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Controlling the kinetic chain length of the crosslinks in photo-polymerized biodegradable networks

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Abstract Biodegradable polymer networks were prepared by photo-initiated radical polymerization of methacrylate functionalized poly(D,L-lactide) oligomers. The kinetic chains formed in this radical polymerization are the multifunctional crosslinks of the networks. These chains are carbon–carbon chains that remain after degradation. If their molecular weight is too high these poly(methacrylic acid) chains can not be excreted by the kidneys. The effect of the photo-initiator concentration and the addition of 2-mercaptoethanol as a chain transfer agent on the molecular weight of the kinetic chains was investigated. It was found that both increasing the initiator concentration and adding 2-mercaptoethanol decrease the kinetic chain length. However, the effect of adding 2-mercaptoethanol was much larger. Some network properties such as the glass transition temperature and the swelling ratio in acetone are affected when the kinetic chain length is decreased.

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1 Introduction

Photo-crosslinked biodegradable polymer networks are an interesting class of materials for biomedical applications, as photo-crosslinking is rapid and can be accomplished with minimal heat generation. Spatial and temporal control over the polymerization process allow for the fabrication of polymer matrices with complex shapes [1]. A wide range of substances and even cells can be entrapped in the networks [2]. Because of these advantageous characteristics, these materials have been studied frequently for application in drug delivery devices [3] and as scaffolding materials for tissue engineering [4].

Biodegradable polymer networks can be prepared by the photo-initiated crosslinking of end-functionalized degradable oligomers. Methacrylate derivatives have been most frequently used in functionalization reactions, although fumaric acid derivatives can also be attractive compounds for end functionalization [5]. A network is formed through photo-initiated polymerization of the end groups via an addition type polymerization reaction. The formed chains, also called kinetic chains, are carbon–carbon chains that remain present after degradation of the network. The size and character of these chains determine whether they can be removed from the body by renal clearance [6]. Water-soluble polymers with molecular weights up to 50 kg/mol

are cleared by the kidneys within a few days [7, 8], while polymers with molecular weights over 200 kg/mol accumulate in the circulatory system and are not excreted by the kidneys [9, 10].

The kinetic chains in photo-crosslinked polymer networks have been the subject of a number of studies. The insoluble nature of the networks complicates these studies. Often the networks are first degraded and the degradation products can then be characterized using different methods. For example, Burkoth and Burdick et al. [11, 12] analyzed the degradation products of their dimethacrylate sebacic acid networks using MALDI-TOF MS (matrix assisted laser desorption ionization—time of flight mass spectrometry) and GPC (gel permeation chromatography) to determine the kinetic chain length. Peters et al. [13] analyzed the degradation products of poly(ethylene glycol)-diacrylate (PEG-diacrylate) 2-ethylhexyl mono-acrylate copolymer networks using GPC to determine the kinetic chain length. Recently Ghaffar et al. [14] investigated the degradation products of polyester urethane acrylate networks in a similar manner. In another recent study Melchels et al. [15] used a different approach and determined the kinetic chain length of poly(D,L-lactic acid)-methacrylate (PDLLA-MA) networks by high resolution magnetic angle spinning nuclear magnetic resonance (HR-MAS NMR) analysis of solvent swollen networks.

As water soluble polymers that exceed a critical molecular weight can accumulate in the body, it is of great importance that the kinetic chain length of these networks can not only be measured, but can also be controlled and reduced. The kinetics of photo-crosslinking reactions are very complex [16] and therefore it is difficult to accurately predict the kinetic chain length [17]. However, several factors are known to affect the kinetic chain length in photo-initiated addition polymerizations. An increased initiation rate results in a decrease in the kinetic chain length [11, 12]. In photo-polymerization reactions the initiation rate can be increased by increasing the initiator concentration or the light intensity. Another parameter that can decrease the kinetic chain length is chain transfer [17, 18]. Thiols are very effective transfer agents, because of the labile S–H bond. In chain transfer to a thiol, the carbon-based radical on the growing chain abstracts a hydrogen atom from a thiol functional group to generate a thiyl radical. Rydholm et al. [17] prepared networks by reacting acrylates with thiols with different functionalities and have shown that an increase in thiol functional group concentration decreases the kinetic chain length.

While Rydholm et al. investigated the diverse properties of their very interesting family of network materials prepared with thiols with different functionalities; in this study our aim is to develop a practical general method, employing a mono-functional thiol, to control the kinetic chain length of photo-polymerized networks without altering other network properties to a large extent. The kinetic chain lengths of the multifunctional crosslinks of PDLLA-MA networks were investigated. The effect of the photo-initiator concentration and 2-mercaptoethanol as a transfer agent on the kinetic chain length was studied. 2-Mercaptoethanol was selected as a transfer agent since it is an antioxidant and is frequently used in cell culture as a medium supplement [19]. The effect of decreasing the kinetic chain length on different network properties was studied.

2 Experimental section

2.1 Materials

D,L-lactide was obtained from PuracBiochem (The Netherlands). Tin 2-ethylhexanoate ($Sn(Oct)_2$), trimethylol propane (TMP), glycerol, methacrylic anhydride, 5,5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent), *N*-methyl dibenzopyrazine methyl sulfate (PMS), deuterated chloroform, trypsin and penicillin–streptomycin solution were purchased from Sigma Aldrich (U.S.A.). Triethylamine (TEA) was obtained from Fluka (Switzerland). Irgacure 2959 (2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone) was obtained from Ciba Specialty Chemicals (Switzerland). 2-Mercaptoethanol was purchased from Acros (Belgium). Phosphate buffered saline (PBS) was obtained from B. Braun (Germany). Deuterated methanol was obtained from Euriso-top (France). Dulbecco's modified Eagle's medium (DMEM) was purchased from Invitrogen (U.S.A.). XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was obtained from Polysciences (U.S.A.). KOH was purchased from Merck (Germany). Newborn calf serum (NCS) was obtained from Lonza (Switzerland). Analytical grade dichloromethane (DCM) was obtained from Biosolve (The Netherlands). DCM was dried over CaH_2 and distilled. Other solvents were of technical grade and were used as received (Biosolve, the Netherlands).

