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Designed biodegradable hydrogel structures prepared by stereolithography using poly(ethylene glycol)/poly(D,L-lactide)-based resins

Tetsu M. Seck [1,2], Ferry P.W. Melchels [2], Jan Feijen [2] and Dirk W. Grijpma [2,3,]*

[1] Institute of Organic Chemistry, Johannes Gutenberg University Mainz, Duesbergweg 10-14, D-55099 Mainz, Germany

[2] MIRA Institute for Biomedical Technology and Technical Medicine, and Department of Biomaterials Science and Technology, University of Twente, P.O. Box 217, 7500 AE, Enschede, The Netherlands

[3] Department of Biomedical Engineering, University Medical Centre Groningen and University of Groningen, P.O. Box 196, 9700 AD Groningen, The Netherlands

[*] Correspondence to Prof. Dr. D.W. Grijpma:

Tel: +31 53 4892966; Fax: +31 53 4892155

E-mail: d.w.grijpma@utwente.nl

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Abstract

Designed three-dimensional biodegradable poly(ethylene glycol)/poly(D,L-lactide) hydrogel structures were prepared for the first time by stereolithography at high resolutions. A photo-polymerisable aqueous resin comprising PDLLA-PEG-PDLLA-based macromer, visible light photo-initiator, dye and inhibitor in DMSO/water was used to build the structures. Porous and non-porous hydrogels with well-defined architectures and good mechanical properties were prepared. Porous hydrogel structures with a gyroid pore network architecture showed narrow pore size distributions, excellent pore interconnectivity and good mechanical properties. The structures showed good cell seeding characteristics, and human mesenchymal stem cells adhered and proliferated well on these materials.

Keywords

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Introduction

The origins of stereolithography date back to the 1980s [1]. Of all rapid prototyping methods, stereolithography is the most developed technique with the highest accuracy. Three-dimensional designed constructs can be precisely fabricated in a layer-by-layer manner. Its working principle is based on the spatially controlled solidification of a liquid photo-polymerisable resin upon illumination with a computer-driven light source. Most commercially available resins for use in stereolithography are conventional epoxy- or acrylate-based resins. In preparing biomedical implants, their use is limited, as these materials are usually not biocompatible or biodegradable. To prepare biodegradable network structures, macromers based on trimethylene carbonate (TMC) and ε-caprolactone [2,3], on poly(propylene fumarate) [4,5] and on poly(D,L-lactide) (PDLLA) [6,7] have been developed. The hydrophobic scaffolds that were obtained from these macromers were well suited for the seeding and culturing of cells.

Hydrogel structures prepared from polyethylene glycol (PEG)-based resins have also been fabricated using stereolithography [8-10]. The biocompatibility of these hydrogel structures was excellent, and even allowing the encapsulation of living cells during the fabrication process. However, the structures were not biodegradable. Polyethylene glycol itself is a stable hydrophilic polymer, but it can be excreted by the kidneys at molecular weights up to 30 kg/mol [11]. Poly(D,L-lactide) is a hydrophobic polyester with a glass transition temperature (T_g) of approximately 55 °C, and in contrast to PEG, can degrade by hydrolytic cleavage of the main chain ester bonds. Highly biocompatible hydrogels that degrade in the body can be prepared by crosslinking macromers based on block copolymers of PEG and PDLLA [12].

In this paper we describe the synthesis of PDLLA-PEG-PDLLA-based macromers, the resin formulation, and the photo-polymerisation process by stereolithography that allows the generation of designed three-dimensional crosslinked structures. The obtained hydrogel

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structures are characterised and seeded with mesenchymal stem cells, to evaluate cell adhesion and proliferation behaviour. By fabricating biodegradable tissue engineering scaffolds that incorporate living cells with these resins, it will be possible to prepare functional tissue engineered constructs with a controlled distribution of cells at high densities.

Materials and Methods

Materials

D,L-Lactide was purchased from Purac Biochem (The Netherlands). Polyethylene glycol (PEG4k, with a molecular weight of 4 kg/mol) was supplied by Fluka. Stannous octoate (tin(II) bis(2-ethylhexanoate), $Sn(Oct)_2$ was obtained from Sigma Aldrich (USA). Methacrylic anhydride (MAAH) was purchased from Merck (Germany). Triethyl amine (TEA) was obtained from Fluka (Switzerland). Lucirin TPO-L (ethyl-2,4,6-trimethylbenzoyl phenylphosphinate) was a gift from BASF (Germany). Phenol red was supplied by Riedel de Haën. Diethyl ether, dimethyl sulfoxide (DMSO), analytical grade isopropanol and dichloromethane (DCM) were supplied by Biosolve (The Netherlands). Dichloromethane was dried over calcium hydride (Acros Organics, Belgium) and filtered prior to use.

Alpha-Modified Eagle's Medium (α-MEM), foetal bovine serum (FBS), L-glutamine, penicillin-streptomycin and trypsin were obtained from Lonza (Belgium). Phosphate-buffered saline (PBS) was purchased from Invitrogen (USA). MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide) was obtained from Sigma Aldrich (USA).

