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Methacrylate-functionalized oligomers based on lactide, ε-caprolactone and trimethylene carbonate for application in stereo-lithography

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Abstract: Photo-curable biodegradable macromers were prepared by ring opening polymerization of D,L-lactide (DLLA), ε-caprolactone (CL) and 1,3-trimethylene carbonate (TMC) in the presence of glycerol or sorbitol as initiator and stannous ^{oc}toate as catalyst, and subsequent methacrylation of the terminal hydroxyl groups. These methacrylated macromers, ranging in molecular weight from approximately 700 to 6000 g/mol, were cross-linked using ultraviolet (UV) light to form biodegradable networks. Homogeneous networks with high gel contents were prepared. One of the resins based on PTMC was used to prepare three-dimensional structures by stereo-lithography using a commercially available apparatus.

Introduction:

Resorbable networks for use in controlled drug delivery, cell encapsulation and tissue engineering can be obtained from functionalized biodegradable oligomers through photo-polymerization [1-3] or thermal radical polymerization [4] methods. Among the advantages of photo-polymerization over other crosslinking techniques are: fast curing rates at room temperature or body temperature, spatial and temporal control of the polymerization, preparation of the gel *in vivo* without surgical intervention and minimal heat production during crosslinking. As a result, photo-polymerization has been much investigated in medical application areas such as in photo-chemically driven wound healing materials [5,6], photo-fabrication of drug delivery devices and surface coatings of implanted devices [6].

Of particular interest is the preparation of complex free-form parts and scaffolds by rapid prototyping techniques. As a rapid prototyping process, stereo-lithography (SL) has received much attention in recent years due to its capability of directly generating physical objects from graphical computer data [7]. This has led to a reduction in the lead time and cost required to introduce new products to the market. In medicine,

stereo-lithography allows the preparation of custom sized implants from computed tomography (CT) data. In this rapid prototyping method, an ultraviolet (UV) laser beam is usually employed to selectively harden successive layers of a photo-curable resin, each layer is built on top of the previous layer. To date, photo-curable resins are mostly acrylic or urethane acrylic based materials and not readily degradable in the body.

In tissue engineering, SL is seen as a key tool, as it allows precise control of the scaffold architecture. This control over the three-dimensional architecture is important to be able to arrange cells and tissues in an appropriate configuration and to present molecular signals in a specific and temporal fashion so that the cells may grow in an appropriate way and form the desired tissue structures. Only limited work has been done in developing degradable materials for use in building rapid prototypes by stereo-lithography [8, 9].

The aim of this work is to develop biodegradable, functionalized oligomers for use in the preparation of medical devices by photo-polymerization and stereo-lithography.

Experimental part:

Materials

D,L-lactide (DLLA, Purac Biochem, The Netherlands) was purified by recrystallization under dry argon from sodium dried toluene. ε-caprolactone (CL, Sigma-Aldrich, USA) was purified by drying over CaH_2 and distilling under reduced argon atmosphere. Polymerization grade 1,3-trimethylene carbonate (1,3-dioxan-2-one, TMC) was obtained from Boehringer Ingelheim, Germany and used as received.

Stannous octoate ($SnOct_2$), glycerol (spectrophotometric grade), vitamin E, camphorquinone (CQ) and 2,2-dimethoxy-2-phenylacetophenone (DMPA) were purchased from Sigma-Aldrich, USA and used without further purification. 2-Hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (HHPMP) was obtained from Ciba Specialty Chemicals, Switzerland and used without purification. D(-)sorbitol was obtained from Merck, Germany and used as received.

Triethylamine (TEA) and methacryloyl chloride (MACl) were purchased from Sigma-Aldrich, USA, and purified by distillation under an argon atmosphere. PA-grade dichloromethane (Biosolve, The Netherlands) was dried over CaH_2.

Syntheses

Star-shaped oligomers with terminal hydroxyl groups were synthesized by ring opening polymerization (ROP) of DLLA, CL or TMC in the presence of either glycerol or sorbitol using $SnOct_2$ as a catalyst. Under an argon atmosphere, monomer, initiator, and catalyst were charged into a glass ampoule, which was heat-sealed after applying vacuum. The polymerization was let to proceed for 40 hrs at 130°C. By adjusting the monomer to initiator ratio, the arm lengths (and thus the molecular weight) of the oligomeric precursors could readily be controlled.