2.2 Synthesis of star-shaped MA-functionalized PDLLA oligomers

Three-armed poly(D,L-lactide) oligomers were synthesized by ring opening polymerization of D,L-lactide in the presence of TMP or glycerol as a trifunctional initiator. D,L-lactide, TMP and $Sn(Oct)_2$ ($\sim$0.2 mmol/mol monomer) as a catalyst were reacted in the melt at 130 °C for 48 h under argon. The monomer-to-initiator ratio was adjusted to yield oligomers with a targeted molecular weight of 1,000 g/mol per arm and a total molecular weight of 3,100 g/mol. The oligomers were functionalized with methacrylate

end-groups by reacting methacrylic anhydride (MAAH) with the hydroxyl end-groups of the oligomers. An amount of oligomer was charged into a three-necked flask and dried for 2 h at 110 °C in vacuo and then cooled to room temperature under argon. The oligomer was dissolved in dry DCM and TEA was added. Methacrylic anhydride was added drop-wise to the vigorously stirred oligomer solution. Per mol of hydroxyl groups 1.2 mol of MAAH and TEA were used. The coupling reaction was continued for 3 days. The macromers were purified by precipitation in cold isopropanol, washing with water and freeze-drying.

The oligomer molecular weights (M_n^-) and the degrees of functionalization were determined from 1H-NMR spectra (Varian Inova 300 MHz NMR spectrometer). Deuterated chloroform was used as a solvent.

2.3 Photo-crosslinking of PDLLA-MA macromers

The macromers were dissolved in dichloromethane (1 g/ml) and different amounts of Irgacure 2959 photo-initiatior and 2-mercaptoethanol were added. Three different initiatior concentrations were used: 0.2, 1 and 3 wt% (all with respect to macromer, corresponding to 1, 5 and 15 mol% with respect to the methacrylate end-groups). Five different 2-mercaptoethanol concentrations were used: 0.4, 0.7, 1.4, 2.9 and 5.8 wt% (all with respect to macromer, corresponding to 5, 10, 20, 40 and 80 mol% with respect to the methacrylate end-groups). These five solutions all contained 1 wt% photo-initiator. The macromer solutions were poured into Teflon moulds, covered with FEP (fluorinated ethylene propylene) foil and crosslinked for 15 min in an Ultralum crosslinking cabinet (365 nm, 3–5 mW/cm^2). Disked-shaped specimens with a diameter of ~10 mm and thickness of ~0.5 mm were obtained.

The specimens were dried overnight at 90 °C under a nitrogen flow. Specimens that were extracted for the studies on cytocompatibility and unreacted thiol concentrations were not dried at elevated temperature (see below).

2.4 Network degradation and analysis of the kinetic chains

Approximately 10 mg of each network specimen was immersed in 1 ml of 1 M KOH in a 10 ml glass vial which was internally lined with PTFE (poly(tetrafluoroethylene)). The mixture was then hydrolyzed at 120 °C, 3 bar for 20 h in a microwave instrument (Discover BenchMate, CEM).

After hydrolysis 1 mL of each hydrolyzed sample was acidified with 200 µL of 37 % HCl at 90 °C for a few minutes. The PMAA (poly(methacrylic acid)) precipitate and the supernatant (containing lactic acid, TMP, etc.) were dried overnight at 40 °C under an air flow. The dried hydrolysates (both precipitate and supernatant together) were re-dissolved in d_4-methanol. Samples of partially-hydrolyzed networks (hydrolyzed in 1 M KOH at room temperature) and networks hydrolyzed for 10 h in the microwave setup were also dissolved in d_4-methanol. ^{1}H-NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer.

For GPC analysis 0.2 ml of the hydrolyzed sample was diluted with 0.2 ml of the mobile phase (see below) and then the samples were neutralized (pH 7.4) with a few drops of 37 % HCl. The samples were filtered through a PTFE (0.45 µm) filter prior to injection.

The GPC experiments were performed on an high performance liquid chromatography (HPLC) (system equipped with an in-line degasser, Model 600 pump, 717 plus TRI-SEC auto-sampler and Model 410 differential refractive index detector at 40 °C (all waters).

The GPC separations were performed on PL aquagel-OH Guard (8 µm, 50 × 7.5 mm), PL aquagel-OH 50, 30 and 10 (each 8 µm, 300 × 7.5 mm) columns connected in series. The column oven temperature was adjusted to 45 °C. The mobile phase (0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH ~7.0) was pumped at a flow rate of 1.0 ml/min. Narrow molecular weight poly(methacrylic acid) sodium salt (PMAA-Na) standards (M_p (the peak value of the molecular weight distribution) varying from 1.22 to 549 × 10^3 g/mol) were used to calibrate the GPC-dRI system. Data were recorded and chromatographic peaks were treated using the Empower 2 software (Waters). Calculations for the molar mass distribution (MMD) on the chromatographic peaks were executed using software written in-house in Excel 2003 (Microsoft). Highly pure water for mobile phase preparation was obtained by means of an Arium® 611 Ultrapure (18.2 MΩ*cm) Water System (Sartorius AG).

Several low molecular weight GPC fractions were collected for analysis by MALDI-TOF MS. MALDI-TOF was performed using a Voyager-DE-RP MALDI-TOF spectrometer (Applied Biosystems) equipped with delayed extraction. A 337 nm UV nitrogen laser producing 2 ns pulses was used. Sinapinic acid was used as the matrix material and negative ions were detected. Collected GPC fractions were protonated with concentrated hydrochloric acid and dried. MALDI-TOF samples were prepared by dissolving the degradation products in a 50:50 mixture of acetonitrile and water containing 0.1 vol% TFA (trifluoroacetic acid) and mixing with the matrix material (~1:1 sample to matrix ratio by volume).

2.5 Network characterization

To determine the gel content and swelling ratio in acetone, a network specimen ($n = 3$) was weighed (m_0), swollen and extracted in acetone and weighed again (m_s). Then the

specimen was dried at 90 °C under a nitrogen flow until constant weight. The mass of the dry network (m_1) was determined. Gel content is defined as:

$$\text{Gel content} = \frac{m_1}{m_0} \times 100\,\% \quad (1)$$

The swelling ratio was calculated using:

$$\text{Swelling ratio} = \frac{m_s}{m_0} \quad (2)$$

The thermal properties of the networks were evaluated using a Perkin–Elmer Pyris 1 differential scanning calorimeter (DSC). Samples were heated from 0 to 100 °C at a heating rate of 10 °C/min, quenched rapidly at 300 °C/min to 0 °C, and then a second heating scan was recorded after 5 min. The glass transition temperature (T_g) was taken as the midpoint of the heat capacity change in the second heating run.

