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Four-order stiffness variation of laser-fabricated photopolymer biodegradable scaffolds by laser parameter modulation

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ABSTRACT

The effects of various fabrication parameters of our Mask Projection Excimer Laser StereoLithography (MPExSL) system were investigated. We demonstrate that laser parameters directly change the physical properties (stiffness, thermal degradation, and height/thickness) of the poly(propylene fumarate) (PFF) scaffold structures. The tested parameters were the number of pulses, fluence per pulse and laser repetition rate. We present a four-order tuning capability of MPExSL-fabricated structures' stiffness without altering the resin composition or using cumbersome post-treatment procedures. Thermogravimetric analysis and differential scanning calorimetry confirmed this tuning capability. Prototype-segmented scaffold designs are presented and analyzed to further expand the concept and exploit this in situ stiffness tuning capability of the scaffolds for tissue engineering and regenerative medicine applications.

1. Introduction

Tissue engineering (TE) [1–4] is an expanding interdisciplinary field with the purpose of growing tissues directly on controlled microenvironments called scaffolds. The design and fabrication of structures are among those parameters that strongly affect the successful outcome of tissue formation. Therefore, before these artificial structures can be considered for medical applications, they must fulfill some general requirements, such as proper porosity and mechanical properties, making them capable of supporting the engineered tissue [5].

One of the major goals of tissue engineering is to provide a rapid and reliable production of well-designed and functional scaffolds, capable of fulfilling the aforementioned requirements. The tuning of these characteristics is also highly desired — in some cases, even within the same structure, in order to achieve multi-phase constructs [6,7].

It is therefore crucial to be able to reliably adjust all physical parameters of the scaffolds, including the stiffness (Young's modulus), the degradation (rate), and the geometry (size and porosity) with a high level of biocompatibility. All structures presented here were fabricated by our novel fabrication process called Mask Projection Excimer Stereolithography (MPExSL) [8] using poly(propylene fumarate) (PFF) resin.

MPExSL is a simple and highly efficient method optimized for rapid prototyping of single-layer (2D) and multilayer (3D) TE scaffolds of various sizes and porosities. It is based on pulsed excimer laser photocuring of a biocompatible photosensitive resin. The biocompatibility of MPExSL-fabricated structures has already been investigated by our group in previous in vitro [9] and in vivo [10] studies. Besides, elastine [11,12] and titanate nanotubes [13,14] were utilized as functional coatings on these PPF scaffolds.

MPExSL applies several variable laser parameters — such as the number of pulses, the pulse fluence and laser repetition rate. The main resin composition parameter is the photoinitiator concentration [15–18]. All of these parameters are potentially capable of tuning the physical properties of the scaffolds [19] due to the changes in the initial photocross-linking density [16], and the reversion occurring under intense exposure [20]. The current study aims at unraveling the effects of these aforementioned parameters on the height, thermal degradation, and stiffness of the fabricated structures.

Scaffolds with properly adjusted stiffness are indispensable to mimic the tissue's extracellular matrix [21–24]. High stiffness is also important for greater structural integrity, for instance, in osteochondral tissue regeneration [25–27]. Usually though, the stiffness tuning capability of these scaffolds is limited, leading to constructs fabricated from various metals, polymers, and ceramics, depending on their functionality. The lack of a facile tuning process becomes apparent when tissue interfaces are repaired using composite scaffolds [25–29] and these structures are made of multiple materials employing lengthy and costly processes.

In this study, we present an *in situ*, 4-order stiffness tuning (4 MPa to 4 GPa) of biodegradable and biocompatible scaffolds without altering the PPF resin composition. The modulation of the scaffold's degradation rate by hydrolysis in 37 °C DMEM is also reported.

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All samples were fabricated by our fast prototyping MPExSL system/technique.

2. Materials and methods

2.1. Stereolithography setup and mask projection

In MPExSL, schematically illustrated in Fig. 1, the image of a mask is projected on the liquid resin defining the geometry of the solidified polymer scaffold. The pulse fluence is controlled by a variable attenuator, while the proper positioning of the resin surface is performed by moving the x-, y-, z-axes using a motorized 3-axis stage. The precise stage movement, the repetition rate and the number of pulses are controlled by a personal computer. A CCD camera is mounted on the top of the optical system to in situ monitor the process.

For 2D scaffold production, a thin layer of resin is sandwiched between a glass holder and a quartz plate as presented in [30].

For 3D scaffolds, the thickness of one layer depends on the energy absorbed in the resin during the exposure time, i.e., the combination of number of pulses, fluence per pulse, repetition rate, light wavelength, and photoinitiator concentration. The overlap between the scaffold building layers is adjusted by a fourth axis, moving the stage vertically in the resin between laser exposures.

A set of interchangeable masks is applied to define the dimensions, porosity, and shape of the fabricated structures. The high-resolution features that can be achieved by our projection technique are demonstrated in Fig. 2a. For 2D scaffolds, in principle, pore diameters can be as low as 5 μm. For 3D structures, due to differences in the fabrication technique meaning the layer-by-layer method and the ordinarily high aspect ratio, some tens of μm in diameter can be achieved depending on the scaffold height. The outer diameter/geometry of the scaffold can be further modulated by an iris positioned in the beam path right in front of the mask(s).