Synthesis of PDLLA-PEG-PDLLA oligomers and -macromers

PDLLA-PEG-PDLLA oligomers were prepared by ring opening polymerisation of D,L-lactide initiated from the terminal hydroxyl groups of PEG4k, in the presence of $Sn(Oct)_2$ as a catalyst (Figure 1A). The targeted molecular weights of the oligomers were between 4500

and 5000 g/mol. Using 600 W microwave irradiation, the PEG oligomer was first molten (3 min), the D,L-lactide monomer and the Sn(Oct)$_2$ catalyst were then mixed in and reacted for another minute at 600 W. The oligomers were precipitated in isopropanol and dried under reduced pressure for 3 days at room temperature. When monomer conversion of the oligomers was less than 95 %, the product was further purified by dissolution in chloroform and precipitation into diethyl ether. Otherwise, the oligomers were used without further purification in the subsequent functionalisation reactions.
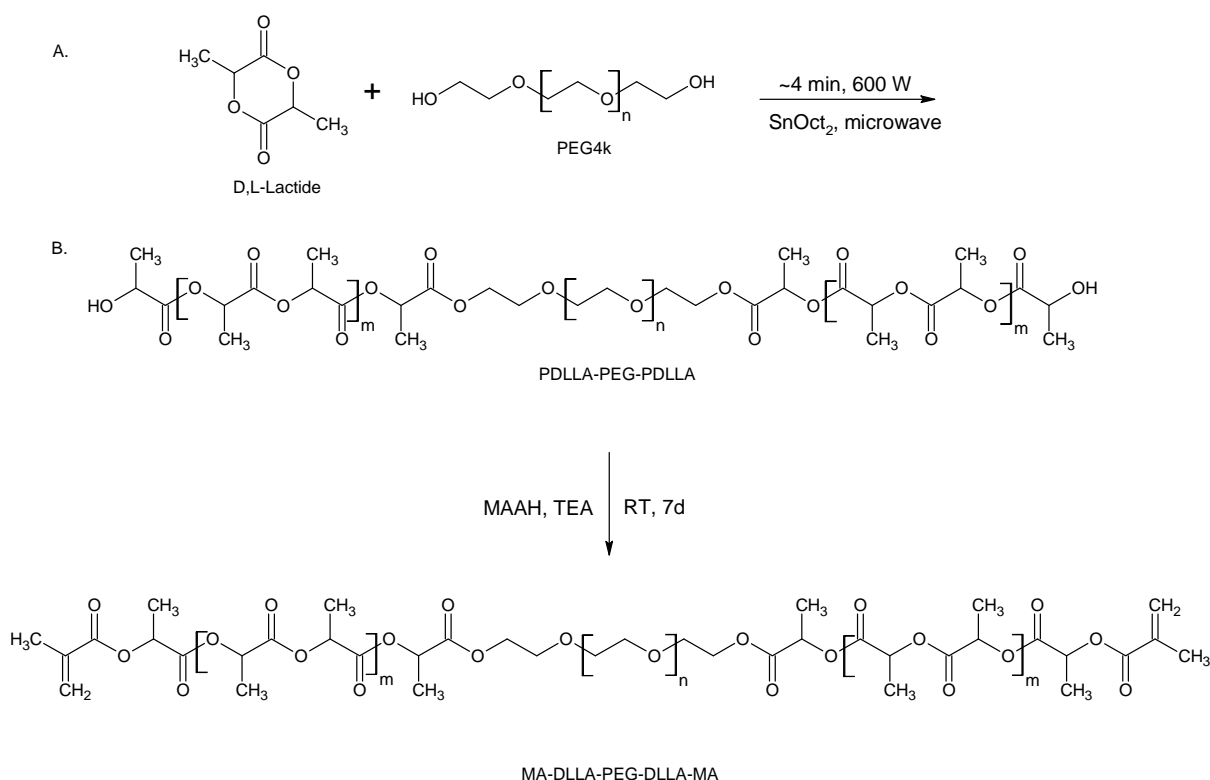

Figure 1: Synthesis of PDLLA-PEG-PDLLA oligomers (A) and subsequent functionalisation with methacrylic anhydride (MAAH) to give MA-PDLLA-PEG-PDLLA-MA macromers (B).

Macromers were prepared by reacting the terminal hydroxyl groups of the PDLLA-PEG-PDLLA oligomers with methacrylic anhydride (Figure 1B). Under an argon atmosphere, the required amounts of oligomer were charged into a three-necked flask and methacrylic anhydride (100 % excess), dry DCM and TEA were added. The reaction was let to proceed at

room temperature for 7 d, while continuously stirring the mixture. The formed MA-DLLA-PEG-DLLA-MA macromers were then precipitated into diethyl ether, and purified by dissolving in chloroform and precipitating once more into diethyl ether.

In a similar manner, MA-PEG-MA macromers were prepared.

Formulation of a stereolithography resin and building of hydrogel structures

To formulate a liquid photo-polymerisable resin for use in stereolithography, the MA-DLLA-PEG-DLLA-MA macromer and the phenol red dye were first dissolved in water. Lucirin TPO-L (visible light photo-initiator) is water-insoluble, and was dissolved in DMSO. Then, the photo-initiator solution was mixed in with the macromer solution and stirred for 20 h at room temperature in order to obtain a clear liquid resin. The resin composition was: 54 wt% water, 33 wt% macromer, 11 wt% DMSO, 1.7 wt% photo-initiator and 0.19 wt% phenol red. To prevent premature crosslinking, 0.1 wt% hydroquinone was added.

The formulated PDLLA-PEG-PDLLA resin was used to prepare biodegradable hydrogel structures by stereolithography. Using a Perfactory Mini Multilens stereolithography apparatus (EnvisionTec, Germany), non-porous hydrogel structures in the shape of disks and films were prepared. Also porous hydrogel structures with a continuous gyroid pore network architecture were built. The structures were designed using Rhinoceros 3D (McNeel Europe) and K3DSurf computer software as described elsewhere [7].

The stereolithography apparatus (SLA) is equipped with a digital micro-mirror device that enables projections of 1280 x 1024 pixels, each measuring 32 x 32 μm^2. Using a build platform step height of 15 μm, layers of resin were sequentially photo-crosslinked by exposure to a pixel pattern of blue light (wavelength 400 - 550 nm, peak intensity at 440 nm). The fabrication of the hydrogel structures was carried out by exposing the photo-polymerisable resin to light intensities of 20 mW/cm^2. The curing time per 15 μm thick layer

was 32.5 s. After photo-polymerisation, the obtained structures were extracted with distilled water to remove soluble compounds and dried at ambient conditions for 3d.