To study degradation of the different PDLLA-MA networks, disk-shaped specimens (diameter = 10 mm, thickness = 0.5 mm) were immersed in 2 ml PBS containing 0.02 wt% NaN_3 as a bactericide. At different time points wet mass, dry mass, swelling ratio in acetone and gel content were determined. At a certain time point four specimens were taken out. Two specimens were weighed to determine the wet mass, dried at 90 °C under a nitrogen flow until constant weight, and then weighed again to determine the dry mass. Two other samples were swollen and extracted in acetone for 1 day, weighed to determine the swollen mass (m_s), and then dried and weighed again (m_1). All samples were also weighed prior to the degradation experiment (m_0). Swelling ratio and gel content were calculated as described above. The PBS was refreshed every 2–3 weeks.

Ellman's reagent was used to determine the 2-mercaptoethanol concentrations in networks extracts [20]. Disk-shaped network specimens were extracted in PBS for 24 h directly after crosslinking. 100 µl of the network extracts and 100 µl of a 300 µM 5,5′-dithiobis(2-nitrobenzoic acid) solution in PBS were mixed in a 96 well plate. A 340 ATTC plate reader (SLT) was used to measure the absorption at a wavelength of 404 nm. A calibration curve was constructed using 2-mercaptoethanol.

2.6 Cytocompatibility of network extracts

Disk-shaped PDLLA-MA network specimens were dried at room temperature under vacuum for 2 days. The materials were disinfected in 70 % isopropanol (2 × 15 min) and washed with sterile PBS (2 × 15 min). Samples were then extracted with serum free culture medium (DMEM), 1 ml per 3 cm^2 surface area) at 37 °C for 24 h. The supernatants were stored at −20 °C until further use. NIH 3T3 fibroblasts were cultured in T75 cell culture flasks in DMEM medium supplemented with 10 % newborn calf serum and 1 % penicillin/streptomycin. The cells were cultured for 1 day at 37 °C in a 5 % CO_2 atmosphere, trypsinized, and redispersed in medium (15×10^4 cells/ml). Cells were then seeded in 96 well tissue culture polystyrene (TCPS) plates at a density of 15×10^3 cells per well and cultured for 1 day. Then the medium was aspirated from the wells, and 50 µl of extract was added to the cells ($n = 3$), followed by 50 µl of culture medium containing 20 % NCS. Normal culture medium (containing 10 % NCS) and 50 % v/v DMSO (dimethylsulfoxide) were used as controls. The cells were cultured at 37 °C, in a 5 % CO_2 atmosphere for 1 day. The metabolic activity of the cells was analyzed using the XTT assay. The medium was aspirated from the cells and replaced by 100 µl of fresh colorless medium and 50 µl of XTT solution (containing 1 mg/ml XTT and 0.38 mg/ml PMS). After 1 h of incubation, the absorbance was read on a 340 ATTC plate reader (SLT) at 450 nm with a reference wavelength of 620 nm.

3 Results and discussion

3.1 Synthesis and characterization of MA-functionalized PDLLA oligomers

Three-armed PDLLA oligomers were prepared by ring opening polymerization of D,L-lactide with a trifunctional initiatior. The obtained molecular weight, determined from the initiator to lactide ratio in 1H-NMR spectra, was 3.4 kg/mol which is close to the intended molecular weight of 3.1 kg/mol.

The PDLLA oligomers were functionalized with methacrylate end-groups through reaction with methacrylic anhydride to obtain crosslinkablemacromers (PDLLA-MA). The successful functionalization was confirmed by the appearance of the $=CH_2$, and $-CH_3$ peaks of the methacrylate at δ 5.65 and δ 6.21, and at δ 1.97 in ^{1}H-NMR spectra. The obtained degree of functionalization calculated from the $-CH_3$ of the methacrylate peak at δ 1.97 and the $-CH_3$ of TMP at δ 0.89 was 93 %.

3.2 Kinetic chains

PDLLA-MA networks were prepared by photo-polymerization of the synthesized macromers (Fig. 1). The amount of photo-initiator and 2-mercaptoethanol, used as a transfer agent, were varied to investigate the effect on the kinetic chain length. It was anticipated that increasing the photo-initiator concentration and adding 2-mercaptoethanol would decrease the kinetic chain length of the networks. 2-mercaptoethanol is

an effective transfer agent because of the labile S–H bond it contains (Fig. 2).

To optimize the hydrolysis method the prepared networks were degraded in 1 M KOH at room temperature and for 10 and 20 h at 120 °C in a microwave instrument [14]. NMR spectroscopy was used to distinguish between lactic acid units attached to the poly(methacrylic acid) backbone and lactic acid units present in solution in the form of free lactic acid and oligomers.

The overlay of ^{1}H-NMR spectra of the three-armed PDLLA oligomers and partially and fully hydrolyzed PDLLA-MA networks (Fig. 3) shows clearly the cleavage of ester bonds as the degradation temperature and time increased. In case of the PDLLA oligomers (Fig. 1a) peaks 4 and 5 belong to the CH group of lactic acid, while peak 3 belongs to the methyl group of lactic acid. The peaks 1 and 2 correspond to glycerol which was used as the initiator for the ring opening polymerization.

