Biomaterials Based on Low Cytotoxic Vinyl Esters for Bone Replacement Application

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Received 29 June 2011; accepted 5 August 2011; published online 7 September 2011
DOI: 10.1002/pola.24933

ABSTRACT: In recent days, additive manufacturing technologies (AMT) based on photopolymerization have also found application in tissue engineering. Although acrylates and methacrylates have excellent photoreactivity and afford photopolymers with good mechanical properties, their cytotoxicity and degradation products disqualify them from medical use. Within this work, (meth)acrylate-based monomers were replaced by vinyl esters with exceptional low cytotoxicity. The main focus of this paper lies on the determination of the photoreactivity and investigations concerning mechanical properties and degradation behavior of the new materials. Tested monomers provide sufficient photoreactivity for processing by AMT. Mechanical properties similar to natural bone could be obtained by adding suitable fillers like hydroxylapatite (HA). The right ratio of hydrophobic and hydrophilic monomers allows the tuning of the degradation behavior. Finally, with the optimum formulation, cellular 3D structures were built using digital light processing. © 2011 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 49: 4927–4934, 2011

KEYWORDS: additive manufacturing technology; biocompatibility; biomaterials; photopolymerization; vinyl ester

INTRODUCTION The research area of bone replacement materials is of tremendous interest for our society as the life expectancy increases. Therefore, higher number of injuries and diseases indicates a need for tissue replacement materials that serve as temporary replacement until healing is completed. Material applied as bone replacement requires certain properties such as biocompatibility, porosity, surface and mechanical properties, osteoinductivity, and controlled biodegradability.[1] Most bioresorbable materials currently used in tissue engineering are based on polycondensates with cleavable ester functionalities from glycolic acid and/or lactic acid.[2] Mechanical properties are mainly determined by the polymer backbone, the crystallinity, and the ratio of D- and L-units in the case of poly(lactic acid) (PLA). PLA is the most frequently used biocompatible and biodegradable polymer. Unfortunately, there are several disadvantages associated with these types of materials: Under hydrolytic degradation, PLA forms acidic groups that induce autocatalytic bulk erosion, leading to a fast loss of mechanical properties. Local decrease of pH value and abrupt release of lactic acid can cause inflammation reactions or in the worst case tissue necrosis.[3,4] Mechanical properties and the degradation rate cannot be easily tuned. Moreover, these types of polymers could only be processed by a limited number of techniques such as evaporation of solvents from polymer solutions (e.g., electrospinning) or melting of polymers (e.g., injection molding, extrusion, or fused deposition modeling).[5] These processing techniques have some major disadvantages for the application in the field of tissue engineering, for example, insufficient feature resolution and the inability to create arbitrary cellular structures, and are therefore not ideally suitable to prepare cellular biomaterials.

Litography-based additive manufacturing technology (AMT), which is based on the photopolymerization of (meth)acrylate-based monomers, has become available for use in the tissue engineering field.[6] These methodologies are computerized fabrication techniques that can produce highly complex three-dimensional (3D) physical objects using data generated by computer assisted design systems, computer-based medical imaging, digitizers, and other data makers. AMT techniques use the underlying concept of layered manufacturing, in which 3D objects made of crosslinked polymers are fabricated layer-by-layer by radical polymerization. Customized design, computer-controlled fabrications, and anisotropic scaffold microstructures are their main advantages for the

use in tissue engineering. Recent work on stereolithography has shown that these techniques are able to shape cellular materials with wall thicknesses well below 200 μm, which is comparable to the average strut diameter in trabecular bone.[7]

Polymers based on acrylates and methacrylates surely have several advantages over other thermoplast-like polymers, especially the processability and the tunable mechanical properties.[8,9] Unfortunately, there are also some major disadvantages: Skin irritancy or toxicity of some monomers currently limits their application in the biomedical field. These drawbacks can be mainly addressed to the reactivity of the acrylate double bond towards Michael Addition reactions with amino or thiol groups of proteins. Nonreacted (meth)acrylic groups give harmful (meth)acrylic acid under degradation. Degradation of crosslinked (meth)acrylates forms high molecular poly(meth)acrylic acid that cannot be transported within the human body. This can lead to a local decrease of pH and, therefore, tissue necrosis might occur in the worst case.