Structures were also prepared by stereolithography using MA-PEG-MA materials.

Characterisation of oligomers, macromers and network structures

Proton nuclear magnetic resonance (^{1}H-NMR) spectra of the reaction products were recorded on a Varian Inova 300 MHz NMR spectrometer and used to determine monomer conversion, oligomer molecular weight and extent of functionalisation. CDCl$_3$ was used as the solvent.

The thermal properties of oligomers, macromers and network structures prepared by stereolithography were evaluated using a Perkin Elmer Pyris 1 differential scanning calorimeter (DSC) connected to a liquid nitrogen cooling accessory. Oligomer, macromer and network specimens were heated from -100 °C to +100 °C at a heating rate of 10 °C/min and then quenched at a cooling rate of 100 °C/min, after which a second scan was run. The melting points and -enthalpies were determined in the first heating scan, while the glass transition temperatures were determined in the second scan.

To determine the degree of swelling of the photo-crosslinked networks, specimens of extracted fabricated structures were dried for 3 d at ambient conditions and weighed (W_0) before placing them in distilled water for 3 d. The specimens were blotted dry and weighed again (W_s). The degree of swelling is defined as:

$$Q = 1 + \frac{w_s - w_0}{w_0} \frac{\rho_p}{\rho_w}$$

here w_s is the weight of the swollen gel (g), w_0 the weight of the dry gel (g), ρ_p the density of the polymer (1.12 g/mL), ρ_w the density of water (1.00 g/mL).

Porous and non-porous hydrogel structures measuring approximately 8 mm in diameter and 5 mm in height were prepared by stereolithography. Their mechanical properties were determined in compression using a Zwick Z020 mechanical tester after swelling in water, and after drying at room temperature for 3 d.

Structural analysis of porous structures built by stereolithography was performed using micro-computed tomography (μCT) on a GE eXplore Locus SP scanner at 14 μm resolution after conditioning the specimens in the ambient environment. GE Healthcare Micro View 2.2 software was used to assess porosities and pore size distributions.

To demonstrate the hydrolytic degradability of the PDLLA-PEG-PDLLA hydrogels, degradation experiments were conducted using 1M NaOH solutions (pH 14) at room temperature. For comparison, photo-crosslinked PEG-dimethacrylate hydrogels, which do not contain a hydrolysable lactide linkage, were also incubated in the NaOH solutions.

Cell culturing experiments

Human mesenchymal stem cells (hMSC) were used in the experiments. The cells were cultured in 175 cm^2 cell culturing flasks that contained alpha-Modified Eagle's Medium (α-MEM) in a humidified 5 % CO_2 atmosphere at 37 °C. The medium was refreshed twice a week until the cells reached confluency, then they were trypsinised and counted.

Porous hydrogel scaffolds and non-porous hydrogel films were sterilised with 70 % ethanol for 3 h, washed 3 times with phosphate buffered saline (PBS) and left overnight in medium containing 10 % FBS. The hydrogel structures were then extensively washed with PBS, and placed in non-treated 24-well plates. The hMSC cells were seeded on the films, and on top of and inside the porous scaffolds (10^6 cells each). Fresh medium was added until the surfaces were fully covered. The cell-seeded films and scaffolds were kept at 37 °C for 1 d to allow cell attachment (porous scaffolds and films) and 5 d (films) to assess cell proliferation. As a

control, the cells were also cultured on tissue culture polystyrene (TCPS) after seeding at the same cell density.

The morphology and distribution of hMSC cells in the attachment and proliferation experiments was assessed by light microscopy and scanning electron microscopy (SEM). The culture medium was removed, after which the samples were washed twice with PBS and fixed with 1.5 % glutaraldehyde in 0.14M sodium cacodylic acid buffer pH 7.3 for 30 min at room temperature. Dehydration was performed with a series of ethanol solutions of increasing concentration beginning with 70 % and progressing through 80 %, 90 %, 96 % and 100 % ethanol. The samples were kept in absolute ethanol, transferred to the critical point dryer and dried using CO_2. Then, the samples were gold-sputtered and analysed using a Philips XL 30 ESEM- FEG.

