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Photopolymers for Rapid Prototyping of Soluble Mold Materials and Molding of Cellular Biomaterials

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Summary. To substitute cross-linked photopolymers in rapid prototyping of mold materials and therefore extend the range of materials which can be casted, organo-soluble photopolymers were developed. Branched bisalkylacrylamides were suitable as base component for such formulations, due to their high reactivity, good mechanical properties, and excellent solubility of the formed polymers. These molding materials were used to prepare cellular biocompatible materials which could be used as bone replacement materials. Biocompatible crosslinkers based on methacrylates from hydrolyzed gelatine or lactic acid ethyleneglycol blockcopolymers and commercially available reactive diluents are the base components of such a formulation. Biocompatibility was investigated by osteoblast-like cells. Cellular biocompatible parts were obtained by thermal polymerization in soluble mould materials prepared by 3D-photoshaping.

Keywords. Polymerization; Photochemistry; Rapid prototyping; Acrylates; Biophotopolymers.

Introduction

In the case of bone fraction or bone tumor synthetic replacements are used to serve as support for the period of time that the host organism needs to regenerate the original structure. Stereolithography is a computerized fabrication technique that offers the possibility to produce such highly complex 3-dimensional replacement materials by photopolymerization. Direct fabrication of cellular structures out of photocurable liquid acrylate-based formulations that cure by radical polymerization is possible but extensive tuning of every formulation is necessary. Indirect

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approaches using sacrificial molds prepared by rapid prototyping (RP) provide a suitable alternative. Thermosetting biopolymers are then filled into the molds which are removed after curing [1, 2].

Commercial RP resins lead to highly cross-linked polymers, which can only be removed by burning at higher temperatures. This limits the range of casting materials and molding of biopolymers is not possible. In the first part of the paper, we aim at the development of a soluble photopolymer formulation which is suitable as mold material for thermosetting biopolymers.

Biopolymers which are currently in use are based on polyesters such as poly(ε-caprolactone) or poly(α-hydroxyacids) such as copolymers of lactic and glycolic acid. These materials are not suitable for the fabrication of bone replacement materials by direct or indirect methods using stereolithography because they can only be processed by different melt or solution techniques. Only few papers have been published that focus on radically polymerizable monomers that lead to biocompatible and biodegradable polymers. Examples are poly(propylene fumarate) [3], block copolymers consisting of oligoethylene glycol and lactic acid terminated with methacrylic moieties [4], a polymerizable lysine based monomer [5], or (meth)acrylate modified oligopeptides [6]. All these oligomers are solid and therefore not useful without reactive diluent.

In our present project we aim at the development of such acrylate based formulations for cellular implants, which can be printed directly by stereolithography or are suitable for thermal curing in molds. To tune the material properties regarding processability, biocompatibility, and mechanical and degradation properties several components such as crosslinkers, reactive diluents, fillers, and photoinitiators have to be considered. To overcome the problem of uncontrolled hydrolytic cleavage of ester containing monomers, biodegradability is preferentially introduced by multifunctional crosslinkers with amide bonds that can be cleaved enzymatically *in vivo*. Processing properties of the formulation and network density of the polymer can be tuned by reactive diluents. In the second part of this paper we discuss the first results on biocompatibility of selected crosslinkers and reactive diluents. Furthermore, a first cellular and biocompatible structure was obtained by molding techniques from organo-soluble photopolymers.

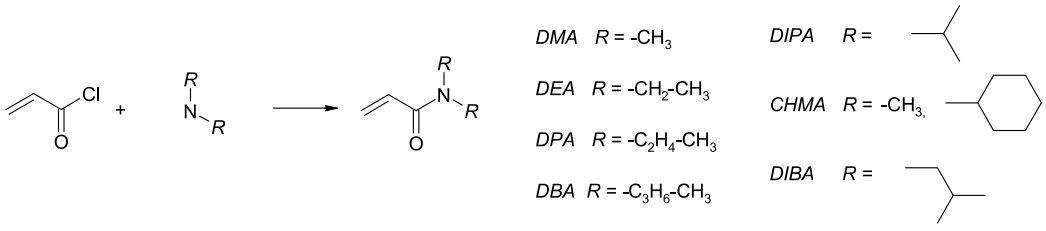
Results and Discussion

Organosoluble Polymers for Rapid Prototyping

Selection and Synthesis of Monomers

For the development of organo-soluble photopolymers it was necessary to find a suitable highly reactive monofunctional base monomer that forms a soluble hydrophobic polymer. Moreover, low shrinkage during polymerization, little swelling of polymer in monomer, and good mechanical properties of the polymer were important.