To prepare reactive macromers, the oligomers were functionalized with methacrylate end groups by reaction of the terminal hydroxyl groups with methacryloyl chloride. An amount of oligomer was charged into a three-necked flask equipped with a magnetic stirrer and dried for 2 hours at 110 °C under vacuum. After purging with argon and cooling to room temperature, dichloromethane was added to dissolve the oligomer, and TEA was added. The solution was cooled to 0 °C with ice water before a MACl

solution in dichloromethane was added drop-wise to the vigorously stirred oligomer solution. In the functionalization reactions, a 20% excess of MACl and TEA was employed. The reaction was continued for 24 hrs, while the temperature was let to increase to room temperature.

After filtration of the formed TEA·HCl salt, the methacrylate-functionalized oligomers were purified by washing with diluted HCl, diluted $NaHCO_3$ and water or, in the case of relatively high molecular weight oligomers, by precipitation into an excess of ethanol cooled to approximately -100 °C and dried in a vacuum oven at room temperature. The macromers were stored in a refrigerator.

Characterizations

Nuclear magnetic resonance (NMR) spectra of oligomer solutions in $CDCl_3$ (Sigma, USA) were recorded using a 300 MHz ^1H apparatus (Varian Inova). Thermal properties of the oligomers, macromers and networks were evaluated by differential scanning calorimeter (DSC). Samples (5-15 mg) placed in stainless pans were analysed with a Perkin Elmer DSC-7 operating at a heating rate of 10 ^oC/min. All samples were heated to 40 ^oC above their melting temperature (when present) or glass transition temperature. The samples were then rapidly quenched (300 ^oC/min) to 40 ^oC below their glass transition temperature and after 5 min a second scan was recorded. Indium and gallium were used as standards for temperature calibration. The glass transition temperature was taken as the midpoint of the heat capacity change, and the peak melting temperatures were determined from the melting endotherms. Unless mentioned otherwise, the data presented were obtained during the second heating scan.

Molecular weights, molecular weight distributions, and intrinsic viscosities of the oligomers and macromers were determined by gel permeation chromatography (GPC) using a Waters Model 510 pump, a HP-TI series 1050 auto sampler, a Waters Model 410 differential refractometer, and a Viscotek H502 Viscometer Detector with 10^5-10^4-10^3-500 Å Waters Ultra-Styragel columns placed in series. Chloroform was used as eluent at a flow rate of 1.5 ml/min. Narrow polystyrene standards were used for calibration. Sample concentrations of approximately 0.5% wt/vol and an injection volume of 30 μl were used. All determinations were performed at 25 ^oC.

Photo-crosslinking of macromers

Dichloromethane or chloroform solutions of the different macromers and DMPA or HHPMP photo-initiators were prepared and cast on glass. After evaporation of the solvent, transparent films were obtained, which were photo-crosslinked at room temperature by exposure to a UV light source (2 Philips TLD-15W tubes, wavelength 300-460 nm with maximum at 365 nm). The distance between the films and the lamps was 10 cm. At different time points, samples were taken to determine the extent of crosslinking. The UV cabinet was purged with nitrogen for 30 min before the start of the photo-crosslinking reactions and for 15 min after each sampling point.

The formed networks were characterized by determination of their gel contents and mass swelling ratios (Q) in chloroform. The gel content is defined as the fraction of insoluble material after extraction in chloroform for 24 hrs at room temperature.

Stereo-lithography

Stereo-lithography (SL) experiments were performed on an EnvisionTec Perfactory SLA apparatus (Germany) operating at room temperature. Characteristic for this piece of equipment is that it operates using blue filtered visible (VIS) light, which

exposes the resin through a glass bottom plate. Therefore, parts are built from the bottom up. By partial masking, the light source can irradiate a whole layer at a time which makes high building speeds possible.

A liquid, three-armed methacrylated PTMC resin of molecular weight 700, to which 6 mol% camphorquinone (CQ) as photo-initiator and 0.06 mol% vitamin E as inhibitor was added, was used to build three dimensional constructs.

As listed in Tab 1. the operating parameters were slightly adjusted from those recommended for the resin supplied with the EnvisionTec apparatus (chemical composition not disclosed). The light intensity could be maximized by inactivating the digital LCD gray mask that is usually applied to correct for intensity variations of the light source.