Therefore, a new concept of biodegradable polymers was recently developed based on vinyl ester-based monomers.[10] Poly(vinyl ester)s form low-toxic poly(vinyl alcohol) as degradation product, which has been approved by the Food and Drug Administration due to well-known biocompatibility.

This article is devoted to the study of a promising formulation consisting of two vinyl ester monomers, namely hexanedioic acid divinyl ester (**4VE**), and 3,6,9-trioxaundecanedioic acid divinyl ester (**DEVE**) (Scheme 1). The choice of vinyl ester monomers is based on a previous study[10] where it was shown that vinyl esters have excellent photoreactivity between those of acrylates and methacrylates. As **4VE** is rather hydrophobic, **DEVE** was designed as a hydrophilic comonomer in order to tune easily the degradation behavior of a formed network. The objectives are the determination of the photoreactivity by photo-DSC as it is a prerequisite for processing by AMT. To get an overview on the reactivity of the new monomers, acrylate **4AC** and methacrylate **4MA** were investigated as reference monomers (Scheme 1). Furthermore, mechanical properties were screened by nanoindentation. To improve the mechanical properties and to promote osteogenesis, HA seems to be reasonable as filler. Finally, the optimized formulation was used for 3D printing using digital light processing (DLP) and the degradation behavior of the polymer specimens was studied.

RESULTS AND DISCUSSION

Synthesis

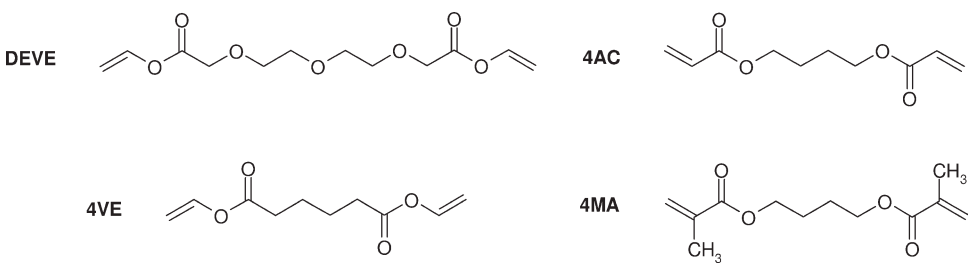
In literature, vinyl acetate is used in most cases as a vinyl group donating agent in the presence of Hg-(II)-acetate[11] or a Pd-(II)-salt[12,13] catalysts for the synthesis of vinyl esters from simple carboxylic acids. Generally, Pd-(II)-salts are rather expensive and the method using toxic Hg-(II)-salts does not tolerate nucleophilic groups due to the deactivation of the intermediately formed Hg-(I)-salt.

In order to obtain the oligo(ethylene glycol)-based vinyl esters, a carboxylic acid terminated precursor is required. The simplest commercially available precursor is the 3,6,9-trioxaundecanedioic acid. Transesterification with vinyl acetate using $Hg(OAc)_2$ as catalyst[11] was unsuccessful. However, transesterification with vinyl acetate using more expensive $Pd(OAc)_2$ catalyst[12] gave **DEVE** in 33% yield after kugelrohr distillation [Scheme 2(a)].