In case of partially degraded networks (Fig. 3b) peak 9 represents the CH group of lactic acid that is still attached to the methacrylate backbone, while peaks 7 and 8 represent free lactic acid (CH group) oligomers in the hydrolysis solution. Peak 6 represents different CH_3 groups of lactic acid units either attached to the methacrylate backbone or present as free monomers or oligomers in the hydrolysis solution. However hydrolysis at an elevated temperature and pressure (120 °C for 10 and 20 h, 3 bars) (Fig. 3c, d) clearly shows only peak 11 for free lactic acid (CH group) and no noticeable peak for lactic acid attached to the methacrylate backbone. Peak 12 and 13 indicate the CH_3

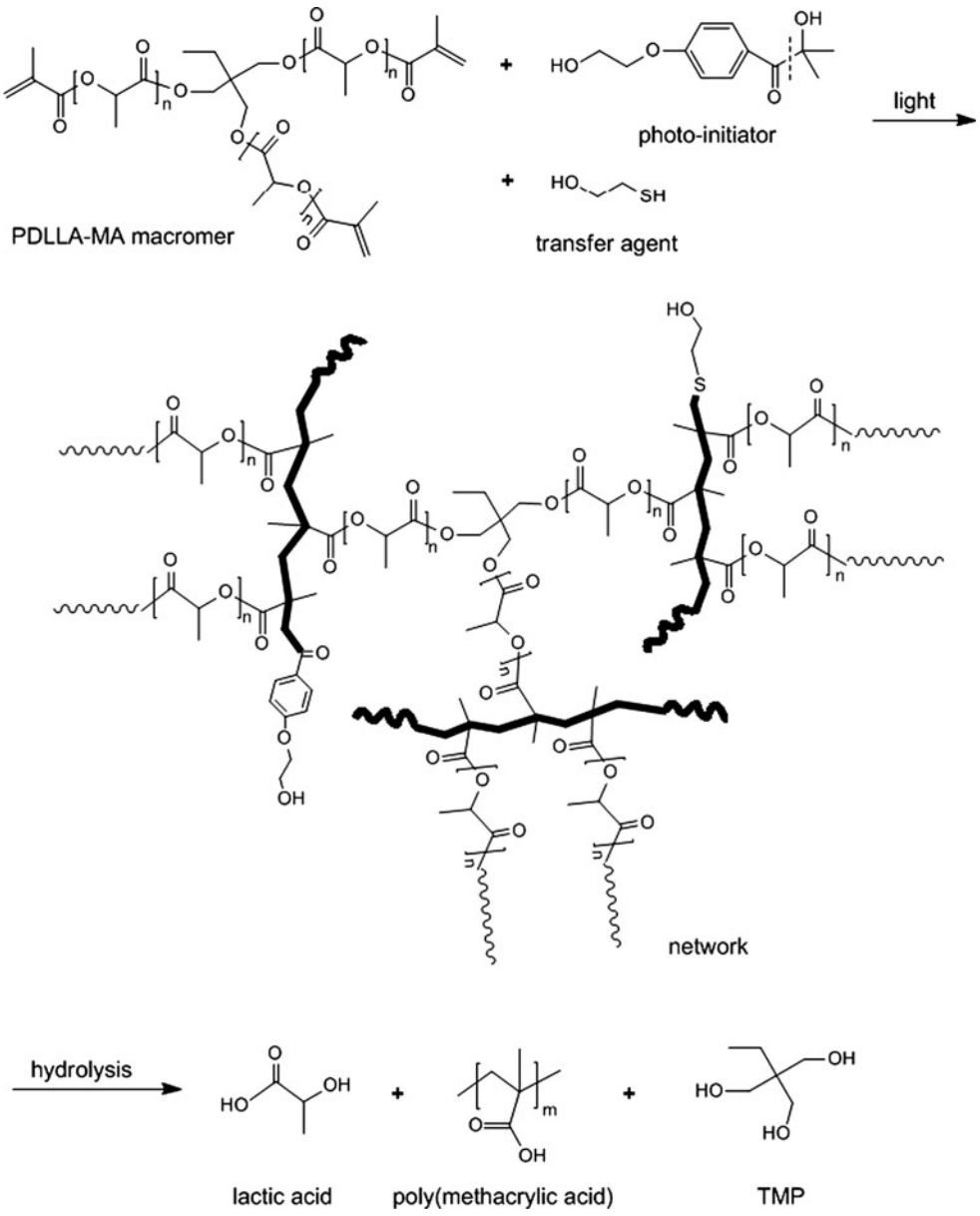

Fig. 1 Network formation and hydrolysis

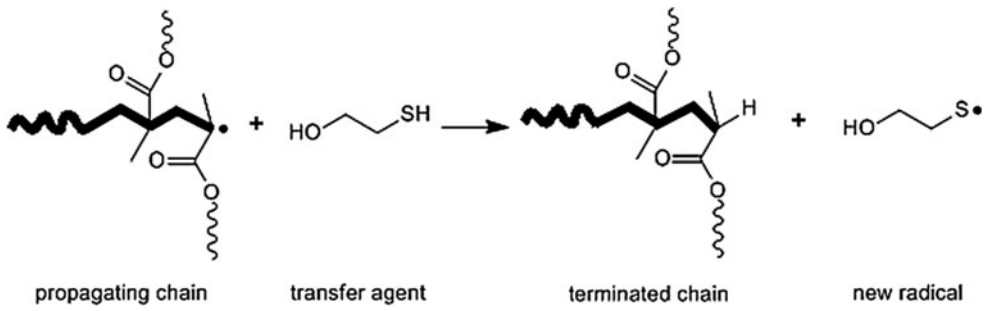

Fig. 2 Chain transfer to 2-mercaptoethanol

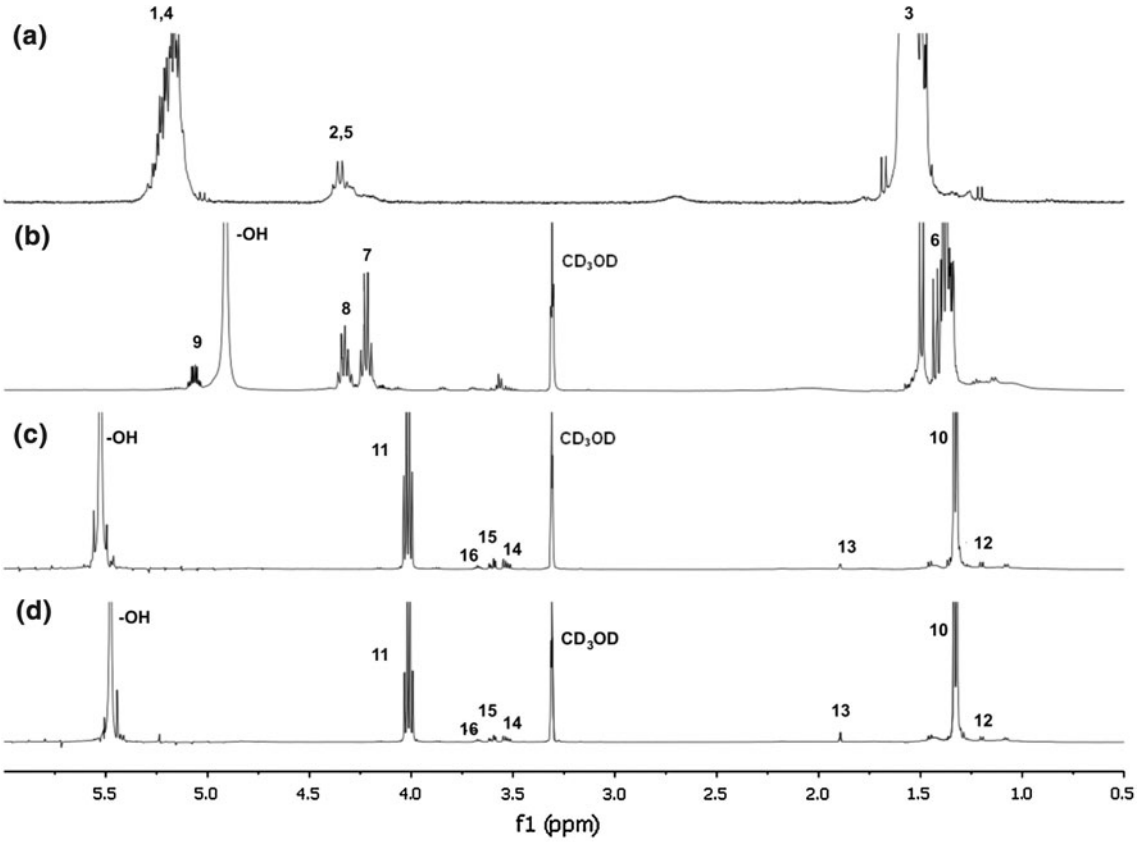

Fig. 3 An overlay of ^{1}H-NMR spectra (in D-chloroform (*a*) and d_4-methanol (*b–d*)) of the three-armed PDLLA oligomer (*a*), PDLLA-MA networks partially hydrolyzed at room temperature for two weeks (*b*) and PDLLA-MA networks hydrolyzed for 10 (*c*) and 20 h (*d*) in a microwave instrument

and CH_2 groups of free polymethacrylic acid chains, respectively. Peaks 14, 15 and 16 indicate the presence of glycerol that in this case was used as an initiator in the ring opening polymerization reaction. Based on these results it was decided to hydrolyze all synthesized networks at 120 °C, 3 bar for 20 h in a microwave instrument to ensure complete hydrolysis.

The network degradation products were analyzed using GPC to determine the molecular weights of the kinetic chains. The results are summarized in Fig. 4 and Table 1.