Tab. 1. Applied operating parameters of the EnvisionTec SLA

Parameter	EnvisionTec resin	PTMC 700 resin
Exposure time (ms)	6500	15000
Intensity (mW/cm^2)	500	1300
Peeling velocity (μm/s)	1500	1000

Results and discussion:

Syntheses

A series of polymerizations was conducted in which DLLA, CL and TMC oligomers were prepared by ring opening polymerizations with glycerol. It is well known that hydroxyl groups are capable of reacting with $SnOct_2$ to form an active Sn-alkoxide bond which initiates the lactone ring opening [10]. By adjusting the ratio of hydroxyl groups to monomer, branched oligomers (or polymers) with well controlled molecular weights can be prepared. In a subsequent functionalization reaction, these hydroxyl group-terminated oligomers are end-functionalized with methacrylate groups that are reactive in radical polymerization or crosslinking reactions.

Fig. 1 shows the preparation route followed to synthesize methacrylate-functionalized DLLA oligomers. Analogous reactions can be carried out to prepare branched CL and TMC macromers.

Oligomeric triols with a predetermined molecular weight could readily be prepared by adjustment of the ratio of glycerol to monomer. Monomer conversion is essentially complete. Tab. 2 shows that after functionalization with methacryloyl chloride the nature of the monomer used determines the physical properties of the macromer to a large extent. In this table the physical properties of macromers with comparable molecular weights is presented.

In the characterisation of the precursor oligomers and macromers, NMR is a very useful tool, as it allows the determination of molecular weights through hydroxyl end group analysis and of the degree of methacrylation after the functionalization reaction. In all cases the degree of methacrylation exceeded 92%.