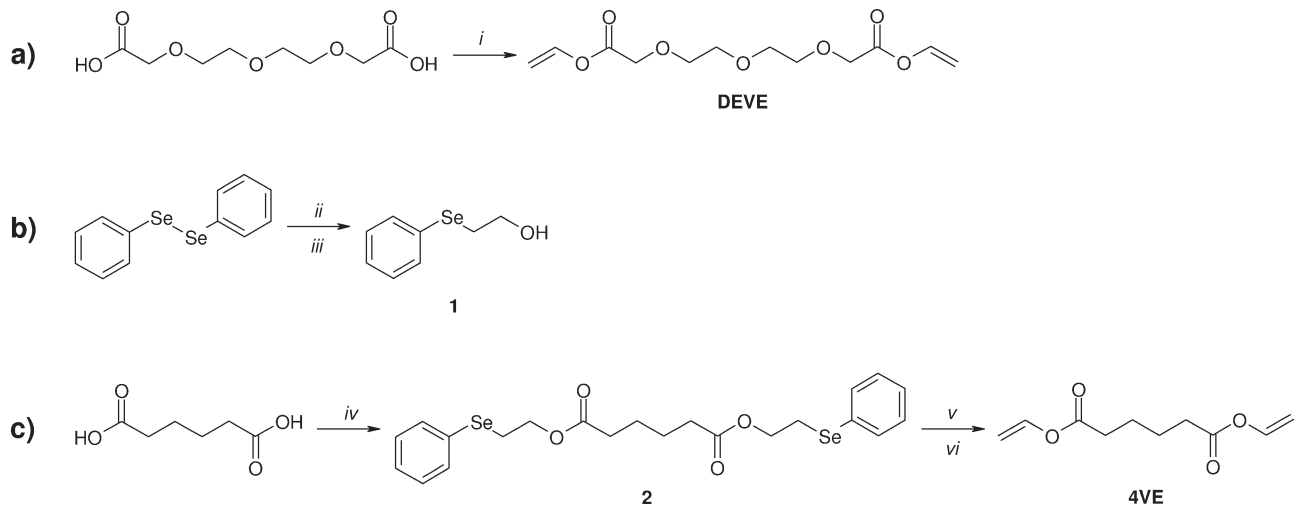
To avoid residual traces of toxic Hg-(II)-salts and use of expensive Pd catalyst, we wanted to verify the efficiency of an alternative three-step synthetic route developed by Weinhouse et al[14] for the preparation of vinyl ester **4VE** [Scheme 2(b,c)]. Phenylselenium ethanol (**1**) was prepared according to literature:[14] Diphenyl diselenide was reduced with $NaBH_4$ in anhydrous ethanol and formed phenyl selenide intermediate was reacted with an equimolar amount of 2-chloroethanol. Purification by column chromatography afforded **1** in 83% yield. This reagent was reacted with hexanedioic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) and of 4-dimethylaminopyridine affording intermediate **2** in 63% yield after chromatographic separation. Then, intermediate **2** was oxidized at the Se-atom with H_2O_2 in THF followed by a subsequent elimination of a phenylselenium derivative in the presence of a sterically hindered base diisopropylamine under reflux. **4VE** was obtained in 60% yield after purification by column chromatography.

Photoreactivity

Photoreactivity is a very important prerequisite for the selection of new monomers to be structured by the means of photolithographic AMT techniques. Double bond conversion (DBC) is a key factor for the practical application: Low values lead to a significant amount of leachable monomers, and also reduced mechanical properties have to be expected. The photopolymerization of the vinyl ester monomers and their corresponding (meth)acrylate references was monitored by



SCHEME 1 Vinyl ester monomers and (meth)acrylate references.