Results and discussion

Synthesis of PDLLA-PEG-PDLLA oligomers and macromers

PDLLA-PEG-PDLLA oligomers could be conveniently be prepared by conducting D,L-lactide ring opening polymerisation initiated by poly(ethylene glycol) using microwave irradiation. At an intensity of 600 W only 4 min reaction time was required. The chemical structure of the synthesised oligomers was confirmed by NMR; a peak at δ 4.2-4.3, corresponding to methylene protons of the PEG connected to PDLLA could be discerned (Figure 2, upper spectrum). The average block length *n* of the PDLLA blocks was calculated by assigning the respective areas of the peaks corresponding to the methyl group of lactyl units (*I* in Figure 2, upper spectrum) and the methylene groups of PEG (*II* in Figure 2, upper spectrum). From the values of *n*, molecular weights (M_n) ranging from 4550 to 4620 g/mol were calculated. The conversion of the PEG hydroxyl groups was 88 to 100 %.
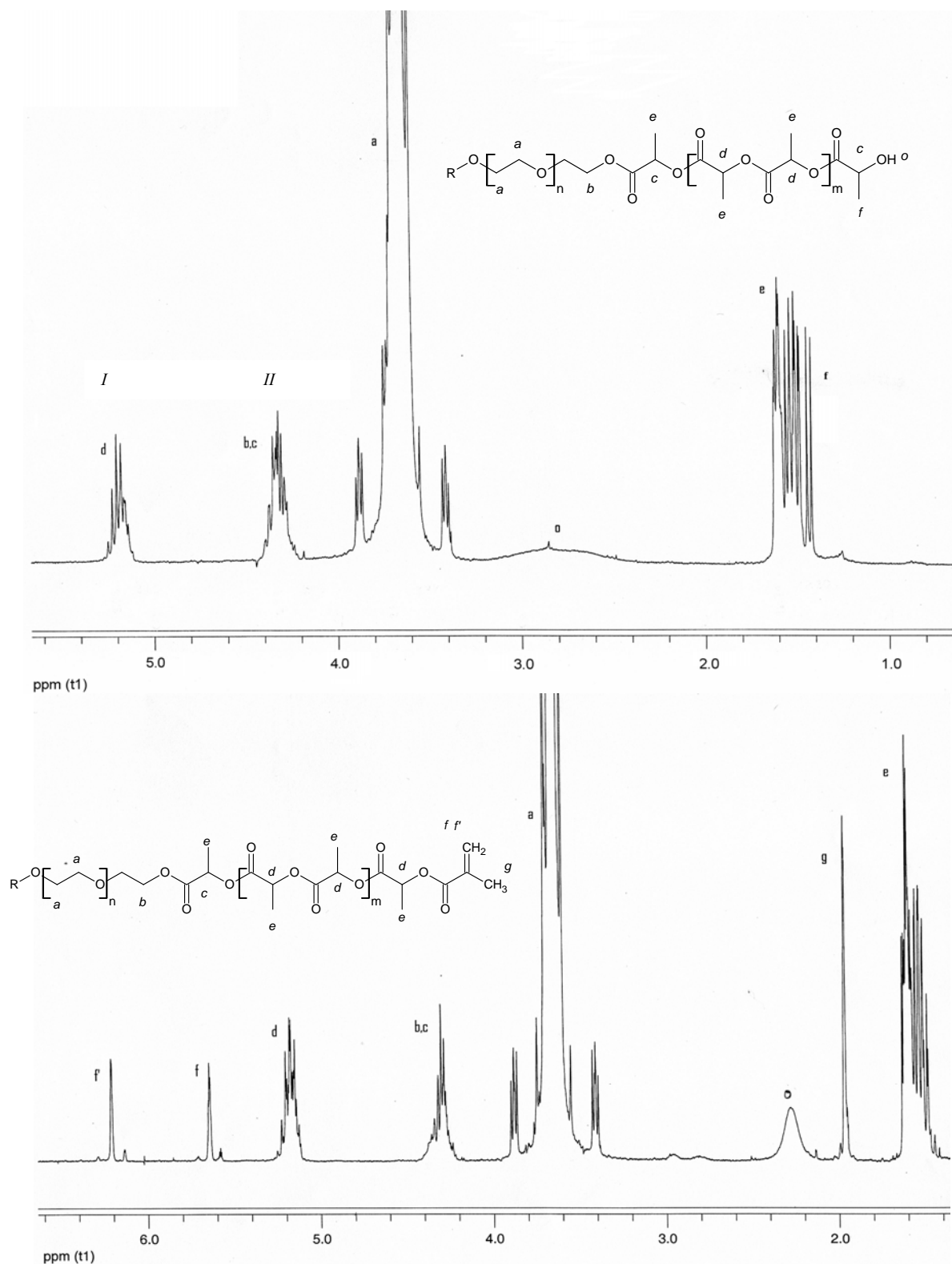
Figure 2: ^{1}H-NMR spectra of a PDLLA-PEG- PDLLA oligomer (upper spectrum) and the corresponding MA-DLLA-PEG-DLLA-MA macromer (lower spectrum) after functionalisation with methacrylic anhydride. In the chemical structures, only half of the molecule is depicted.

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The hydroxyl end groups of the synthesised PDLLA-PEG-PDLLA oligomers were reacted with methacrylic anhydride to obtain MA-DLLA-PEG-DLLA-MA macromers. The coupling of MAAH to PDLLA-PEG-PDLLA was confirmed by the appearance of three peaks in the spectra of the macromers: at δ 6.21 (f'), δ 5.64 (f) and δ 1.97 (g) corresponding to the $=CH_2$ (f', f) and $-CH_3$ (g) of the methacrylate groups (Figure 2, lower spectrum). The degree of functionalisation was determined by comparing the integrals of the peaks corresponding to the methylene protons of the methacrylate group (δ 6.21 and 5.64) with those of the methylene protons of PEG (δ 3.65). A degree of functionalisation of 100 % was achieved. This implies that besides MA-PDLLA-PEG-PDLLA-MA triblock macromers, also small fractions of MA-DLLA-PEG-MA diblock copolymers and MA-PEG-MA macromers are formed.

The thermal properties of the oligomers and macromers were determined by DSC. Values of molecular weights (M_n, determined by NMR) and of melting points (T_m), melting enthalpies (ΔH_m) and glass transition temperatures (T_g) of the compounds are presented in Table 1. The block copolymerisation of PEG with short D,L-lactide blocks and the subsequent functionalisation with methacrylate groups leads to a small increase in molecular weight of the oligomers. The D,L-lactide block copolymerisation hinders crystallisation, which leads to lower melting temperatures and heats of fusion. As the T_g of PDLLA is approximately 55 °C, this value increases somewhat. Upon functionalisation using MAAH, T_g of the macromer decreases only slightly.

Table 1: Molecular weights (M_n), melting points (T_m), melting enthalpies (ΔH_m) and glass transition temperatures (T_g) of synthesised PDLLA-PEG-PDLLA based oligomers and macromers. The values of PEG4k are given as a reference.