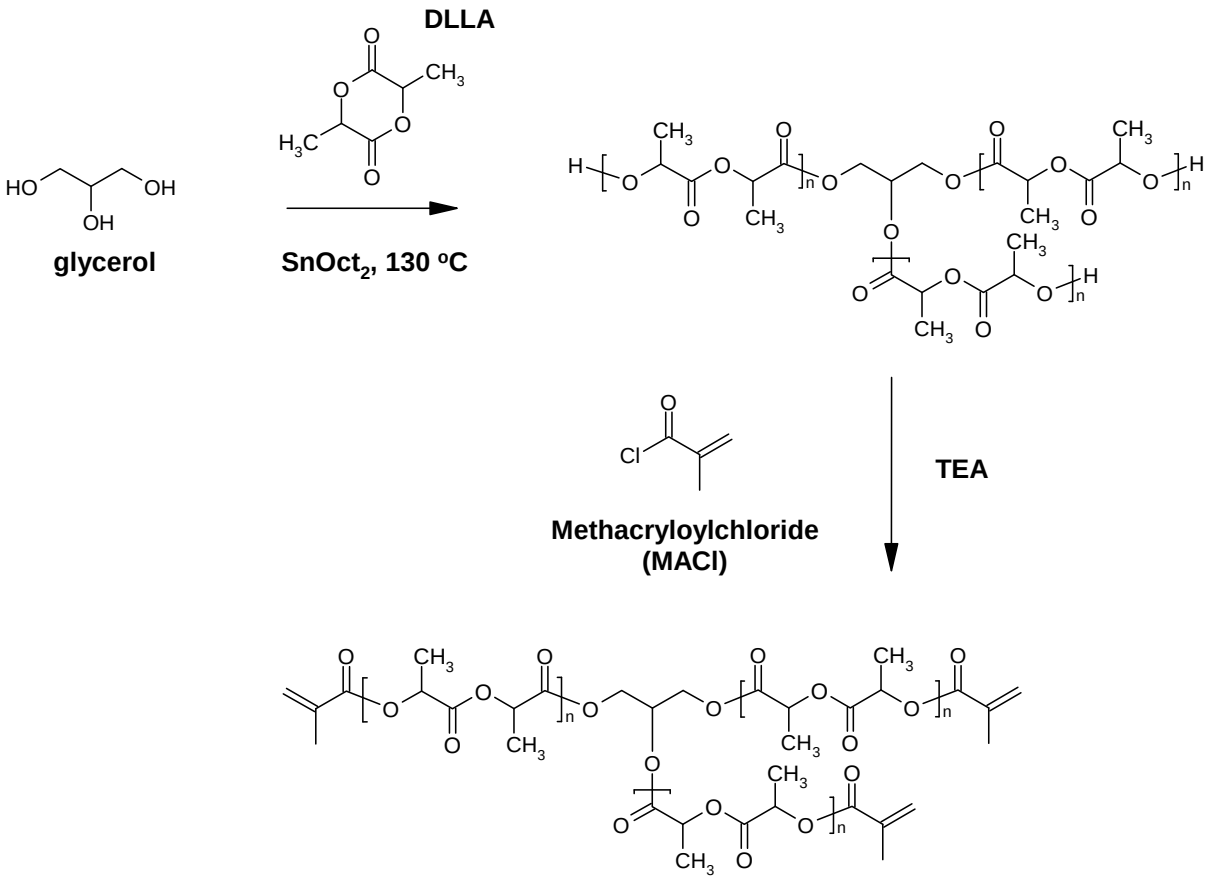

Fig 1. Preparation of methacrylate functionalized DLLA oligomers (macromers) by ring opening polymerization and subsequent functionalization with methacryloyl chloride. Analogous reactions were carried out with CL and TMC.

Tab. 2. Characteristics of selected macromers prepared by functionalization of three-armed DLLA, CL and TMC oligomers with methacryloyl chloride.

3-armed macromer	*Mn* targeted	*Mn* by GPC	*Mn* by NMR	Appearance at RT
PDLLA	2000	2200	2000	Sticky transparent solid
PCL	2100	2400	2300	Waxy opaque solid
PTMC	2000	-	2200	Viscous transparent liquid

First photo-polymerization and crosslinking reactions were carried out at 365 nm with these macromers using DMPA as a photo-initiator at a concentration of 1 wt% relative to the macromer (which is approximately 2.5 mol% per methacrylate end-group) and a photo-polymerization time of one hr. Tab. 3 shows that networks with high gel percentages can readily be prepared from all macromers. Furthermore, upon crosslinking an increase in the glass transition temperatures can be discerned.

Tab. 3. Characteristics of selected macromers prepared by functionalization of three-armed DLLA, CL and TMC oligomers with methacryloyl chloride.

3-armed macromer	*Mn* [a)] of macromer	*Tg* of macromer (*Tm* of macromer) (^{o}C)	Network gel (%)	*Tg* [b)] of network (*Tm* of network) (^{o}C)
PDLLA	2000	19	87	26
PCL	2300	-70 (37)	83	-65 (36)
PTMC	2200	-30	80	-5

[a)] *Mn* determined by NMR

[b)] *Tg* and *Tm* determined after extraction of the sol fraction and drying.

Although DMPA has often been used in photo-polymerizations for the preparation of medical devices, the use of HHPMP as a UV photo-initiator might be preferred as it has been shown that it is less toxic to cells [11]. Therefore, in investigating the effects of architecture and molecular weight of the different macromers on the kinetics of network formation, we employed this initiator.

Network properties and crosslinking kinetics

In photo-polymerizations of cast films into which an amount of 2 mol% HHPMP relative to the double bonds present in the macromer was mixed, typical exposure times to the UV light source required to reach maximal gel contents were 15 to 20 min. Tab 4. gives an overview of the macromers investigated and the properties of the networks obtained after crosslinking. It is shown that high gel percentages are reached quite rapidly.

First the behaviour of PDLLA macromers in which the number of arms was varied (by carrying out ring opening polymerizations with either glycerol or sorbitol) but the molecular weight of each arm remained constant was investigated. The table shows that in both cases a glass transition temperature not higher than the ambient temperature at which the photo-polymerization is carried out is reached. As the *Tg* of linear, high molecular weight PDLLA is approximately 55 ^{o}C, this implies that during curing, vitrification of the network occurs [12]. As a result of this, the network, although relatively high in gel content, is likely to contain many dangling chain ends. These unreacted chain ends can significantly deteriorate the mechanical properties of the formed networks. To overcome this, the photo-polymerization or subsequent post-curing reactions should be carried out at higher temperatures or in the presence of (reactive) diluents. Although not shown in the table, the PDLLA macromer with the highest *Tg*, which is close to room temperature, reacted at the lowest rate.

