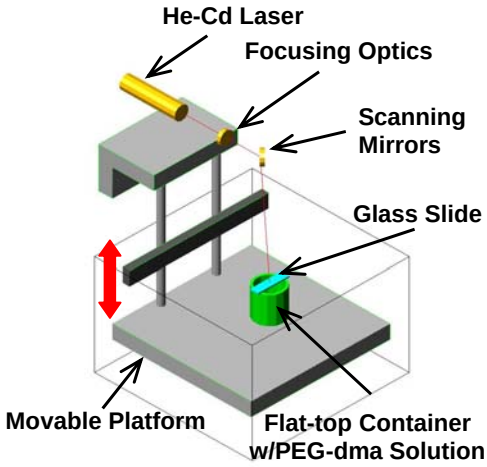

Figure 1. Schematic of SL showing the setup to perform the gel thickness experiments.

polymer solution was added to the flat-bottom container in order to construct a new layer and maintain the laser beam diameter at ~250 μm. Fabrication of multiple material scaffolds is also feasible using this setup by adding and withdrawing materials as each layer is fabricated.

2.3. Fabrication of Hydrogel Scaffolds Containing Live Cells

Live cells and bioactive agents were combined with PEG-dma photoreactive solution and photocrosslinked using SL. For these experiments, human dermal fibroblasts (HDF) cells were encapsulated in bioactive PEG scaffolds. The tetrapeptide RGDS was conjugated to PEG and added in small concentrations to the photoreactive solution to promote cell adhesion. The following sections describe the experimental methods and procedures carried out to create cell-containing scaffolds.

2.3.1. Cell Maintenance

Human dermal fibroblasts (HDFs) were obtained from Cambrex Bio Science (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 incubated at 37ºC in a 5% CO_2 environment. Cells at passage four were used in the experiments after 80-90% confluence was reached.

2.3.2. Peptide Conjugation to PEG

The tetrapeptide Arg-Gly-Asp-Ser (RGDS) was conjugated to PEG 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 h. The mixture was then lyophilized and stored frozen. The RDGS sequence is a known cell adhesion ligand (Mann *et al*., 2001; Mann and West, 2002). The resulting acryloyl-PEG-RGDS was used to promote cell attachment and fabricate bioactive scaffolds.

2.3.3. Bioactive Scaffold Fabrication

First, the PEG-dma and the photoinitiator I-2959 were dissolved in a 10 mM HEPES (N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) buffered saline solution (pH 7.4). A small concentration (5-10 mg/mL) of acryloyl-PEG-RGDS was then added to the photoreactive PEG-buffered solution, and the solution was filter sterilized. A cell suspension in complete media was mixed in equivolume amount with the PEG solution to form a solution with the following concentrations: 0.25 x 10^6 cells/mL, 30% (w/v) PEG-dma, 0.5% I-2959, and 5 mg/mL of acryloyl-PEG-RGDS. The final PEG-cells solution was then pipetted inside the flat-bottom container, and the SL machine was commanded to photocrosslink a simple pattern (ID = 3 mm, OD = 5mm). The scaffolds were fabricated with three different conditions: (a) single layer (1-mm thick), (b) single layer – pattern drawn twice (1-mm thick), and (c) double layer (2-mm thick). Eight scaffolds were fabricated for each condition in batches of four scaffolds.

Once the cell-containing scaffold was fabricated, it was placed in a 96-well plate with media and placed in an incubator at 37ºC and 5% CO_2. Cell viability of the encapsulated cells was tested with the LIVE probe of the LIVE/DEAD® assay (Molecular Probes, LIVE/DEAD® Viability/Cytotoxicity Kit L-3224, Eugene, OR) at 48 hours. To perform the viability assay the old media was removed, and the gels were rinsed twice with Dulbecco's phosphate buffered saline solution (D-PBS). After that, the gels were stained with Calcein AM 0.4 μM and observed using a confocal microscope with fluorescence capabilities.