When comparing the results for the different initiator concentrations it can be observed that increasing the initiator concentration results in a decrease in the kinetic chain length, but the effect is relatively small. Increasing the photo-initiator concentration from 0.2 to 3 wt% results in a decrease of M_n of the kinetic chains from 44 to 23×10^3 g/mol.

The effect of adding 2-mercaptoethanol as a transfer agent on the kinetic chain length is much larger. Adding 5 mol% 2-mercaptoethanol, 1 molecule per 20 methacrylate end-groups, results in a decrease in Mn of from 32 to

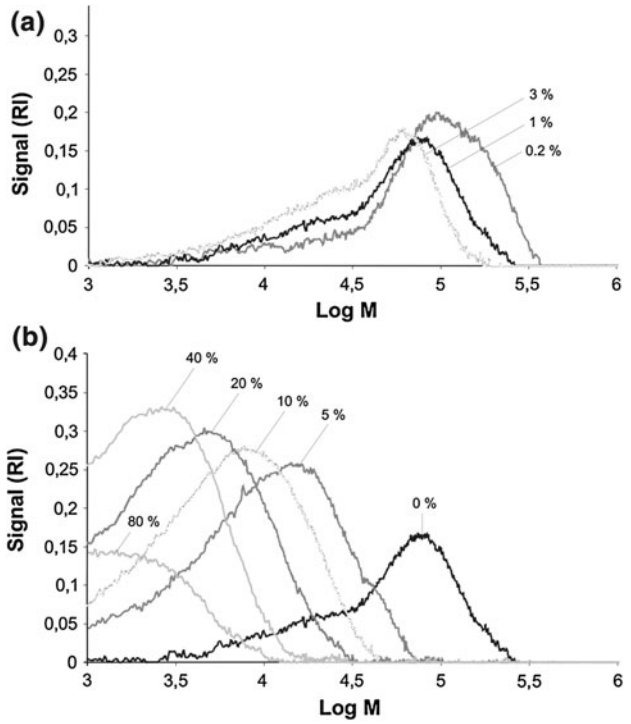

Fig. 4 GPC analysis of the products of microwave hydrolysis of PDLLA-MA networks prepared with different photo-initiator concentrations (**a**) and different 2-mercaptoethanol concentrations (**b**). Photo-initiator concentrations are indicated in wt% with respect to macromer. 2-Mercaptoethanol concentrations are given in mol% with respect to the methacrylate end-groups

8×10^3 g/mol. The addition of more 2-mercaptoethanol decreases the molecular weight even further. So clearly, by adding small amounts of 2-mercaptoethanol as a transfer agent, the kinetic chain length of PDLLA-MA networks can be decreased.

The effect of chain transfer on the polymer molecular weight can be described by the Mayo equation. A plot of $1/X_n$ (degree of polymerization) against the transfer agent concentration [S] divided by the monomer concentration [M] should result in a straight line [18]:

$$\frac{1}{X_n} = \frac{1}{(X_n)_0} + C_s \frac{[S]}{[M]} \quad (3)$$

The slope of the straight line is the chain transfer constant (C_s). $1/(X_n)_0$ is the value of $1/X_n^-$ in the absence of the transfer agent. When our data are plotted in this way (Fig. 5), indeed a straight line is obtained ($R^2 = 0.995$). The chain transfer constant obtained for 2-mercaptoethanol and our PDLLA-MA macromer is 0.079.