SCHEME 2 Synthesis of **DEVE** and **4VE**. Reaction conditions: (i) vinyl acetate, $Pd(OAc)_2$, KOH; (ii) $NaBH_4$, EtOH; (iii) $ClCH_2CH_2OH$, EtOH; (iv) **1**, EDC.HCl, 4-dimethylaminopyridine, DMF; (v) H_2O_2, $CHCl_3$; (vi) $NH(iPr)_2$, $CHCl_3$.

means of photo-DSC to investigate the polymerization kinetics and the conversion of the double bonds as a function of exposure time (Fig. 1). The experiments were done using 5 wt % of Irgacure® 819 as photoinitiator as this compound has extraordinary high photoreactivity and no adverse effects in cell culture experiments of photopolymerized test specimens were found recently.[15]

The rate of polymerization (R_p) of the different divinyl monomers having the same spacer length between the polymerizable groups decreased in the following order: acrylate **4AC** > vinyl ester **4VE** > methacrylate **4MA**. Also, the DBCs decreased in the same order giving conversions of 74, 66, and 60%, respectively. For biomedical applications such as bone cements, methacrylates are usually applied because of the lower cytoxicity. Based on the photoreactivity results, vinyl esters are a suitable alternative.

To enhance the hydrophilicity of the formulations and therefore, an easier access for water and hydrolysis (*vide infra*), the formulations consisting of hydrophobic **4VE** with hydrophilic **DEVE** were tested. The influence of the hydrophilic crosslinker on the rather high photoreactivity of **4VE** was investigated.

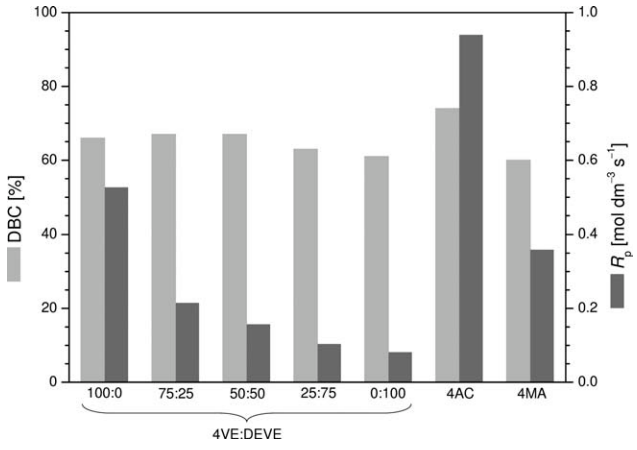


FIGURE 1 Photoreactivity of **4VE**:**DEVE** formulations compared to **4AC** and **4MA**.

Expectably, the values for R_p decreased with increasing **DEVE** content due to the higher molecular weight of **DEVE** compared to **4VE**. Additionally, oligo(ethylene glycol) units are more sensitive to hydrogen abstraction reactions by the highly reactive vinyl ester radicals, forming radicals with significantly lower reactivity toward relatively low-reactive vinyl ester double bonds.

Despite the lower photoreactivity that has to be accepted in conjunction with the use of hydrophilic **DEVE**, the curing quality of the mixtures, expressed by similar percentages for DBC (>60%), only slightly decreases and is of the same order of magnitude as those of methacrylates.

Nevertheless, there is a major advantage of residual vinyl ester group compared to methacrylates. While the latter one forms unwanted methacrylic acid under degradation, vinyl esters form acetaldehyde. The human body can easily convert this molecule to harmless acetic acid with acetaldehyde dehydrogenase.

Cytotoxicity

Photopolymers are known to potentially release residual monomers into the environment. For this reason, it is of significant interest how these chemicals influence bone cell proliferation and differentiation. These parameters were addressed by measuring cell viability. To compare the toxicity of the monomers, MC3T3-E1 cells were incubated with increasing concentrations of the monomers up to 10 mmol L^{-1} and approximated the concentration where half of the cells survived after 5 days. This concentration was denoted as LC_{50} (Table 1).

As expected, 50% of the cells did not survive even at the lowest used concentration (0.63 mmol L^{-1}) of the acrylates **4AC** as well as **4MA**, while **4MA** seemed less toxic than **4AC**.