As *N*,*N*-dimethylacrylamide (*DMA*) is a highly reactive monomer and was used in formulations that lead to water-soluble photopolymers [1], different dialkylacrylamides with increasing side chain length were considered as suitable components.



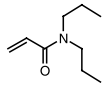
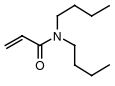
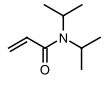
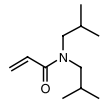
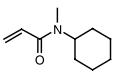
Scheme 1. Synthesis of dialkylacrylamides

With longer side chains of the monomers, hydrophobicity and therefore organosolubility of the formed polymer increases (*DMA* < *DEA* < *DPA* < *DBA*; Scheme 1). Branched or cyclic side chains should improve the mechanical properties (*DIPA*, *CHMA*, *DIBA*). With the exception of *DMA*, which is commercially available, the investigated monomers were synthesized by reaction of 1 equivalent of acrylic acid chloride with 2 equivalents of dialkylamine (Scheme 1) as described in Ref. [7].

Photo-DSC

The photoreactivity of the different dialkylacrylamides was investigated by photo-DSC as described earlier [1]. Therefore, 0.5 *wt%* of a bisacylphosphinoxide based photoinitiator (*Irgacure 819*) was used. Absorption in the visible range of the spectrum was essential, because the stereolithographic process is based on the DLP (digital light processing) principle. The time to reach the maximum polymerization

Table 1. Photo-DSC data of dialkylacrylamides

Monomer	Abbr.	t_{max} / s	*DBC* / %	R_P / mol l^{-1} s^{-1}
	*DMA*	10.8	78	0.46
	*DEA*	10.2	83	0.35
	*DPA*	9.6	82	0.28
	*DBA*	8.4	84	0.26
	*DIPA*	18.0	63	0.11
	*DIBA*	6.6	75	0.23
	*CHMA*	7.2	72	0.28

heat (t_{max}), the double bond conversion (*DBC*), and the rate of polymerization (R_p) were determined. Generally, most acrylamides showed sufficiently high reactivity for stereolithographic applications (Table 1).

Generally, the R_P decreases with increasing molecular weight. With the exception of the sterically hindered *DIPA* with very low reactivity, the branched monomers showed a higher reactivity expressed by t_{max}. Acrylamides with linear side chains had similar values for the *DBC* whereas the *DBC* of the branched acrylamides was significantly lower.

Properties of the Polymers

In addition to the photopolymerization activity of the monomer, mechanical properties and solubility of the polymer were also of interest. Test specimens ($60 \times 10 \times 3\,mm^3$) were prepared by irradiation of the formulation in a suitable silicone mold using 0.5 *wt% Irgacure 819* as photoinitiator. Shrinkage should be low to obtain good feature resolution during the RP process and avoid warping of the final part. Calculation of the shrinkage was done by comparison of monomer densities given from the literature and polymer densities obtained from the buoyancy in glycerol. Swelling of polymer in monomer during RP processing should be minimized in order to obtain hard RP parts with suitable feature resolution. The degree of swelling was measured by the gain of weight of a polymer in its monomer (50°C, 24 h). Shore hardness *D* was used to characterize the mechanical properties. Solubility, one of the most important properties, was determined by stirring a specimen of defined form and size in *THF* at 50°C and determination of the time needed for entire dissolution.

As expected, the shrinkage during polymerization decreases with increasing molecular weight of the monomers (Table 2). In case of polymers of *DEA*, *DPA*, and *DIBA*, swelling decreased with increasing side chain length of the monomers. But no exact conclusion can be drawn because some test specimens were highly swollen and sticky that they could not be evaluated. Monomers with short linear side chains formed hard polymers. As expected, hardness significantly decreased

Table 2. Properties of homopolymers and copolymers

	Homopolymer				Copolymer with 10 *wt% MSA*		
Monomer	Shrinkage %	Swelling in monomer %	Shore hardness *D*	Solubility min[a]	Swelling in monomer %	Shore hardness *D*	Solubility min[b]
DMA	13.5	[c]	79	55	238	80	90
DEA	11.6	599	70	swelling	166	77	swelling
DPA	9.6	377	61	80	88	70	30
DBA	8.0	[c]	26	40	69	58	60
DIPA	9.0	268	70	10	48	74	15
CHMA	7.4	59	83	85	16	85	60
DIBA	7.8	122	73	190	19	77	65

[a] In *THF*; [b] in *THF*/*n*-butylamin ($v/v = 4/1$); [c] swelling exceeds 1000%

with growing linear side chains, but highly branched and cyclic side chains gave again hard polymers.