Low molecular weight fractions were collected from GPC for analysis by MALDI-TOF–MS. Only low molecular weight GPC fractions were analyzed by MALDI-TOF–MS since it is known that MALDI-TOF–MS of high molecular weight poly(methacrylic acid) is hindered by the so-called mass discrimination effect [21]. The obtained spectra show a clear molecular weight distribution in agreement with the molecular weight of the fractions determined by GPC (Fig. 6). For example, the GPC molecular weights of the fraction for which the MALDI-TOF–MS spectrum is shown in Fig. 6 were below approximately 4.6×10^3 g/mol. Furthermore, the distance between the peaks corresponds to the molecular weight of methacrylic acid (86 g/mol), confirming that the degradation products analyzed by GPC were indeed fully hydrolyzed poly(methacrylic acid) kinetic chains. No peaks with intervals corresponding to lactic acid (72 g/mol) were found indicating no evidence of the presence of lactic acid esters.

Table 1 Molecular weights of the kinetic chains of PDLLA-MA networks prepared with different photo-initiator and 2-mercaptoethanol concentrations

Initiator concentration (wt%)[a]	2-Mercaptoethanol concentration (mol%)[b]	M_n (g/mol)	M_w (g/mol)	PDI[c]	M_p (g/mol)[d]
0.2	–	44×10^3	110×10^3	2.5	97×10^3
1.0	–	32×10^3	72×10^3	2.3	74×10^3
3.0	–	23×10^3	52×10^3	2.3	62×10^3
1.0	5	8.0×10^3	18×10^3	2.2	14×10^3
1.0	10	5.3×10^3	10×10^3	2.0	7.7×10^3
1.0	20	[e]	[e]	[e]	4.6×10^3
1.0	40	[e]	[e]	[e]	2.6×10^3
1.0	80	[e]	[e]	[e]	$<1 \times 10^3$

[a] With respect to macromer

[b] With respect to methacrylate end-groups

[c] Polydispersity index

[d] M_p is the peak value of the molecular weight distribution

[e] Could not be determined accurately due to low molecular weight resulting in incomplete separation by the columns (see Fig. 4)

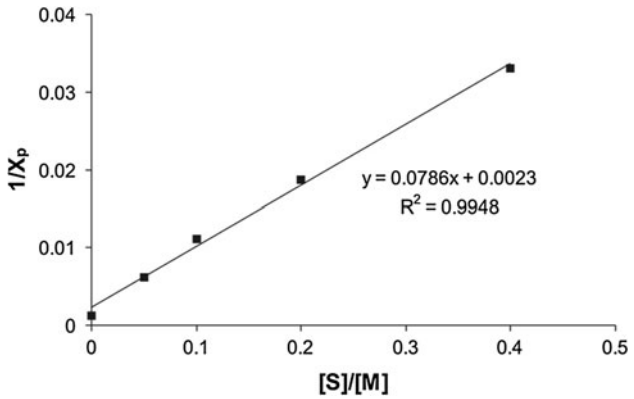

Fig. 5 Plot of $1/X_p$ against [S]/[M] to obtain the chain transfer constant for PDLLA-MA macromers and 2-mercaptoethanol. X_p is the peak value of the degree of polymerization distribution. These values were used since for the higher 2-mercaptoethanol concentrations X_n values could not be determined (Table 1)

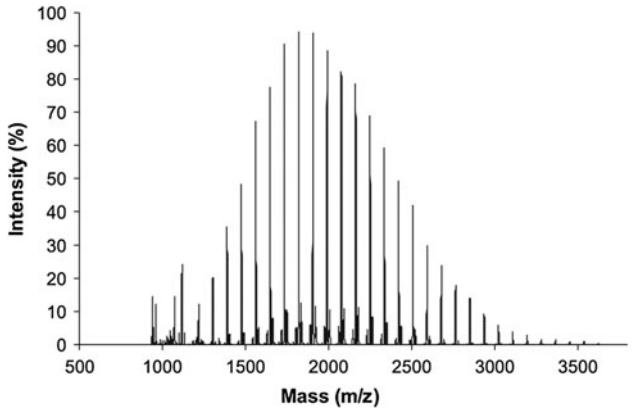

Fig. 6 MALDI-TOF MS spectrum of a low molecular weight GPC fraction (molecular weights below approximately 4.6×10^3 g/mol) of the degradation products of PDLLA-MA networks prepared with 20 mol% 2-mercaptoethanol

3.3 Network properties

Although controlling the kinetic chain length is very important when biodegradable photo-crosslinked polymer networks are used in biomedical applications, decreasing the kinetic chain length may affect important network properties. The effect of varying the photo-initiator concentration and adding 2-mercaptoethanol on the gel content, swelling behavior and glass transition temperature of the networks was investigated. The results are summarized in Table 2.

All networks showed gel contents above 90 % and no clear trend as a function of photo-initiator or 2-mercaptoethanol concentration was observed. From this it can be concluded that under all used conditions the photo-crosslinking process was effective and most macromer molecules were incorporated in the networks. The swelling ratio in acetone was similar for the networks with varying photo-initiator concentration. The photo-initiator concentration does not affect the crosslink density significantly. This can be explained by the fact that altering the photo-initiator concentration only has a small effect on the kinetic chain length. However, increasing the 2-mercaptoethanol concentration leads to a pronounced increase in swelling ratio and decrease in crosslink density. The significant decrease in the kinetic chain length upon addition of 2-mercaptoethanol can explain this. The functionality of the crosslinks is lower (less polylactide chains attached to one poly(methacrylic acid) chain) for shorter kinetic chains resulting in a lower crosslink density. With respect to the glass transition temperature the same trends are observed. The T_g is a measure of crosslink density. A higher crosslink density results in a higher T_g [22]. For networks containing 2-mercaptoethanol it can be observed that the T_g decreases with increasing 2-mercaptoethanol concentrations. Any unreacted 2-mercaptoethanol present in the networks that was not removed during drying of the specimen at 90 °C may act as a plasticizer and also decrease the T_g somewhat.

Network degradation behavior is important for many biomedical applications. For example, in drug delivery systems, the degradation behavior of the networks can influence the release kinetics. Therefore, the influence of the kinetic chain length on network degradation was investigated. Degradation of crosslinked polymer networks is influenced by the network structure. For example, it has been shown that the mass loss behavior of thiol-acrylate networks, consisting of PEG-diacrylate and thiols of varying functionality, is influenced by the length of the poly(acrylic acid) kinetic chains [23].