In the case of PTMC macromers, all glass transition temperatures are well below room temperature, although the viscosity increases with *Mn*. Here also networks with high gel percentages are obtained within 20 min. Although the resulting gel percentages seem lower than in the case of PDLLA macromers, it is believed that the effect is not significant as the error in these single experiment determinations can be quite large. More detailed future research will address possible differences between PDLLA and PTMC macromers in their photo-polymerization and gelation behaviour.

Tab. 4. Properties of networks prepared from macromers of different composition, architectures and molecular weights.

macromer	*Mn* [a)] of macromer	*Tg* of macromer (^{o}C)	Appearance at RT	Network gel (%)	Q	*Tg* [b)] of network (^{o}C)
3-armed PDLLA	1850	9	viscous liquid	99	2.3	19
6-armed PDLLA	3600	19	sticky solid	91	3.0	25
3-armed PTMC	700	-57	viscous liquid	90	-	-
3-armed PTMC	1300	-42	viscous liquid	85	-	-
3-armed PTMC	3000	-30	viscous liquid	84	3.3	-16
6-armed PTMC	6000	-32	viscous liquid	85	3.0	-11

[a)] *Mn* determined by NMR

[b)] *Tg* determined after extraction of the sol fraction and drying.

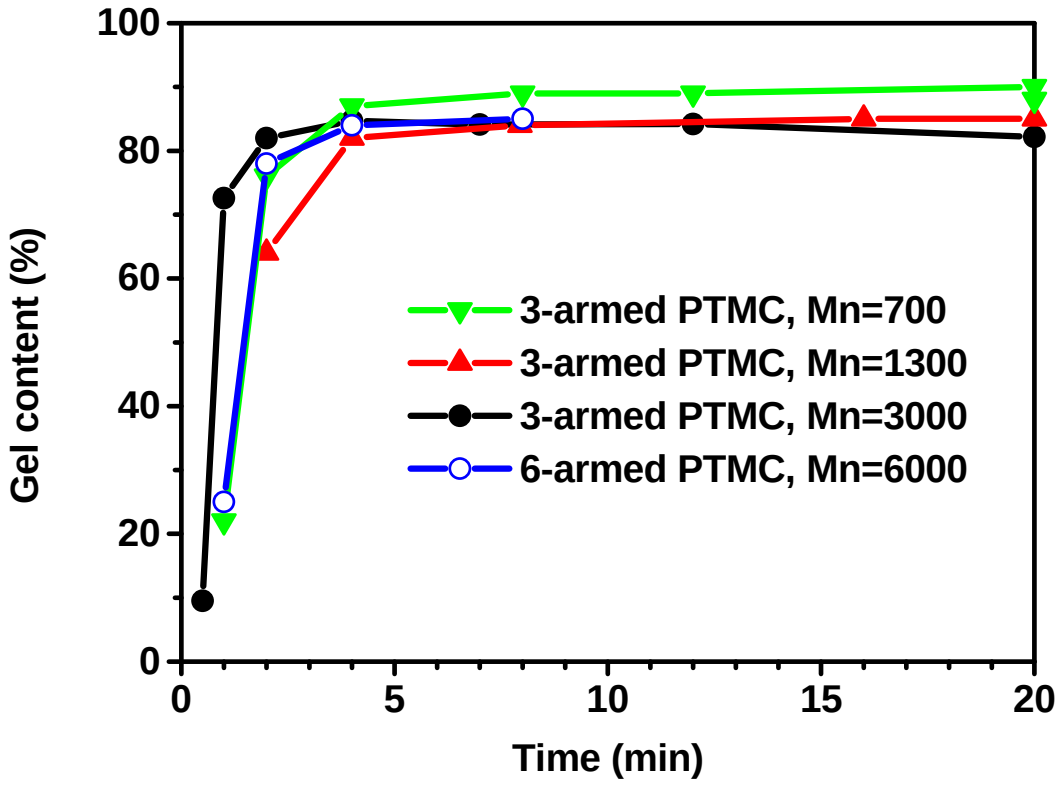

Fig 2. Kinetics of the photo-crosslinking reactions upon UV irradiation of PTMC macromers varying in molecular weight and number of branches. As an initiator HHPMP at a concentration of 2 mol% per methacrylate end-group was employed.

Fig. 2 shows that in all cases complete gelation occurs within 5 to 10 minutes of irradiation of the PTMC macromers. It seems that neither the molecular weight nor the number of arms of the macromer is a factor in determining the curing rate or the gel content that can be reached.