In previous investigations methacrylic anhydride (*MSA*) turned out to be a suitable alkaline cleavable crosslinker for water dissolvable formulations [1]. Improved mechanical properties and feature resolution as well as decreased swelling of the polymer in the monomer have been obtained. Therefore, *MSA* was also used as a cleavable crosslinker for organo-soluble formulations. In an appropriate organic solvent like *THF* it can easily be cleaved by *n*-butylamine. Table 2 shows some properties of the homopolymers in comparison with their copolymers with 10 *wt% MSA*.

Influence of MSA Content on Copolymers of CHMA and DIBA

CHMA and *DIBA* were of special interest for further investigations because their homopolymers showed good mechanical properties and low swelling in the monomer. Therefore the optimum concentration of *MSA* was evaluated.

As expected, swelling of the polymer in the monomer decreased by addition of *MSA* and reached a minimum at 15 to 20 *wt%* of the crosslinker (Fig. 1). By using 5 to 15 *wt% MSA* we obtained a significant increase of hardness for the *DIBA*-copolymer. The hardness of *CHMA* had already a level similar to *MSA*. Surprisingly, crosslinking with 10–20 *wt%* of *MSA* improved the dissolution behavior of the copolymers in a mixture of *THF*/*n*-butylamine (4/1). The reason for that might be the formation of butylamide units with excellent solubility.

Optimization of Formulation

For further experiments *DIBA* was chosen as suitable base monomer because of its best overall performance. To tune the formulation with respect to the resolution, mechanical properties, solubility, and shrinkage a series of experiments with different co-monomers was carried out. Diacetone acrylamide (*DAA*) was found to be a highly reactive and well soluble comonomer. Besides showing improved solubility of the mold material, *DAA* reduced diffusion into the silicone-vat. 10 to 15 *wt%* of crosslinker *MSA* proved to be effective for improving mechanical properties and to reduce swelling of the polymer in the monomer. To find the optimum PI concentration, photo-DSC experiments with 0.1 to 3.0 *wt%* of PI in *DIBA* were carried out. 1 to 3 *wt%* PI were sufficient for high reactivity (t_{max}) and for almost complete double bond conversion (*DBC*).

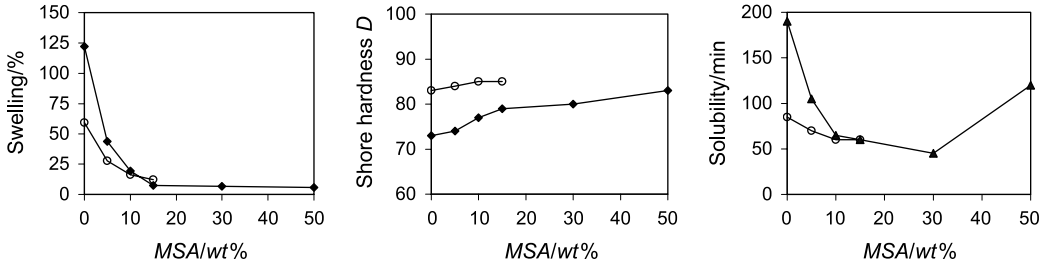


Fig. 1. Properties of *DIBA* (--◆--) and *CHMA* (--○--) with different *MSA* content

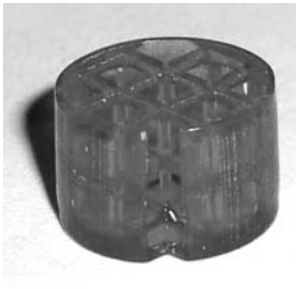


Fig. 2. Cellular structure obtained by stereolithography

Cellulose acetate butyrate was used to increase the viscosity of the resin, which is important for good processing properties by RP and to decrease radical diffusion. It was found that the optimum formulation consists of 72 *wt% DIBA*, 11 *wt% MSA*, 10 *wt% DAA*, 4 *wt%* cellulose acetate butyrate, and 3.0 *wt%* PI *Irgacure 819*. Figure 2 shows a cellular structure obtained by rapid prototyping using stereolithography.