To investigate the degradation behavior, the wet mass, dry mass, swelling ratio in acetone and gel content of the different networks were followed in time (Fig. 7). As was found in previous experiments, networks with shorter kinetic chains showed a higher swelling ratio in acetone. The networks containing different amounts of photo-initiator all showed a similar swelling ratio in acetone, since the amount of photo-initiator did not affect the kinetic chain length much. All materials show similar degradation behavior until ~10 week. The wet mass of the networks steadily increases, while the dry mass stays more or less constant. After 14 and especially 20 week differences between the materials start to appear. Considering the networks prepared with different photo-initiator concentrations it can be seen that networks prepared with 3 wt% photo-initiatior seem to degrade somewhat faster than the networks prepared with 0.2 and 1 wt% photo-initiator. For the networks prepared with different amounts of 2-mercaptoethanol, especially the networks with the highest 2-mercaptoethanol concentrations, 40 and 80 mol%, show fast degradation. These two materials show

Table 2 Gel contents, equilibrium swelling ratios (in acetone) and glass transition temperatures of PDLLA-MA networks prepared with different concentrations of photo-initiator and 2-mercaptoethanol

Initiator concentration (wt%)[a]	2-Mercapto-ethanol concentration (mol%)[b]	Gel content (%)	Swelling ratio	Tg (°C)
0.2	–	94.4 ± 0.5	1.99 ± 0.05	57.0
1.0	–	97.4 ± 1.4	2.20 ± 0.08	57.2
3.0	–	97.2 ± 0.8	2.07 ± 0.07	57.5
1.0	5	90.5 ± 0.8	2.07 ± 0.03	55.6
1.0	10	95.8 ± 1.4	2.34 ± 0.19	54.0
1.0	20	96.1 ± 1.4	2.70 ± 0.04	53.8
1.0	40	92.6 ± 3.0	3.01 ± 0.03	52.7
1.0	80	95.9 ± 1.8	3.27 ± 0.12	49.5

[a] With respect to macromer

[b] With respect to methacrylate end-groups

the lowest dry mass and gel content after 20 week of all the investigated materials. Lower molecular weight poly (methacrylic acid) chains have a smaller number of PDLLA chains that connect them to the rest of the network. Therefore, for networks prepared with higher 2-mercaptoethanol concentrations, the extent of degradation required before these poly(methacrylic acid) chains can go into solution is less. The degradation behavior of the networks prepared with 5, 10 and 20 wt% 2-mercaptoethanol is more similar to that of the networks prepared without 2-mercaptoethanol, although the networks containing 10 and 20 wt% 2-mercaptoethanol show a large increase in wet mass after 20 week.

In a previous study a stronger dependence of mass loss on the kinetic chain length of the networks was reported [23]. This might be because, in the networks prepared in this study, multifunctional thiols were used, so changing the thiol concentration greatly alters the crosslink density of the networks. Furthermore the prepared PEG-based networks are much more hydrophilic than our PDLLA networks and therefore the length of the kinetic chain has a more direct influence on degree of swelling of the networks in water. For many applications it may be an advantage that for the more hydrophobic networks described in this study, the kinetic chain length can be decreased substantially without altering the degradation behavior of the material to a large extent.

It is possible that not all 2-mercaptoethanol added has reacted into the network. Therefore the prepared networks were extracted in PBS and the 2-mercaptoethanol concentrations in these extracts were determined (Table 3).

No 2-mercaptoethanol was detected in extracts from networks prepared with 5 or 10 mol% 2-mercaptoethanol. Most likely here all the 2-mercaptoethanol was incorporated into the networks, although there might be some unreacted 2-mercaptoethanol entrapped in the networks. In extracts from networks prepared with 20, 40 or 80 mol% 2-mercaptoethanol, 2-mercaptoethanol was indeed detected. For the networks prepared with more 2-mercaptoethanol the concentration in the extracts was higher. For these networks, clearly not al 2-mercaptoethanol was incorporated in the network. These results are in agreement with the study of Reddy et al. [24] who concluded from their modeling study on thiol-acrylate polymerizations that the final thiol conversion decreases with increasing thiol/acrylate ratio in the initial mixture. Although only one to three percent of the 2-mercaptoethanol initially present was extracted from the networks, it is possible that more 2-mercaptoethanol did not react. It may be that 2-mercaptoethanol was physically entrapped in the hydrophobic networks and was therefore not extracted out of the networks. Extractable 2-mercaptoethanol may play a role in network cytocompatibility. While 2-mercaptoethanol is an antioxidant and is frequently used in cell culture as a medium supplement [19], it can reduce disulfide bonds and can therefore denature proteins.

PDLLA-MA networks are known to be cytocompatible [25]. To investigate whether this changes when the photo-initiator concentration is altered or 2-mercaptoethanol is added, a cytocompatibility experiment was performed. Network samples were extracted in culture medium. NIH 3T3 fibroblasts were cultured in the presence of this medium and the metabolic activity of the cells was determined using the XTT assay. The results are summarized in Fig. 8.

As expected, cells cultured in the presence of DMSO showed significantly lower metabolic activity than cells cultured in normal culture medium. Although it is known that photo-initiators can be cytotoxic [26, 27] cells in contact with networks prepared with different photo-initiator concentrations showed metabolic activities comparable to the control. Most likely even for the networks prepared with 3 wt% photo-initiator the concentration of initiator in the extracts is low. Furthermore, Irgacure 2959