3. Results

3.1. Cure Depth Curves

The experimental measurements of gel thickness as a function of PI concentration and energy dosage are shown in Figures 2 and 3. The gel thickness curves as a function of PI concentration shown in Figure 2 illustrate that the gel thickness begins at zero for zero PI concentration, has a maximum at low PI concentrations, and decreases asymptotically to a non-zero value as PI concentrations increase. The gel thickness starts at zero due to the presence of polymerization inhibitors, including monomethyl ether hydroquinone (MEHQ) and butylated hydroxytoluene (BHT), added by the manufacturer. Measurements in the region between zero and the maximum gel thickness are not presented here as the gel is loosely crosslinked at these PI concentrations and the measurements are highly uncertain (and thus, the maximum gel thickness presented here should not be viewed as an absolute maximum). The level of crosslinking is also related to the PI concentration. In general, for a given laser energy, low PI concentrations correspond to thicker, loosely crosslinked gels, while high PI concentrations correspond to highly crosslinked and thinner gels.

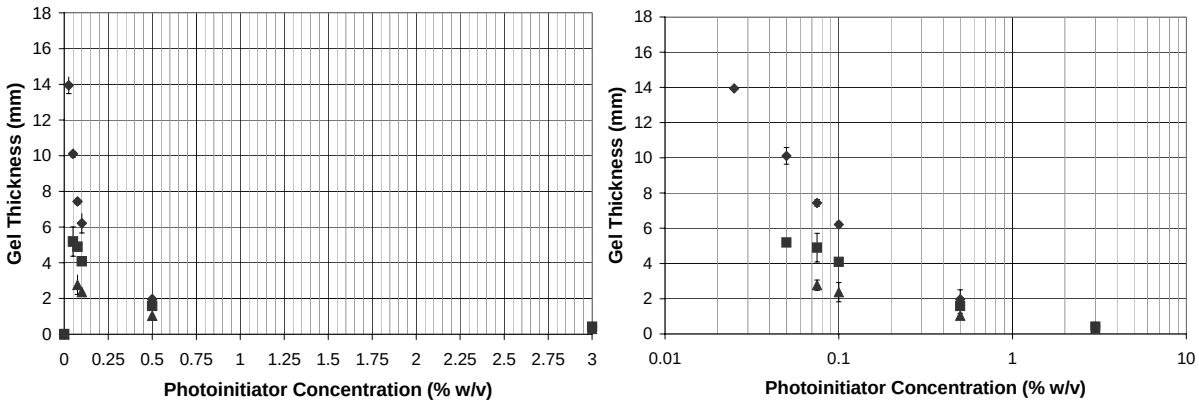

Figure 2. Gel thickness curves versus photoinitiator concentration for a 30% (w/v) PEG-dma solution in distilled water: linear scale (left) and log scale (right). The three different types of markers correspond to three different energy dosages (♦ 3.604 J/cm^2 [0.258 IPS], ■ 1.640 J/cm^2 [0.567 IPS], ▲ 0.586 J/cm^2 [1.585 IPS]). Error bars not shown are contained within the symbol.

The energy dosage significantly affects gel thickness. High energy dosages generally produce thicker gels. Figure 3 reports the relationship between energy dosage and gel thickness, for a PEG solution with 0.5% (w/v) I-2959. For PI concentrations smaller than 0.05%, small energy dosages (fast scanning speeds) produce thick and loosely crosslinked gels.

3.2. Scaffold Fabrication Demonstration

Complex 3D hydrogel structures were fabricated using the cure depth data by selecting a combination of layer thickness and energy dosage (scan speed and laser energy) that provided adequate resolution for the desired part and produced sufficiently crosslinked gels, respectively. The pictures in Figure 4 show two chess rooks manufactured using SL. The rook on the right in each picture was manufactured using the 3D Systems Viper™ Si^2 SL apparatus using DSM Somos® WaterShed™ 11120 resin. The rook on the left in each picture was built in the modified SL 250/50 apparatus with a 30% (w/v) PEG-dma solution with 3% (w/v) of I-2959. As