Biopolymers

Reactive Diluents

Different commercially available reactive diluents either monofunctional or multifunctional (Fig. 3) were investigated concerning biocompatibility. Selection of

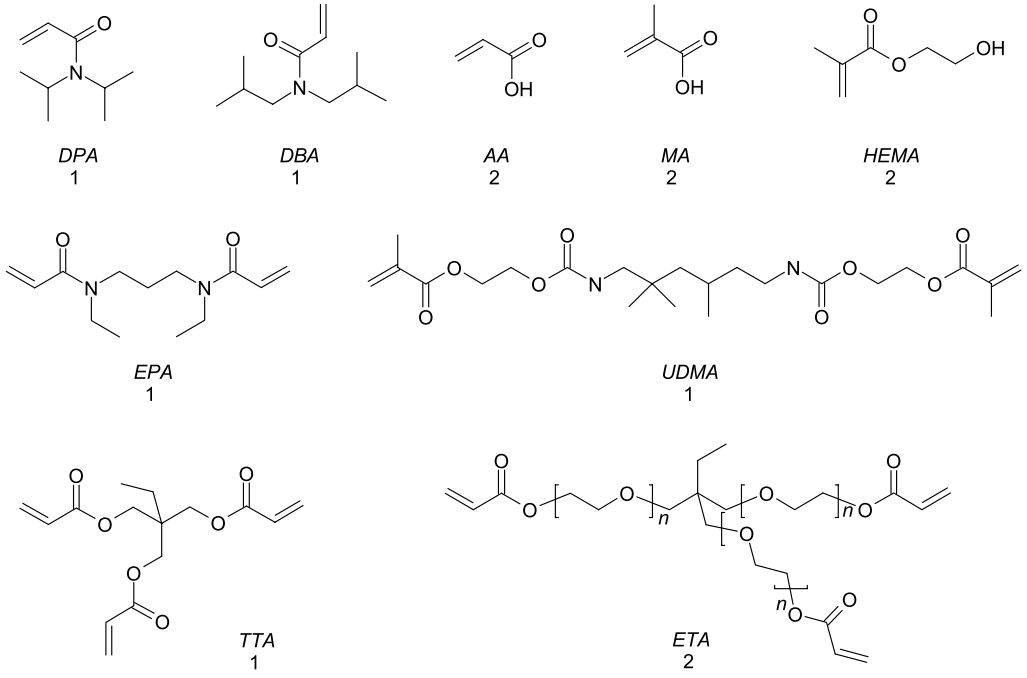


Fig. 3. Structure of reactive diluents and biocompatibility (1 . . . good biocompatibility and cell adhesion; 2 . . . good biocompatibility, less cell adhesion; 3 . . . no biocompatibility)

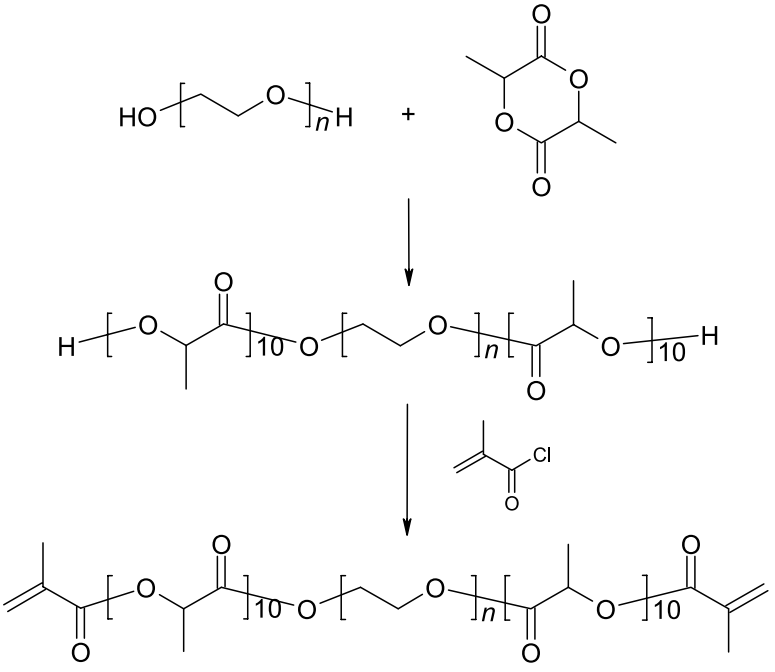
monomers was carried out under consideration of different functional groups (COOH, OH, oligoethylene glycol, acrylates, and methacrylates). Additionally, hydrolysable esters based on (meth)acrylates and more stable acrylamides were investigated.