	M_n (kg/mol)	T_m (°C)	ΔH_m (J/g)	T_g (°C)
PEG4k	4.1	63.0	190	-40
PDLLA-PEG-PDLLA oligomer	4.6	49.4	136	-30
MA-PDLLA-PEG-PDLLA-MA macromer	4.8	49.9	135	-32

Biodegradable hydrogel structures prepared by stereolithography

In order to build designed biodegradable hydrogel structures, a PDLLA-PEG-PDLLA based photo-polymerisable resin was formulated. The mixture contained 33 wt% MA-DLLA-PEG-DLLA-MA macromer, 1.7 wt% Lucirin TPO-L (photo-initiator), 0.19 wt% phenol red (colorant), 0.1 wt% hydroquinone (to prevent premature crosslinking), 54 wt% water and 11 wt% DMSO.

In stereolithography, the liquid photo-polymerisable resin is exposed to a light source in order to be cured in a layer-by–layer manner. The thickness of a solidified layer (cure depth C_d in µm) is controlled by the light irradiation dose E (mJ/cm^2). A plot of the cure depth versus the natural logarithm of the energy ln(E) yields a straight line, with slope D_P and intercept E_C. This curve is known as a working curve and is represented by the following equation [13]:

$$C_d = D_p \ln\left(\frac{E}{E_C}\right)$$

The critical exposure or critical energy (E_C in mJ/cm^2) and penetration depth (D_P in µm) are considered fundamental properties of each photo-polymerisable resin formulation. E_C

represents the minimum energy level required to transform the resin from liquid to solid. D_P describes the penetration depth of light into the photo-polymer resin, and therefore the increase of cured layer thickness with exposed energy [14]. If a layer is not cured deep enough, the interlayer bonding fails and delamination occurs. On the other hand, if the exposure is too much, inaccuracies and deviations from the designed architecture occur. To determine the most suitable parameters for the building process, E_C and D_P need to be evaluated. This can be done experimentally by measuring the depth of the solidified photo-polymer at different exposure values.
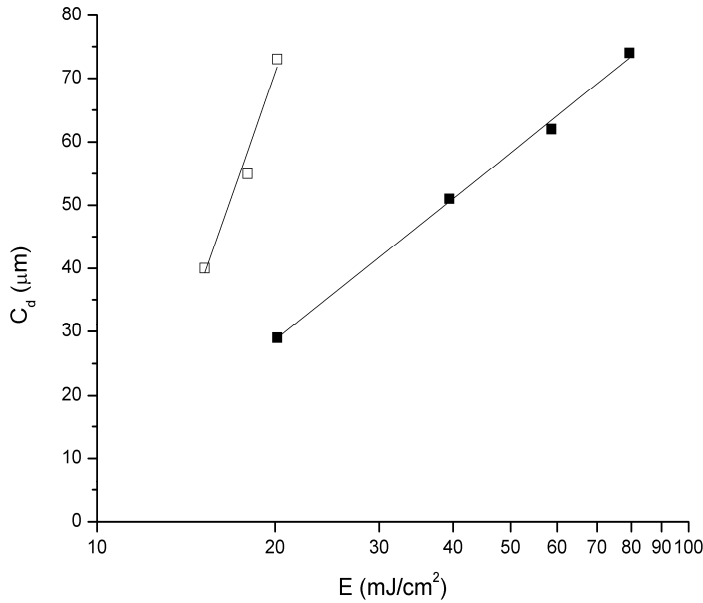

Figure 3: Stereolithography working curves of PDLLA-PEG-PDLLA macromer-based resins containing 0.19 wt% dye (phenol red ■) and not containing dye (□). Note the logarithmic scale of the x-axis.

Working curves for different resin compositions were determined by varying the light exposure at the resin surface (E) and measuring the resulting cure depths (C_d) of the resins. We observed that the penetration depth (D_P) of the resin decreased from 58 μm to 15 μm upon addition of 0.19 wt% phenol red (Figure 3). The addition of the dye to the resin is very

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important, as the penetration depth decreases control over the layer thickness becomes better and more precise. The required curing time per 15 µm thick layer was 32.5 seconds at a light intensity of 20 mW/cm^2.

Designed hydrogel structures were prepared in the stereolithography apparatus using the MA-PDLLA-PEG-PDLLA-MA resin containing 0.19 wt% phenol red dye. Solid structures as well as porous scaffolds were built. Figure 4 shows photographs of the structures extracted with water to remove soluble compounds. Due to the phenol red, the colour of the hydrogels scaffolds varies between orange and yellow. The intensity of the colour depends on the time of extraction. The built hydrogel structures were soft and highly flexible, their shape and structure precisely matched their design.