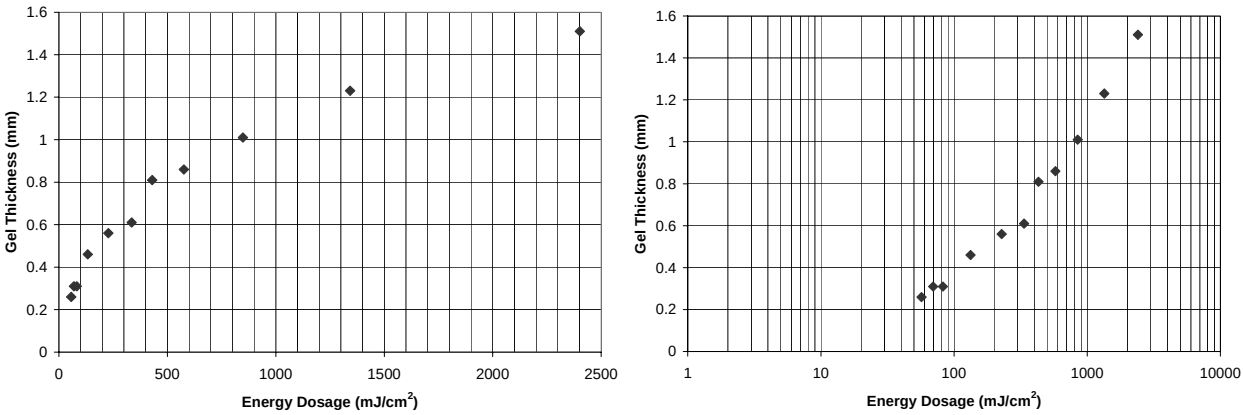

Figure 3. Gel thickness vs. energy dosage for a 30% (w/v) PEG-dma solution with 0.5% (w/v) of I-2959: linear scale (left) and log scale (right).

shown in the picture in the middle of Figure 4, the PEG-based chess rook is elastic and can be easily deformed. The PEG-based chess rook includes all the details of the resin-based rook such as the staircase contained within the walls, the windows, and the bricks in the facade. The PEG-based piece measures approximately 23 mm, and was fabricated in 0.010-in layers. The picture on the right illustrates the shrinkage experienced by PEG when dry.

Other complex PEG-based structures with embedded channels can also be fabricated using SL. The pictures in Figure 5 show a block with a single embedded channel that bifurcates into two branches. These two branches then bifurcate again into two channels (each) in a different plane of the gel (each bifurcation is oriented 90^{o} from one another). This bifurcating geometry is representative of the arterial system and a gel such as this one may potentially be used as a tissue construct in angiogenesis studies (along with the appropriate bioactive agents and cells). The dimensions of the PEG block are 1 by 1 by 1.6 cm, and the diameters of the embedded channels are 1 mm. The scaffold was built with a 30% (w/v) PEG solution containing 0.5% I-2959. Scaffolds of virtually any length and geometry can be fabricated as demonstrated by the scaffold in the bottom of Figure 5, which starts as a single channel and bifurcates twice with an overall length of approximately 5 cm.

Figure 4. Complex 3D structures manufactured using SL, the chess rook on the right in each picture was made with DSM Somos® WaterShed™ 11120 resin while the rook on the left is PEG-based. The picture on the left shows a PEG-based structure immediately after it was built. The picture in the center shows how easily a PEG-based structure can be deformed. The picture on the right shows a dry PEG-based structure, illustrating the shrinkage experienced by PEG when dry.

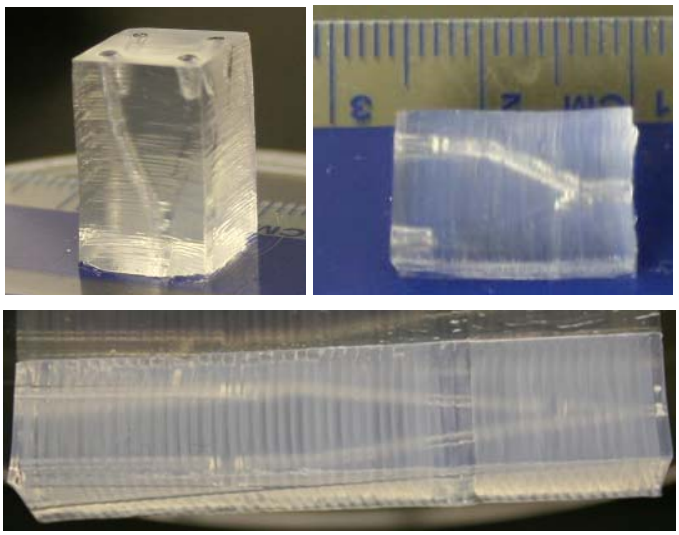

Figure 5. PEG-based scaffolds with embedded channels of different sizes and orientations: isometric view (top left) side view (top right) of a 1 by 1 by 1.6 cm block, isometric view of a 1 by 1 by 5 cm block.

