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# Stereolithography of spatially controlled multi-material bioactive poly(ethylene glycol) scaffolds

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ABSTRACT

Challenges remain in tissue engineering to control the spatial, mechanical, temporal 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, a mini-vat setup was designed allowing for self-aligning X–Y registration during fabrication. The mini-vat setup allowed the part to be easily removed and rinsed, and different photocrosslinkable solutions to be easily removed and added to the vat. Two photocrosslinkable hydrogel biopolymers, poly(ethylene glycol) dimethacrylate (PEG-dma, MW 1000) and poly(ethylene glycol) diacrylate (PEG-da, MW 3400), were used as the primary scaffold materials. Multi-material scaffolds were fabricated by including controlled concentrations of fluorescently labeled dextran, fluorescently labeled bioactive PEG or bioactive PEG in different regions of the scaffold. The presence of the fluorescent component in specific regions of the scaffold was analyzed with fluorescent microscopy, while 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. Multi-material spatial control was successfully demonstrated in features down to 500 μm. In addition, 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. 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.



## 1. 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 (3-D) structures has grown rapidly in the last 5 years [1–11]. Different RP technologies and their modified versions have been used in combination with the available biomaterials for the creation of 3-D scaffolds. Deposition technologies, such as 3-D plotting, were early SFF technologies used in TE. Viscous solutions or pastes of agarose, chitosan, hydroxyapatite and poly(lactic-co-glycolic acid) are some of the biomaterials used in 3-D plotters [1,6,10]. Biomaterials in a powder form, such as microspheres of poly(caprolactone), have been used with selective laser sintering RP technology [11]. Photolithographic methods involving the use of masks and a source of ultraviolet (UV) radiation have been used along with photoreactive hydrogel materials to produce 2-D [7,12] and 3-D [13] micro-patterned scaffolds. Although feature sizes of less than 50 μm have been achieved using the developed photolithographic methods, the technique is challenging to employ for large 3-D complex scaffold primarily because of the masking requirements. These techniques are similar to the RP technology of stereolithography (SL), as a photoreactive material and a UV source are used to create 3-D features in a layered fashion. SL has a potential use in TE for fabricating 3-D tissue engineered scaffolds for both soft and hard TE applications. SL is an accurate and easy-to-use technology to fabricate complex structures individually or in mass production. SL has open and easy access to the building chamber, and the building envelope can be easily adapted on demand, either in size or to create a clean, particulate-free environment, or for accurate registration between materials in multi-material fabrication [14,15]. The SL system laser and optics are relatively easy to control, with potential for high accuracy in micro-fabrication. Despite these advantages, the use of SL in TE has not been explored to any great extent, perhaps because of the lack of commercially available implantable or biocompatible materials from the SL industry. Advancements in the chemistry of biomaterials have developed photocrosslinkable biopolymers with potential use in SL. Photocrosslinkable non-toxic formulations have been developed recently for use in SL [16–20].

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For example, mixtures of photocrosslinkable poly(propylene fumarate) and diethyl fumarate [8,18] and mixtures of polyfunctional methacrylic oligomers and hydroxyapatite [19,20] have been used in SL to create complex porous scaffolds for bone regeneration. Poly(ethylene glycol) (PEG) with photoreactive end groups such as acrylates or methacrylates is being used in SL for soft TE applications [2–5,9]. Other biopolymers, such as the natural polysaccharide hyaluronic acid, have also been modified with photocrosslinkable groups [21–23], and therefore may find potential use in SL.

The use of RP technologies and biomaterials has allowed the creation of biocompatible 3-D 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 microenvironment with specified bioactivity that promotes regeneration. In this work, we explore the use of SL to create 3-D multi-material PEG scaffolds with specified spatially controlled characteristics.

PEG is a biocompatible material with numerous applications in medicine, biological sciences and tissue engineering. It is 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 [24]. These photoreactive groups, in the presence of a photoinitiator and upon exposure to UV light, serve to crosslink the PEG into a hydrogel. Photopolymerizable PEG hydrogels have been investigated for a variety of TE applications including cell encapsulation [25–29], creation of synthetic extracellular matrix analogs [28,30,31], and for the formation of substrates with patterned arrays of immobilized proteins and/or cells [7,12]. Research has been performed to understand the photocrosslinking of PEG hydrogels in SL and to successfully create complex 3-D structures [2–5], although further research is necessary to fabricate complex multi-material PEG structures with accurate dimensions.