For the determination of the biocompatibility test specimens (diameter 7 mm, thickness 3 mm) were made in a silicone mold from the monomers with 1 *wt%* of an equimolar mixture of camphorquinone (*CQ*) and *N*,*N*-dimethylaminobenzoic acid ethylester (*DMAB*), which is known to be a biocompatible photoinitiating system [4]. Monofunctional monomers were used with 20 *wt%* of a crosslinker to avoid swelling and dissolution. *EPA* was chosen because first results indicated excellent biocompatibility. Photocuring was performed with a high-pressure mercury lamp (1000 W, distance 15 cm) under nitrogen atmosphere for several minutes. After extraction with different organic solvents ($CHCl_3$, *Me*OH, *Et*OH), phosphate buffered saline (*PBS*), and water in an ultrasonic bath and sterilization of the test specimens, osteoblast like cells (*MC3T3-E1*) were seeded on the polymers. If the cells could adhere and survive, the material was stated to be biocompatible.

Figure 3 shows the first results of the biocompatibility tests. Both test specimens made from *AA* and *MA*, each with 20 *wt% EPA* as crosslinker, were not stable in cell culture and could not be tested. Therefore, new test specimens with 80% crosslinker *EPA* and 20% monofunctional monomer were prepared. Polymers of *AA* and *MA* can be considered as degradation products and therefore it is important that they are not cytotoxic. In our experiments polymers made from *HEMA* showed only average biocompatibility although their application is often described in the literature [8]. The reason might be the release of ethylene glycol, whose metabolite oxalate is cytotoxic [9]. Polymers from acrylamides *DBA* and *DPA* have not been considered as biopolymers so far. Herein, excellent cell adhesion was found.

In the case of difunctional monomers, *EPA* [10] and *UDMA* are well known from dental applications, giving polymers with outstanding mechanical properties. All multifunctional reactive diluents, especially *UDMA*, *EPA* (Fig. 4), and *TTA*, are suitable for a photopolymerizable biopolymer formulation. The difference in cell adhesion on the resins made from multifunctional monomers *TTA* and *ETA*, can be addressed to the poly(ethylene glycol) spacer, which is known to be resistant against the attachment of cells.

Fig. 4. *MC3T3-E1* osteoblast like cells adhere and proliferate on p*EPA*



Scheme 2. Synthesis of crosslinkers based on lactic acid

Crosslinkers

Crosslinkers with ester or amide groups were considered which degrade by hydrolytic or enzymatic mechanisms. Polymers with amide linkages tend to degrade more slowly and therefore provide more durable mechanical support for the healing bone, and are thus preferred as crosslinker in a biodegradable bone replacement material.

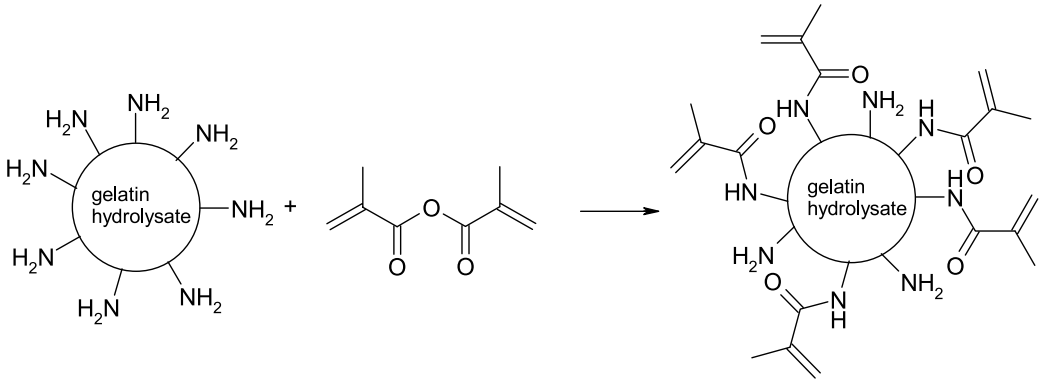
Similar to *Anseth et al*. [4] block copolymers of di(ethylene glycol) (*E2-L20-M*) and poly(ethylene glycol) (*E8 L20-M*) with lactic acid were prepared by ring opening polymerization and subsequent reaction with methacryloyl chloride (Scheme 2).