The scaffolds shown in Figure 6 illustrate examples of multi-material fabrication. Each different color corresponds to two layers of 1 mm thickness each. The design corresponds to a multi-lumen nerve guidance conduit (NGC) with end caps for suturing to the severed nerve stumps. The dimensions of the end caps are OD = 5 mm, ID = 3 mm, and the multi-lumen section contains twelve lumens, each 500 μm in diameter. Commercially available NGCs are artificial tubes, made out of biocompatible polymers such as silicone and polyglycolic acid (PGA) and their water content is similar to human tissue. These single-lumen NGCs are designed for permanent implantation (in the case of PGA, the polymer is eventually bio-absorbed) and have proven to be effective in repairing damaged nerves. Occasionally these tubes require removal due to local discomfort caused by inflammation at the severed nerve ends (Lundborg *et al.*, 2004). Research in TE nerve regeneration is now introducing bio-absorbable, bio-degradable materials, growth factors, and cells into the NGCs to promote faster healing over longer lengths. Current research is examining conduit designs with multiple lumens in order to better mimic the natural structure of nerves (Hadlock *et al.*, 2000, Sundback *et al.*, 2003, Lundborg *et al.*, 2004). A low-pressure injection molding, thermally induced phase transition technique was recently developed to fabricate multi-lumen biodegradable NGCs (Sundback et al., 2003). These five-lumen conduits are made out of polylactide-co-glycolide (PLGA) and coated with laminin to promote the adhesion of Schwann cells that are seeded into the scaffolds prior to implantation in rats. After six weeks, *in vivo* regeneration in these conduits was favorably compared with autografts. Even though these conduits were shown to be effective in the regeneration of nerves, the low-pressure injection molding fabrication technique would be difficult to scale-up to a manufacturing level. The combined use of SL and photocrosslinkable biomaterials (like PEG) makes it possible for the bulk fabrication of complex 3D scaffolds. In the case of NGCs, SL successfully fabricates multi-lumen and multi-material NGCs that along with precisely placed bioactive agents and cells could be used to regenerate peripheral nerves faster and over longer distances. Our group is investing considerable effort in this area of research at the present time (Arcaute *et al.*, 2005b).

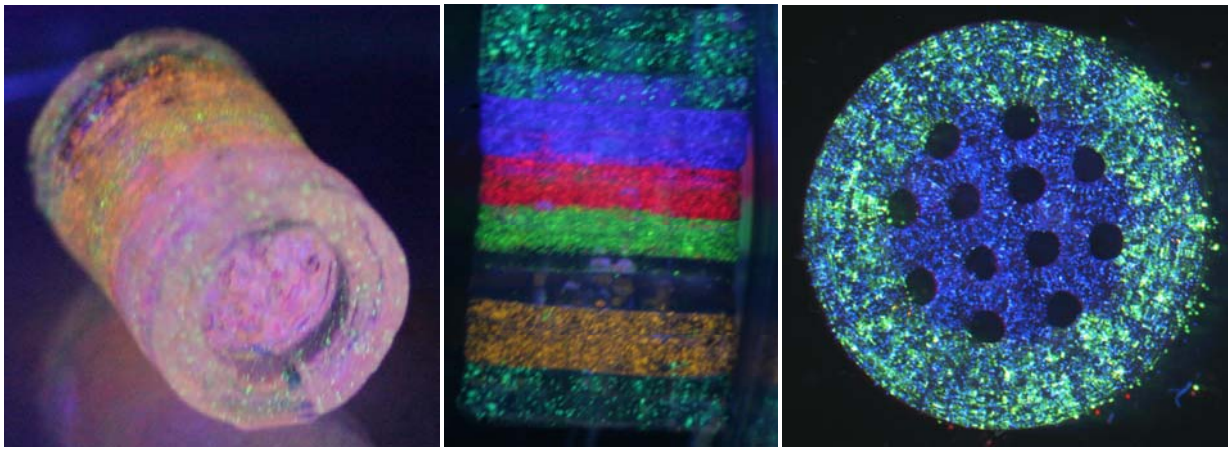

Figure 6. PEG-based multi-lumen scaffolds containing different colors of 15-µm fluorescent particles: isometric view (left), side view (center), and top view (right).