The present work describes the use of SL to fabricate multi-material 3-D constructs with specified spatially controlled characteristics. Equilibrium swelling dimensional factors were determined in order to properly design scaffolds with specified final swollen size features. Then controlled concentrations of fluorescently labeled dextran, bioactive PEG or fluorescently labeled bioactive PEG were 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 microscopy was used to confirm the presence of the fluorescent component (physically trapped or covalently attached to hydrogel) in the specific regions of the scaffolds. Furthermore, cells were seeded onto scaffolds to demonstrate specific localization of bioactive PEG.

## 2. Materials and methods

### 2.1. Photopolymer solution

Two biocompatible photopolymers – poly(ethylene glycol) dimethacrylate (PEG-dma, MW 1000; Polysciences, Inc., Warrington, PA) and poly(ethylene glycol) diacrylate (PEG-da, MW 3400; Laysan Bio Inc., Arab, AL) – were the primary materials used to prepare the photoreactive solutions. The photopolymers were dissolved in HEPES-buffered saline at a concentration of 20 wt.%. The photoinitiator Irgacure 2959 (I-2959, Ciba Speciality Chemicals Corp., Tarrytown, NY) was added to the photopolymer solution at a concentration of 0.5 wt.%.

To demonstrate the unique capabilities of SL for fabricating multi-material spatially controlled scaffolds, fluorescently labeled (490/520 nm) dextran (fluorescein isothiocyanate (FITC)–dextran, Sigma–Aldrich, St. Louis, MO), bioactive PEG or fluorescently labeled bioactive PEG was added to the photopolymer solution. The bioactive PEG contained the tetrapeptide Arg–Gly–Asp–Ser (RGDS; Sigma, St. Louis, MO) and was prepared by reacting the peptide with acrylate–PEG–succinimidyl carboxymethyl (ACRL–PEG–SCM, MW 3400; Laysan Bio Inc.) at a 1:2 M ratio in 50 mM sodium bicarbonate buffer (pH 8.5) for 2 h and subsequent freeze-drying [31]. FITC (Sigma, St. Louis, MO) was used to fluorescently label the bioactive PEG (PEG–RGDS). FITC was dissolved in anhydrous dimethyl sulfoxide (5 mg $ml^{-1}$) and added to PEG–RGDS dissolved in 50 mM sodium bicarbonate buffer (pH 8.5) at a 1:10 M ratio. The reaction mixture was allowed to react for 8 h in the dark at 4 °C. The desired product (PEG–RGDS–FITC) was purified by dialysis and then freeze-dried. The photopolymer solutions with or without FITC–dextran, PEG–RGDS or PEG–RGDS–FITC were used to fabricate multi-material scaffolds as described below.

### 2.2. Cell maintenance

Cell culture reagents were obtained from Sigma–Aldrich (St. Louis, MO) unless otherwise specified. Human dermal fibroblasts (HDFs) were obtained from Cambrex BioScience (Walkersville, MD) and maintained on Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, MD), 200 mM L-glutamine, 10,000 units $ml^{-1}$ of penicillin and 10 mg $ml^{-1}$ of streptomycin. Cells were maintained at 37 °C in a 5% $CO_2$ environment. Cell seeding experiments were conducted using cultures at passage 12 or less.

### 2.3. Material characterization

The dimensions of swollen hydrogel scaffolds at equilibrium were measured to determine the dimensional swelling factor (DSF). Hydrogel samples of the two different photopolymer solutions were crosslinked in SL. A simple ring pattern (inside diameter (ID) = 1.72 mm, outside diameter (OD) = 2.94 mm) was drawn by the laser to crosslink the samples. 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 ODs and IDs of different samples ($n \geqslant 4$) were 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 (one measurement per dimension per sample) to determine the DSF at equilibrium according to the following equation:

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

### 2.4. Multi-material fabrication

A modified 3-D 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. Fig. 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: a

| ID | Title | Pages |
|---|---|---|
| 1678 | Stereolithography of spatially controlled multi-material bioactive poly(ethylene glycol) scaffolds | 8 |