Multifunctional macromers with amide linkages (*GHM*) were prepared from gelatin hydrolysate, by modification of lysine groups (0.38 mmol/g) with methacrylic anhydride (Scheme 3) [11].

For determination of the biocompatibility, test specimens were made as described for the reactive diluents. In the case of *GHM*, which is solid, test specimens were made with 80% of reactive diluent *EPA*. Crosslinkers based on *GHM* and *E2-L20-M* showed very good biocompatibility. Fewer cells could adhere on *E8-L20-M*, which relies on the longer *PEG* chain that is known to be a poor substrate for cell adhesion [12].

3D Molding

First 3-dimensional cellular structures were prepared by molding (Fig. 5). An organo-soluble sacrificial mold was made by rapid prototyping (1–3) and filled (4)



Scheme 3. Synthesis of *GHM*

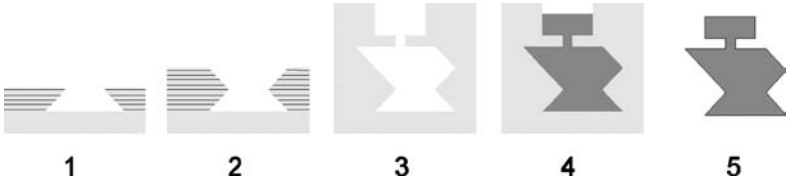


Fig. 5. Rapid prototyping of mold material and molding technique

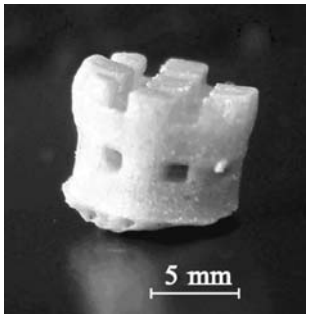


Fig. 6. Cellular structure prepared by molding

with a biocompatible monomer formulation consisting of 20 *wt%* crosslinker *E2-L20-M*, 80 *wt%* reactive diluent *EPA*, and 1 *wt%* thermal initiator (benzoyl peroxide and *DMAB*). Curing was carried out at 65°C. The sacrificial mold was then removed (5) by dissolving in *THF*/*n*-butylamine. The resulting cellular structure is shown in Fig. 6.

Experimental

All reagents were purchased from Sigma-Aldrich and were used as received. Photoinitiator *Irgacure 819* was received from Ciba SC as a gift. All solvents were dried and purified by standard laboratory methods. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-E-200 FT-NMR-spectrometer.

Photo-DSC experiments, preparation and testing of test specimens, and stereolithography were carried out as recently described [1].

Dialkylacrylamides (DEA, DPA, DBA, DIPA, CHMA, DIBA)

The dialkylacrylamides where synthesized according to the work of *Maier* [13] with diethyl ether as solvent. ^{1}H NMR spectra of *DEA*, *DPA*, *DBA*, and *DIPA*, were found to be identical with those described in the Refs. [13–15].

CHMA [16]: bp 90.0–91.0°C (0.25 mbar); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.56$ (dd, 1H, CH_2=C*H*–), 6.25 (d, 1H, CH_2=CH–), 5.63 (d, 1H, CH_2=CH–), 4.65–4.30 (m, 0.5H, $H^1{}_{cycl}$), 3.90–3.45 (m, 0.5H, $H^1{}_{cycl}$), 3.00–2.75 (s, 3H, N–CH_3), 1.95–0.95 (m, 10H, $H^{2,3,4,5,6}{}_{cycl}$) ppm.