Stereo-lithography

With stereo-lithography (SL), custom made objects with precisely determined shapes can be manufactured from CT scans or from graphical computer data. When prepared from biodegradable polymers, such pre-designed objects can find application as scaffolds in tissue engineering and in numerous other biomedical devices.

The resin we employed for use in the EnvisionTec SLA apparatus was based on PTMC. The reached network glass transition temperatures in the crosslinking reactions are near those of high molecular weight PTMC (-17 ^{o}C), therefore, it can be expected that a low amount of dangling ends is present in the network and most suitable mechanical properties can be achieved. Furthermore, we chose the macromer with the lowest molecular weight, *Mn* = 700, as it has the lowest viscosity and should result in objects with the highest resolution.

The methacrylate macromers are highly reactive and, as it was observed that in some cases premature crosslinking could occur, small amounts of vitamin E were added to the resin. At a concentration of 200 ppm (with respect to the methacrylate groups) it proved a suitable inhibitor that prevents premature crosslinking without significantly interfering in the photo-polymerization process.

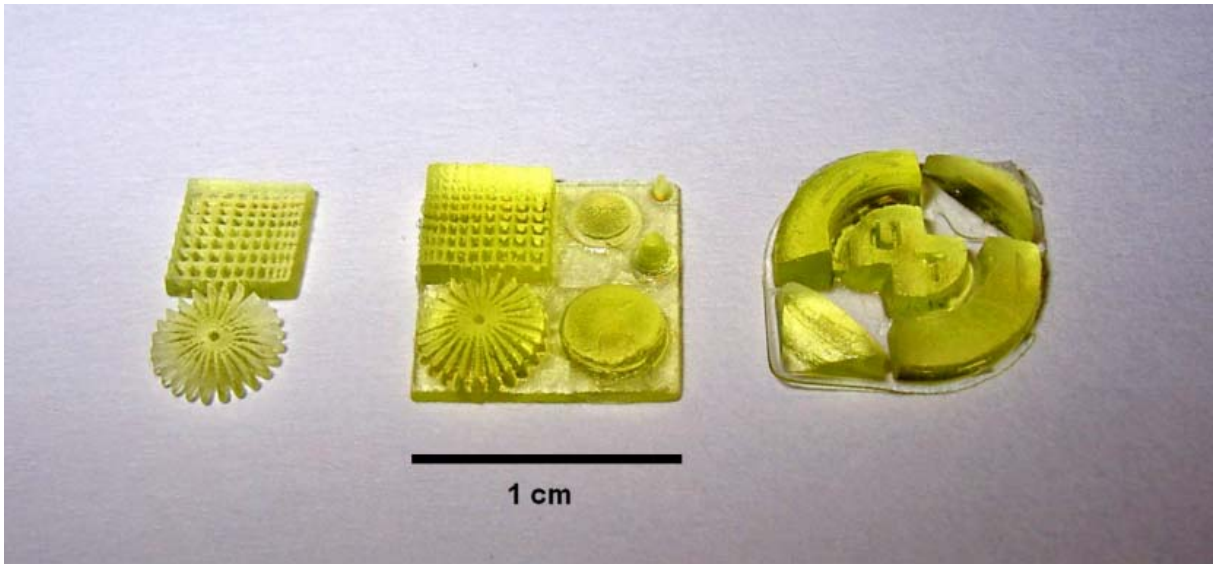

Fig. 3. Three-dimensional structures prepared from a PTMC macromer by stereo-lithography using an EnvisionTec SLA.

Fig. 3 is a photograph of structures prepared with the PTMC 700 resin containing CQ as a blue light initiator and vitamin E as an inhibitor. The geometries were created using computer software and illustrate the capabilities of the SLA apparatus and the suitability of the resin. By operating the equipment at conditions that are comparable to those recommended when using the non-degradable resin supplied by the

manufacturer, highly detailed specimens could readily be created. The obtained resolution is approximately 25 μm.

Conclusions:

Functionalization of branched oligomers prepared by ring opening polymerization of lactide, ε-caprolactone and trimethylene carbonate with methacryloyl chloride gives macromers which can readily be photo-crosslinked with UV or visible light. Employing commercially available stereo-lithography equipment, well-defined degradable implants can readily be prepared from computer data.

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