3.3. Scaffolds Containing Live Cells and Bioactive Factors

HDF cells encapsulated inside PEG scaffolds fabricated in SL successfully survived the fabrication process. The photocrosslinked samples containing cells were stained with the LIVE probe after 48 hours, and observed using a confocal microscope with fluorescence capabilities. The LIVE probe distinguishes live cells by measuring intracellular esterase activity. The non-fluorescent calcein, present in the LIVE probe, enters the cell membrane and is converted by enzymes in living cells to fluorescent calcein. The pictures shown in Figure 7 show that the hydrogel samples fabricated in SL have an even distribution of live cells. The pictures were taken at low (2.5X) magnification, and show approximately half of the cell-containing scaffold.

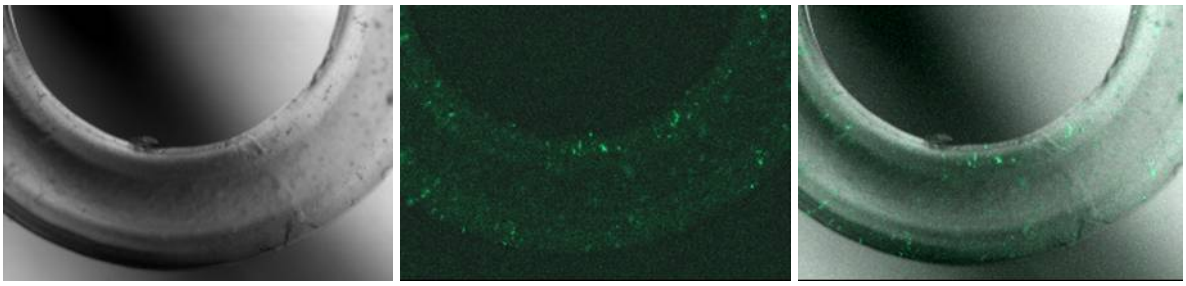

Figure 7. Live stain of the cell-containing scaffold after 48 hr: visible light picture (left), fluorescent light picture (middle), and visible and fluorescent light pictures overlapped (right). These pictures were taken at low (2.5X) magnification.

Figure 8 shows 10X magnification pictures of scaffolds fabricated at the three different conditions: single layer scaffold (1-mm thick) in the top row, single layer scaffold with laser pattern drawn twice (1-mm thick) in the middle row, and double layer scaffold (2-mm thick) in the bottom row. Qualitatively, increased numbers of live cells are observed in the single and double layer scaffolds (top and bottom rows) compared with the single layer scaffold fabricated with twice the UV laser energy. The decreased number of cells in the successive exposure gel could be due to the increased UV exposure (at the laser wavelength of 325 nm). Work is ongoing to test the effect of successive UV exposure as well as other SL fabrication parameters on cell viability.