DIBA: bp 78.5–80.0°C (0.05 mbar) (Ref. [17] 103–105°C (1 Torr)); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 9.50$–7.50 (m, 12H, –CH–(CH_3)$_2$), 6.54 (dd, 1H, CH_2=C*H*–), 6.27 (dd, 1H, CH_2=CH–), 5.59 (dd, 1H, CH_2=CH–), 3.25–3.00 (m, 4H, –N–CH_2–CH–), 2.10–1.70 (m, 2H, –CH_2–C*H*–(CH_3)$_2$) ppm.

Methacrylated Ethylene Glycol Lactic Acid Block Copolymers (E2-L20-M, E8-L20-M)

Synthesis of *E2-L20-M* was carried out as described. Spectral data were in agreement with reported data [4]. Preparation of *E8-L20-M* was done in a similar manner.

E8-L20-M: ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 6.18$ (s, 2H, *H*CH=C), 5.62 (s, 2H, HC*H*=C), 5.13 (m, 20H, CH–CO), 4.25 (m, 4H, CH_2–O), 3.65 (s, 28H, CH_2–O), 1.94 (s, 6H, CH_3–C=C), 1.55 (m, 60H, CH_3–C–O) ppm; ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 169.51$ (C=O), 169.27 (C=O), 135.40 (*C*=CH_2), 126.59 (C=*C*H_2), 68.89 (CH_2), 68.60 (*C*H–CH_3), 18.05 (CH_3), 16.54 (CH–*C*H_3) ppm; IR (KBr): $\bar{\nu} = 2995$, 2945, 2881, 1755, 1636, 1453, 1382, 1363, 1272, 1190, 1132, 1096, 1063 cm^{-1}.

Methacrylated Gelatin Hydrolysate (GHM)

For the synthesis of *GHM*, 1 g (9.4 mmol lysin) of gelatin hydrolysate was dissolved in 30 cm^3 of phosphate buffered saline (18.3 g $Na_2HPO_4 * 2H_2O$, 2 g KH_2PO_4, 2 g KCl, 80 g NaCl, 1000 cm^3 H_2O) at 40°C. Methacrylic anhydride (1 g, 6.7 mmol) was added and the reaction mixture was stirred for 4 h. The resulting product was purified by dialysis (molecular weight cut off 3500) against distilled water giving 0.9 g of *GHM*. An average degree of derivatization of 50% (^{1}H NMR: $\delta = 5.75$ (d, 1H, =CH_2), 5.46 (d, 1H, =CH_2)) based on lysine units was obtained.

Acknowledgements

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References

[1] Liska R, Schwager F, Maier C, Cano-Vives R, Stampfl J (2005) J Appl Polym Sci **97**: 2286
[2] Woesz A, Rumpler M, Manjubala I, Pilz C, Varga F, Stampfl J, Fratzl P (2005) Mater Res Soc Symp Proc **874**: L7.9.1
[3] Cooke M, Fisher JP, Dean D, Rimnac C, Mikos A (2003) J Biomed Mater – Part B Appl Biomater **64**: 65
[4] Davis KA, Burdick JA, Anseth KS (2003) Biomaterials **24**: 2485
[5] Müh E, Zimmermann J, Kneser U, Marquardt J, Mülhaupt R, Stark B (2002) Biomaterials **23**: 2849
[6] Zimmermann J, Bittner K, Stark B, Mülhaupt R (2002) Biomaterials **23**: 2127
[7] Maier L (1973) Helvetia Chimica Acta **56**: 1252
[8] Montheard JP, Chatzopoulus M, Chappard D (1992) J Macromol Sci, Rev Macrom Chem Phys **C32**: 1

[9] Guo C, McMartin KE (2005) Toxicology **208**: 347
[10] Moszner N, Zeuner F, Angermann J, Fischer UK, Rheinberger V (2003) Macromol Mater Eng **288**: 621
[11] Schacht E (1998) WO Pat Appl WO 98/55161
[12] Hern DL, Hubbell JA (1998) J Biomed Mat Res **39**: 266
[13] Maier L (1973) Helvetia Chimica Acta **56**: 1252
[14] Iizawa T, Ono H, Okatome K, Sato Y (1994) Polym J **26**: 977
[15] Sheng S, Zhou W, Sang XY, Liu XL, Wang QY (2004) J Chem Res **9**: 626
[16] Griffith M, Carlsson DJ, Li F (2004) WO 2004014969
[17] Kogyo AK (1970) Fr 2046122