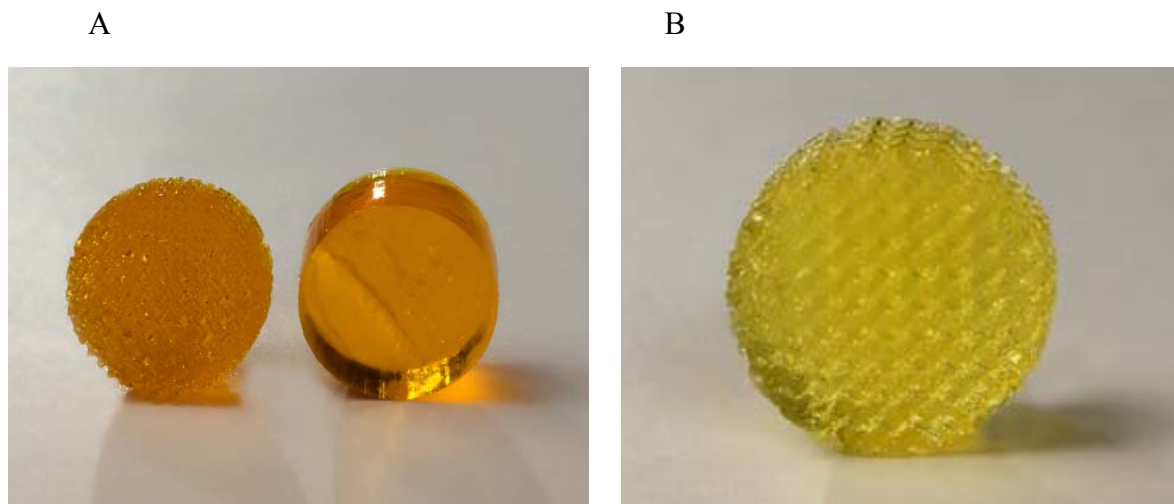A   B

Figure 4: Hydrogel structures built by stereolithography using an MA-PDLLA-PEG-PDLLA-MA based resin. (A) Disk-shaped porous (left) and solid (right) hydrogel scaffolds after extraction for several hours. (B) Hydrogel scaffold with gyroid pore network design after several days of extraction. The diameter of the disks is 8 mm.

In the photographs, the porosity and the regularity of the gyroid pore network structure of the porous hydrogel scaffolds can readily be recognised. The gyroid architecture is mathematically defined, allowing precise control of porosity and pore size of a fully interconnected pore network [15]. Gyroid structures have a large specific surface area and

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high pore interconnectivity. These characteristics are quite important for cell-seeding and proliferation at high cell densities, ensuring homogeneous distributions of cells and unhindered transport of nutrients throughout the scaffolds.

Micro-computed tomography (µCT) was used to assess structural parameters of the pore network architecture of the built scaffolds. Figure 5 depicts µCT images of two built porous structures with gyroid pore network architectures after drying at ambient conditions. As can be clearly seen in the images, this architectural design and the fidelity of the stereolithography technique in building the designed structures has allowed precise control of porosity and pore size of the fully interconnected pore network of the porous hydrogel structure. From the µCT data a porosity of 52 % was determined, while the porosity of the designed architecture was 55 %.
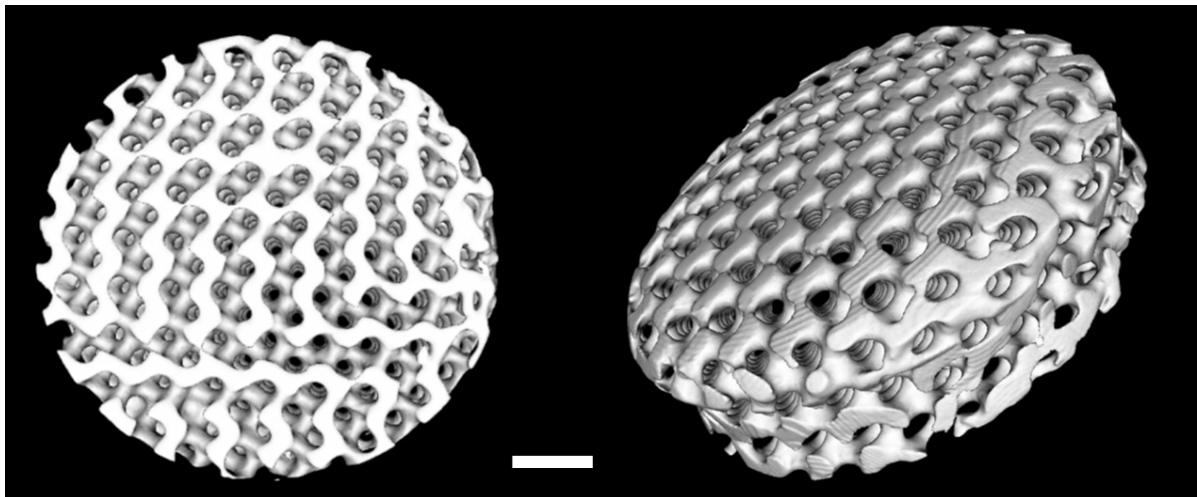

Figure 5: Three dimensional images reconstructed from µCT analyses of designed hydrogel structures prepared from PDLLA-PEG-PDLLA based macromers by stereolithography. Scalebar is 1 mm.

The pore size distribution of the gyroid pore network architecture in the built hydrogel structures is visualised in Figure 6. As a result of the regularity of the design and the precision of the building technique employed, the histogram shows a narrow pore size

distribution. The average pore size was 423 μm, and pore sizes ranging from 387 to 558 μm account for more than 76 % of the pore volume.
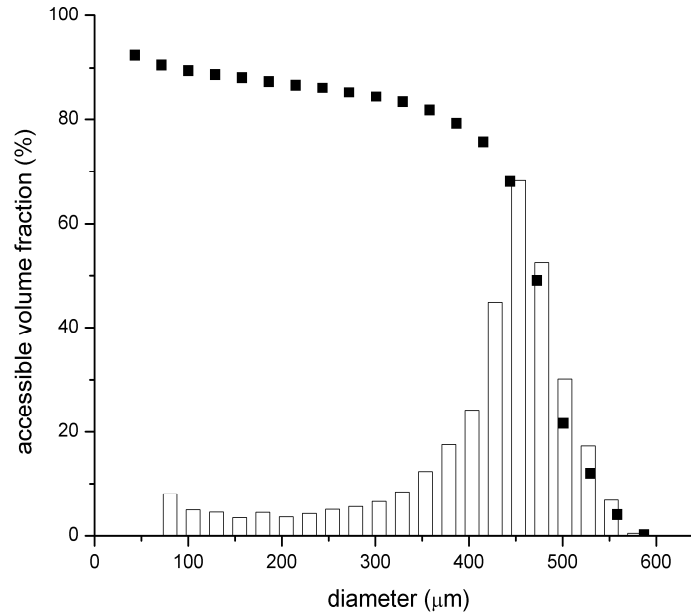

Figure 6: Pore size distribution (histogram) and accessible pore volume (black squares) of porous hydrogel structures with a gyroid pore network architecture prepared by stereolithography.