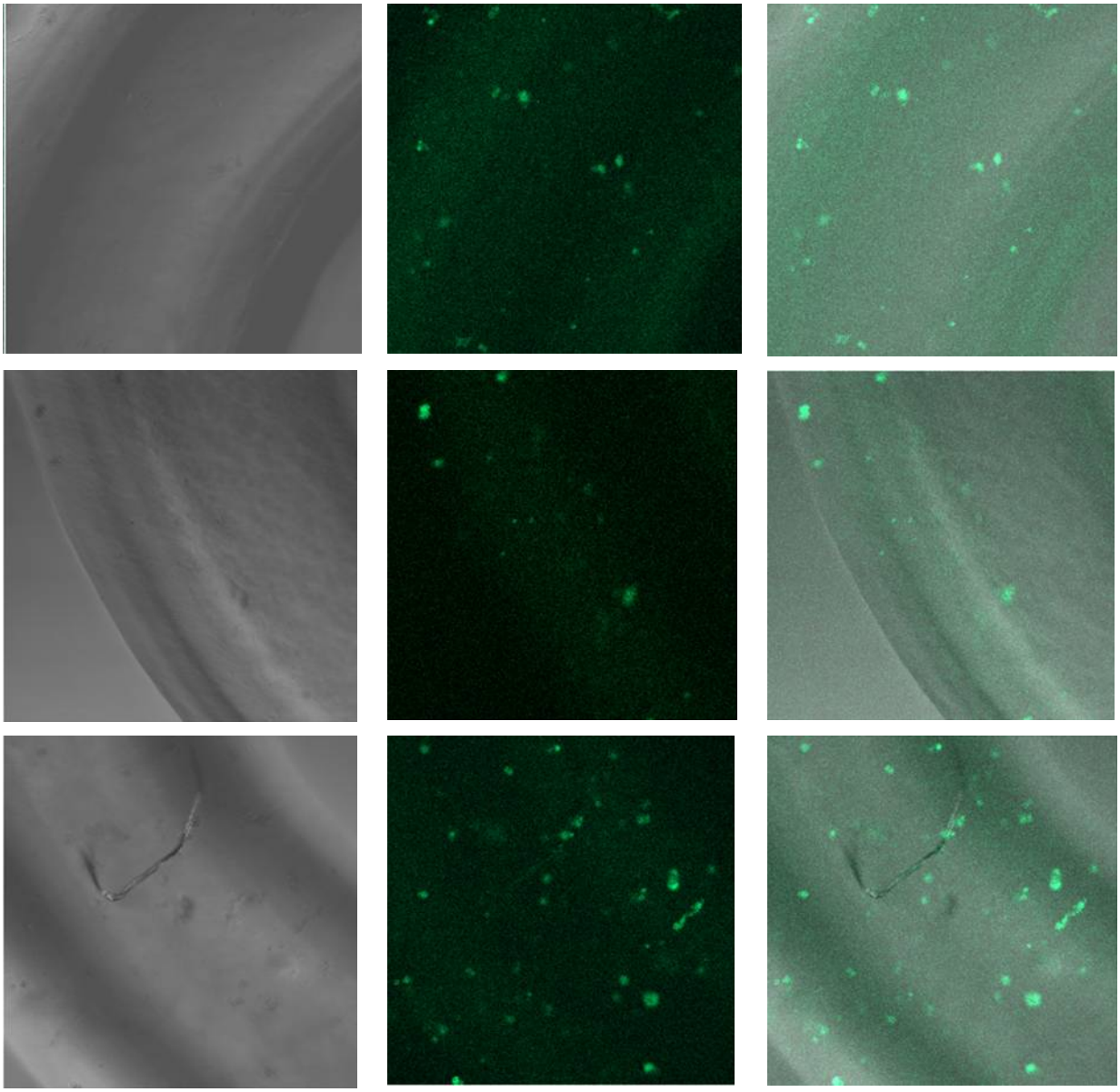

Figure 8. Live stain (after 48 hours) of the cell-containing scaffolds fabricated in SL. Top row: single layer scaffold (1-mm thick); middle row: single layer scaffold with laser pattern drawn twice; bottom row: double layer scaffold (2-mm thick). Left column: visible light pictures, middle column: fluorescent light pictures, and right column: visible and fluorescent light pictures overlapped.

4. Conclusions and Future Work

The use of SL for fabricating complex 3D tissue engineered PEG scaffolds was presented. The photopolymerization reaction of the materials used in SL should be sufficiently fast to produce thin polymer networks with adequate mechanical strength to be handled. A practical method was described which determined the cure depth, or gel thickness, of a photoreactive hydrogel solution. The gel thickness curves obtained with this approach were utilized to successfully fabricate PEG-based scaffolds using SL. Gel thickness can be controlled by varying

the PI concentration or the energy dosage (using SL scan parameters and laser energy) for a given PI concentration. Curves of hydrogel thickness were utilized to successfully plan, perform, and demonstrate layered manufacturing of highly complex hydrogel scaffold structures, including structures with internal channels of various orientations. Successful fabrication of 3D, multi-layered bioactive PEG scaffolds containing cells was accomplished using a slightly modified commercial SL system and procedure. Human dermal fibroblast (HDF) cells were encapsulated in bioactive PEG hydrogels fabricated using SL and successfully demonstrated to survive the fabrication process.

The combined use of SL and photocrosslinkable biomaterials such as PEG makes it possible to fabricate complex 3D scaffolds that provide site-specific and tailored mechanical properties (using multiple polymer materials) with a polymer matrix that allows transport of nutrients and waste at the macroscale and facilitates cellular processes at the microscale through precisely placed bioactive agents. The preliminary results presented here demonstrate that SL is a capable hydrogel layered manufacturing technology with potential for fabricating TE scaffolds incorporating precisely placed bioactive agents and live cells. Additional work is necessary to quantify cell viability in the fabricated scaffolds, and to determine what effects other SL factors (such as laser wavelength, successive energy exposure, radical formation during photocrosslinking, and others) have on cell viability and proliferation.

Acknowledgments

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