Certain flexibility emerges from the programmable feature of the MPExSL's XYZ stage control illustrated in Fig. 2b, where a "star" shape was used as a standard unit for the fabrication.

2.2. The applied resin and light source

Poly(propylene fumarate) (PPF) [31–33] is a good and versatile candidate for tissue engineering due to its (i) biodegradability, (ii) cytocompatibility of the degradation products, (iii) high Young's modulus and (iv) the unsaturation in the backbone of the polymer.

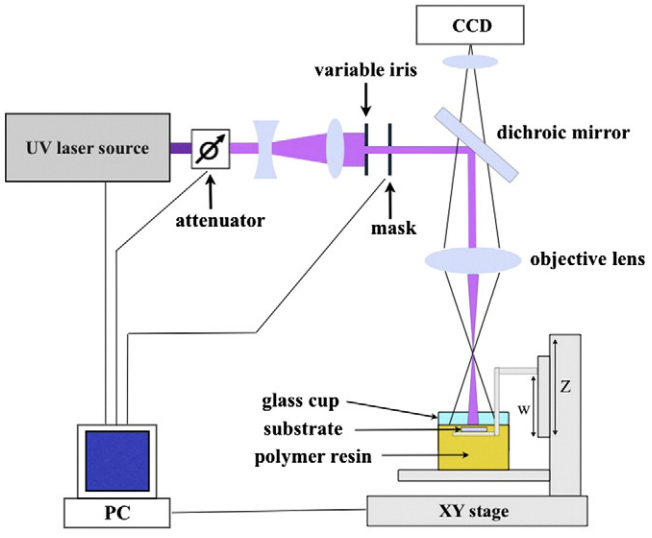

Fig. 1. Schematic of the MPExSL setup for 3D scaffold fabrication.

Pure PPF scaffolds can also be fabricated, albeit at the expense of time due to its high viscosity. In order to attain a high enough fabrication speed with MPExSL, the PPF viscosity has to be decreased. This is achieved by blending the PPF with diethyl(fumarate) (DEF) in a 7:3 weight/weight ratio.

The photocross-linking density is tuned by photoinitiator phenylbis (2,4,6-trimethylbenzoyl) phosphine oxide (BaPO) concentration.

It is important to note that since the light absorption of both the resin and the photoinitiator varies with the wavelength [30], the applied wavelength determines the curing (penetration) depth of the laser pulses into the polymer, thus the time needed for the fabrication of constructs. Compared to the 248-nm-light (KrF excimer laser), the 308 nm has a far greater penetration depth in the PPF resin, which results in a higher scaffold production capacity [30]. For this reason, in MPExSL a XeCl excimer laser operating at 308 nm is used (energy per pulse 70–260 mJ, pulse duration 20 ns, repetition rate 1–100 Hz).

All reagents and materials were purchased from Sigma-Aldrich and used as received.

2.3. Mechanical and thermal testing, FTIR

Stiffness measurements in both 2D and 3D were performed by means of nanoindentation on non-porous samples. The indenter was a Micro Materials Ltd. NanoTest, tests were conducted by using a Berkovich-tip with a maximum load of 0.6 mN, a dwell time at maximum load of 30 s, loading and unloading periods of 30 s and 15 s, respectively. Every sample has been measured at 16 different points (in a matrix of 4 × 4, distance between measurements 50 μm). Young's modulus was calculated through the Oliver and Pharr method each time. The stiffness for the whole sample was acquired eventually by having the mean value of these aforementioned 16 points. It is important to note that this nanoindentation setup (with the Berkovich-tip) could not be reliably used on samples with stiffness less than 4–5 MPa or when there was adhesion between the tip and the sample. Height measurements were carried out by a Veeco Dektak 150 profiler.

Thermogravimetric analysis (TGA) was conducted in a TGA Q500 from TA Instruments. The sample was placed in a platinum pan with an equilibrating step at 30 °C. Heating went to 800 °C with a 10 °C/min rate. Nitrogen flow was 50 ml/min.

PerkinElmer Diamond DSC was used for differential scanning calorimetry (DSC). In an aluminum pan, the sample was heated from −40 °C to 120 °C with 20 °C/min ramp, followed by a cool down with the same rate and a 5 min hold step at −40 °C. This was preferable so as to eliminate any thermal history on the samples. The temperature hold step was succeeded by a ramp-up process with the same parameters as before. Nitrogen gas flow was 20 ml/min.

Fourier transformed infrared spectroscopy (FTIR) was conducted using a Bruker Vertex 70v.

3. Results and discussion

In order to test the effects of different parameters, first 2D (i.e. single-layers) structures have to be characterized (Section 3.1). They are the basic building blocks of 3D structures and thus a good control over their properties is essential to successfully build uniform and stable multilayer constructs. Fundamental correlations of the light-polymer interaction presented in detail later in this article could already be observed here.

It is important to note the existing disparity between the 2D/sandwiched and the 3D/stereolithography processes though. In 2D, the quartz sheet diffracts a portion of the incoming laser pulse, resulting in a thin, partially-cured layer covering both the quartz and the sample. This low stiffness film can alter all nanoindentation measurements conducted on single-layers, preventing a reliable comparison to any 3D sample considering the mechanical properties. Thus, 3D structures