To quantify the interconnectivity of the pore network, the accessible pore volume was determined. This was done by simulating the permeation of a spherical particle [6]. The figure shows the accessible pore volume (as a fraction of the total volume) versus the diameter of the permeating particle. More than 90 % of the pore volume in the built structures is connected to the outsides of the scaffolds with pores and channels of sizes of the order of magnitude of cells. The specific surface area of the porous structures, to which cells can attach upon cell seeding in tissue engineering applications, could also be obtained from the μCT-data: a high value of 6.23 mm^2/mm^3 was determined for these scaffolds.

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With these results we have demonstrated that using a MA-PDLLA-PEG-PDLLA-MA macromer based resin, designed porous hydrogel structures with narrow pore size distributions and high pore interconnectivities can be prepared by stereolithography.

Properties of biodegradable PDLLA-PEG-PDLLA-based hydrogel structures built by stereolithography

The thermal properties of the prepared network structures were measured in the dry and in the wet state. By DSC, the melting temperature of the dry network was 45 ^{o}C and the heat of fusion 68 J/g, a glass transition temperature could not be detected. No crystallinity could be detected in the wet state, but a glass transition temperature of -67 ^{o}C could be determined. Upon equilibration in water, large amounts of water are taken up by the built hydrogel structures. The water content of the non-porous PDLLA-PEG-PDLLA hydrogel structures was 86.5 ± 0.2 %, while that of the porous hydrogel structures was 91.3 ± 0.2 %. (Note that in the latter determinations water may still have been present within the pore network of the porous gyroid structures.) The volume degrees of swelling were respectively 7.39 ± 0.1 and 11.5 ± 0.3.

When the hydrogel structures prepared from the PDLLA-PEG-PDLLA based macromers were immersed in a 1M NaOH solution (pH 14), the networks very rapidly degraded and clear solutions were obtained in approximately 5 s. This implies that the ester bonds in the short PDLLA blocks are available to water and can be readily hydrolysed. In demineralised water, hydrolysis is much slower and the hydrogels remained unchanged for several weeks. Hydrogel structures prepared from MA-PEG-MA macromers that do not contain degradable PDLLA ester bonds were very stable, and did not degrade at pH 14 in the course of several days.
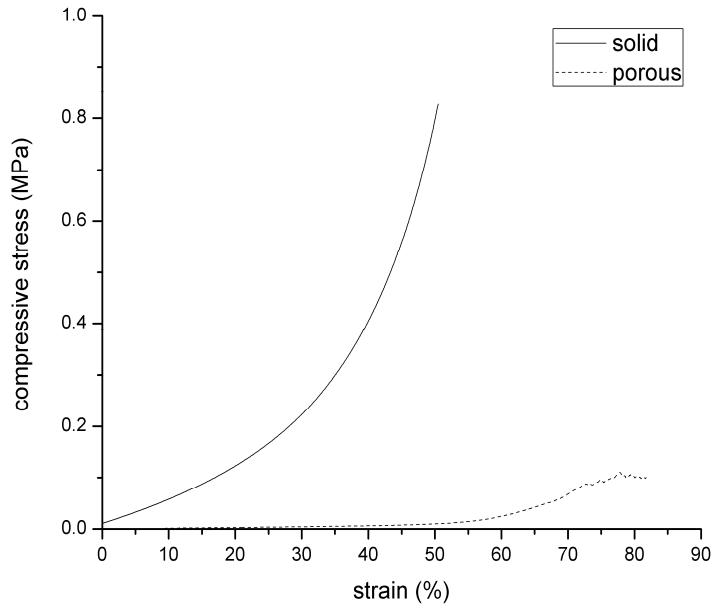
Figure 7: Mechanical properties in the wet state of non-porous and porous hydrogel structures prepared by stereolithography using PDLLA-PEG-PDLLA based macromers. E-modulus values: non-porous structures 420 kPa, porous structures with gyroid pore network architecture 14 kPa

The mechanical properties of the porous and non-porous hydrogel structures fabricated by stereolithography were evaluated in the wet and in the dry state in compression tests. The compressive stress-strain curves are presented in Figure 7. The porosity of a structure has a large influence on its mechanical properties, it can be observed that the E-modulus and compressive strength of a non-porous water-swollen hydrogel is very much higher than that of the hydrated porous hydrogels with gyroid pore network architecture. (The compression modulus values for the non-porous and the porous water swollen hydrogels are respectively 420 kPa and 14.0 kPa.) However, the porous structures show higher maximal deformations than the solid structures; the solid scaffolds fragmented into small pieces at 50 % strain, while the porous scaffolds could be compressed to more than 80 % (first damage occurring after 70 %, the structures behave elastically before reaching this value).

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In the dry state (after drying at 90 °C for 2 d) the mechanical properties of the built PDLLA-PEG-PDLLA structures are very different than those in the wet state, although here too porosity has a large effect on the mechanical properties. The E-modulus values for the dry non-porous and porous structures are respectively 154 MPa and 7.35 MPa. It is evident that regarding their use as implants, the properties of the network structures in their wet state are most relevant.