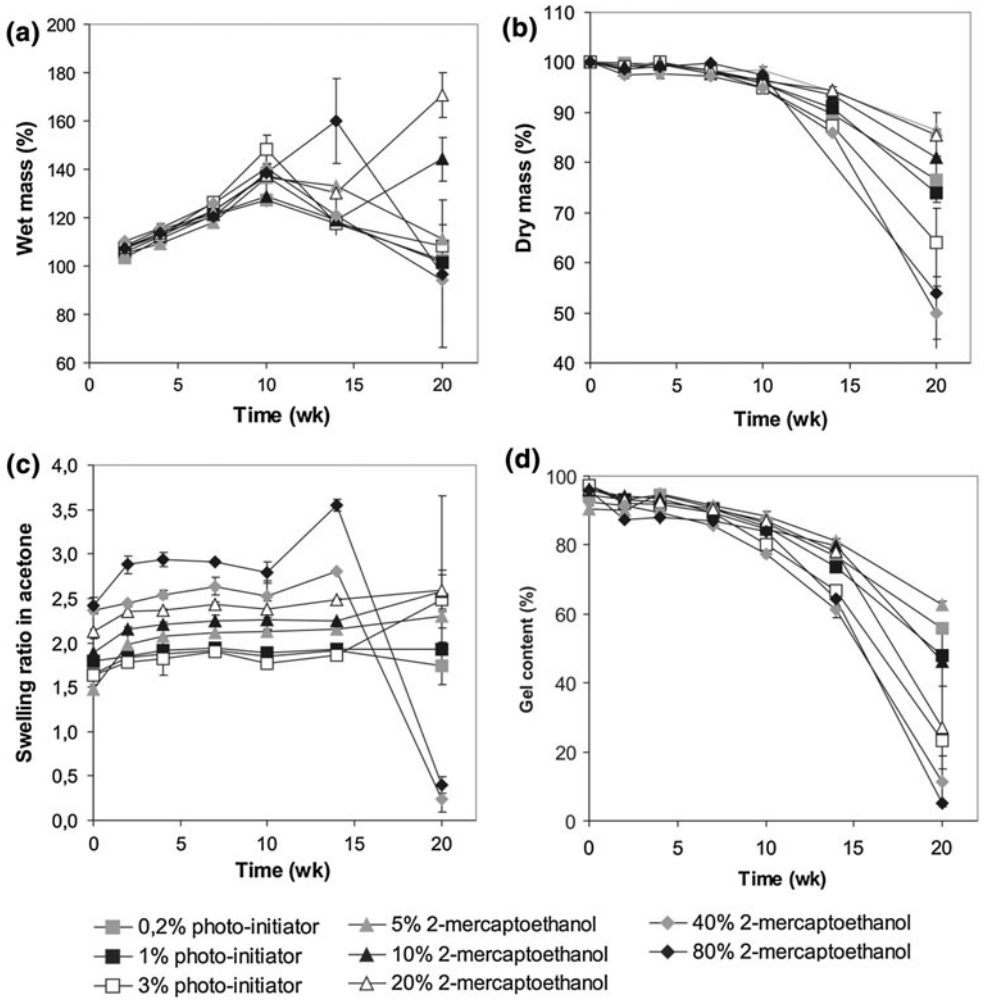

Fig. 7 Degradation of PDLLA-MA networks containing different amounts of photo-initiator (mass% with respect to macromer) and 2-mercaptoethanol (mol% with respect to methacrylate end-groups, all containing 1 wt% photo-initiator). Wet mass (**a**), dry mass (**b**), swelling ratio in acetone (**c**) and gel content (**d**) as a function of time

Table 3 2-Mercaptoethanol concentrations in network extracts

[2-Mercapto-ethanol] in network (mol%)[a]	[2-Mercapto-ethanol] in network extracts (mM)	% Of initial 2-mercaptoethanol
0	0	0
5	0	0
10	0	0
20	0.28	1.0
40	1.55	2.9
80	1.64	1.5

[a] With respect to methacrylate end-groups

has been indicated as a relatively cytocompatible UV photo-initiator [26, 27].

For NIH 3T3 fibroblasts cultured in the presence of extracts from networks prepared with 5, 10 or 20 mol% 2-mercaptoethanol high metabolic activity was observed, comparable to cells cultured in normal culture medium. However, cells cultured in the presence of extracts from networks prepared with 40 and especially, 80 mol% 2-mercaptoethanol, showed lower metabolic activity. These are also the materials for which significant amounts of 2-mercaptoethanol could be extracted from the networks (Table 3). Therefore is seems very likely that 2-mercaptoethanol released from the networks causes the toxicity of the extracts. However, also for networks prepared with only 5 or 10 mol% of 2-mercaptoethanol a significant decrease of the kinetic chain length was found (Table 1). Importantly, for these low concentrations no unreacted 2-mercaptoethanol was extracted from the networks (Table 3) and network extracts were found to be cyto-compatible (Fig. 8). If higher 2-mercaptoethanol concentrations are used, it may also be possible to extract unreacted 2-mercaptoethanol prior to implantation or to dry the samples at elevated temperature. If however, for a certain application the networks are loaded with a drug, this might not be feasible.

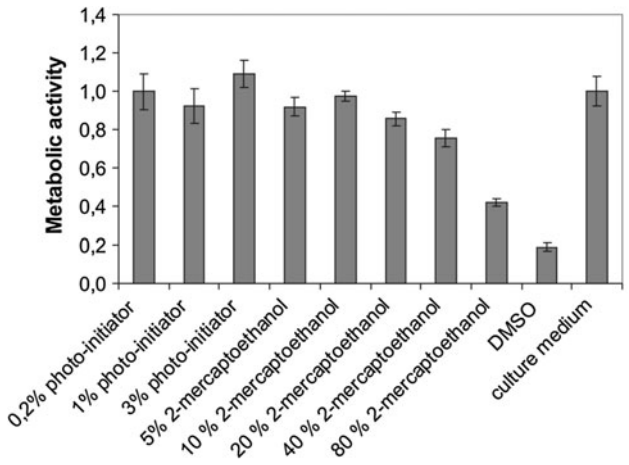

Fig. 8 XTT assay results. NIH 3T3 fibroblasts were cultured in medium containing extracts of PDLLA-MA networks. The networks were prepared with different amounts of photo-initiator and 2-mercaptoethanol. Values are relative to the control (culture medium). Photo-initiator concentrations are indicated in wt% with respect to macromer. 2-Mercaptoethanol concentrations are given in mol% with respect to the methacrylate end-groups

4 Conclusions

The kinetic chain length of PDLLA-MA networks can be decreased in a controlled way by adding 2-mercaptoethanol as a transfer agent. Some network properties such as the glass transition temperature and the swelling ratio in acetone (crosslink density) are affected when the kinetic chain length is decreased. Only small amounts of 2-mercaptoethanol are required to significantly decrease the kinetic chain length. Large amounts should be avoided, since unreacted 2-mercaptoethanol that is extracted from the networks may be toxic to cells.

Upon implantation of these degradable networks in the body, the accumulation of (non-degradable) kinetic chains of high molecular weight may be prevented in this way. To determine exactly which PMAA chain lengths are still excreted by the kidneys, in vivo studies with labeled PMAA are required.

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