Cell adhesion and proliferation on PDLLA-PEG-PDLLA-based structures built by stereolithography

To evaluate their compatibility with cells, human mesenchymal stem cells were cultured on non-porous hydrogel films and on porous hydrogel structures prepared from PDLLA-PEG-PDLLA based macromer resins by stereolithography. Cell morphology was assessed by scanning electron microscopy SEM.

Although PEG is a polymer that is known to minimise the adhesion of cells to surfaces, we observed that hMSCs were able to attach to the surfaces of MA-PDLLA-PEG-PDLLA-MA based structures prepared by stereolithography. Before cell-seeding, the hydrogel structures were incubated in culture medium that contained 10 % FBS overnight. This could have lead to adsorption of proteins in the medium that might provide attachment sites for the cells. Nevertheless, the presence of hydrophobic PDLLA segments in the network structure might also have diminished the suppressive effect of the hydrophilic PEG on the adhesion and proliferation of cells on the network surfaces.

Figures 8A and 8B show light microscopy images of hMSCs attaching to the surface of the porous hydrogel scaffolds after 24 h. The pores are black, while the bright areas show light coming through the walls of the scaffold. The attaching cells are better recognised in the right picture (Figure 8B).
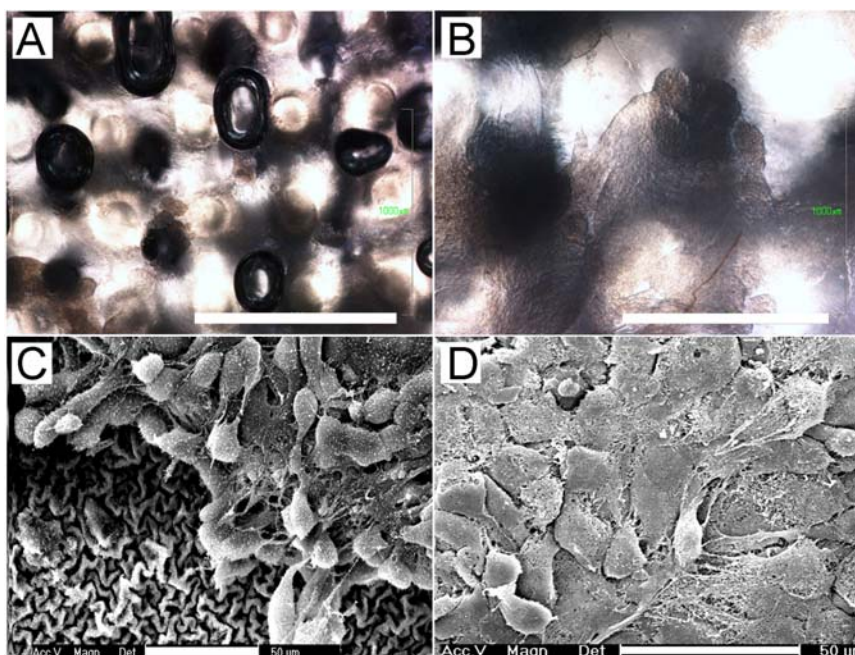
Figure 8: Microscopy images of the attachment of hMSCs on porous PDLLA-PEG-PDLLA-based hydrogel scaffolds prepared by stereolithography. (A, B) Light microscopy images after 24 hrs (C) SEM image after 24 hrs (D) Cell proliferation; SEM image after 5 days. Scale bars are 1 mm (A, B) and 50 μm (C, D).

To distinguish their morphology in more detail, SEM pictures were made of hMSCs seeded on hydrogel films that were also prepared by stereolithography. In this experiment with PDLLA-PEG-PDLLA hydrogel films, the attachment of hMSCs and proliferation was evaluated. Figure 8C illustrates the attachment of cells on hydrogel films at day 1, while Figure 8D shows their proliferation after five days. The adhering hMSCs cells showed a spread morphology, although it seems that the cells have a tendency to aggregate. This might be due to the lack of proteins that support cell attachment; Stupack *et al*. have shown that in the absence of interaction with the matrix, cells undergo apoptotic progression [16].

We have demonstrated the suitability of these PDLLA-PEG-PDLLA-based hydrogel structures prepared by stereolithography in cell culturing. To ensure even better cell

attachment and cell proliferation within the pore network of the hydrogel structures, cell-binding ligands such as RGD containing peptide sequences could be incorporated. This can be done for example by Michael addition reactions of cystein thiol groups with unreacted methacrylate groups present in the network.

Future work will address the encapsulation of living cells within the matrix of the designed hydrogel networks. This seems quite feasible, as preliminary experiments showed that the viability of hMSC cells that were exposed to the non-crosslinked resin for 24 h was approximately 70%.

Conclusions

We have successfully used stereolithography to fabricate designed three-dimensional porous and non-porous biodegradable hydrogel structures at high resolutions. For this an aqueous photo-curable resin based on methacrylate-functionalised poly(ethylene glycol)/poly(D,L-lactide) macromers and Lucirin TPO-L as visible light photo-initiator was developed.

After the building process and extracting with water to remove unreacted components, the crosslinked network structures closely matched their design. In compression experiments, the specimens showed very good mechanical properties similar to those of soft tissues. The porous hydrogel structures had a well-defined pore network architecture, with narrow pore size distribution and high interconnectivity of the pores. Good pore interconnectivity is a prerequisite for efficient cell-seeding and -proliferation, and transport of nutrients and metabolites during cell culturing in three-dimensional structures.

The resin and the built structures were compatible with cells. Upon seeding, human mesenchymal stem cells attached to the surfaces of the hydrogel structures and showed a spread morphology. After five days in culture, proliferation of the cells was observed.

Suitable applications of these hydrogel structures could therefore be in tissue engineering, drug delivery, cell-transplantation and other biomedical applications.

Acknowledgements

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