TABLE 1 Cytotoxic Influence of the Monomers on Osteoblasts

Monomers	Viability (LC_{50}) (mmol L^{-1})
4VE	≥10
DEVE	6.4
4AC	<0.63
4MA	<0.63

Compared to the methacrylate **4MA** and especially to the acrylate **4AC** reference, the vinyl ester **DEVE** showed significantly better tolerance as demonstrated by cell viability. **4VE** even exceeded the test range. In summary, compared to the (meth)acrylic components, the vinyl esters clearly demonstrated one to two orders of magnitude lower toxicity on the osteoblasts.

Viscosity

Appropriate viscosity of the formulation is a prerequisite for suitableness for AMT. Low-viscosity materials allow the coating of reasonably thin layers and faster production of the parts. Viscosities were determined at 25 °C at a shear rate of 100 s^{-1}. **4VE** has lower viscosity than its (meth)acrylate analogues **4AC** and **4MA** (Fig. 2). All tested formulations **4VE**:**DEVE** consist of low molecular weight monomers and, therefore, the viscosities are two to three orders of magnitude lower than commercial stereolithography resins (≈1–5 Pa s). This allows the incorporation of various types of fillers, for example, HA (*vide infra*) or tricalcium phosphate.

Mechanical Properties

Nanoindentation allows a very fast and material-saving comparison of some basic mechanical properties of the investigated polymers. The indentation hardness and modulus were determined for selected formulations and compared to PLA and poly(ε-caprolactone) (PCL) (Fig. 3).

Polymer networks from **4VE** and **4AC** show comparable values of the indentation modulus and a higher value of the indentation hardness for **4VE**. This indicated the suitability of vinyl esters for photopolymerization-based AMT techniques. Higher values for the modulus and hardness of **4MA** are due to the additional methyl group.

Longer spacer length leads to a lower crosslink density of the polymers, which decreases the elastic modulus and hardness. As the difference of the molecular weights between **4VE** (198 g mol^{-1}) and **DEVE** (274 g mol^{-1}) is not very large, the values for the modulus and the hardness do not vary much with increasing **DEVE** content. Additionally, these results also confirm the similar curing quality expressed by the DBC. Consequently, the mechanical properties should play only a minor role in the material selection of the **4VE**:**DEVE** mixtures. Additionally, the hydrophilic **4VE**:**DEVE** 1:1 mixture was equipped with 20 wt % of the osteoconductive HA nanopowder (<200 nm particle size) as an inorganic filler. This formulation exhibits an increase of 30–40% in mechanical properties compared to the unfilled polymer. The moduli of all photopolymers were between the values of thermoplastic reference materials PLA and PCL.

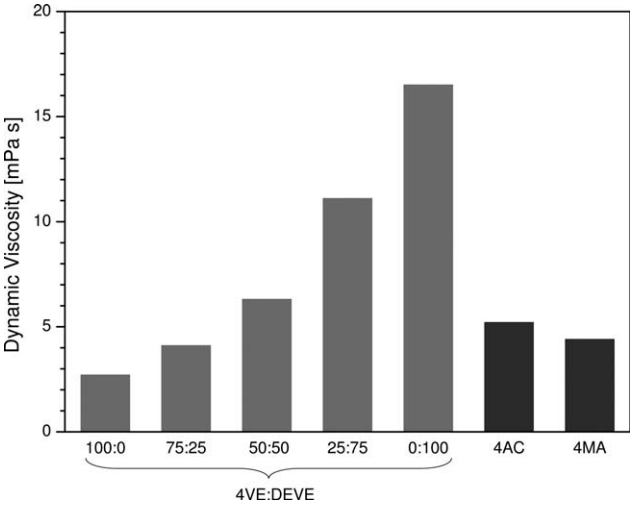


FIGURE 2 Viscosities of **4VE**:**DEVE** formulations.

Degradation Study

Degradation of the scaffold *in vivo* is a key feature that circumvents revision surgery after many years due to a failure of the implant or long-term adverse effects. Obviously, comparison of *in vivo* and *in vitro* degradation is complicated due to numerous factors influencing the degradation behavior in a living body. Nevertheless, *in vivo* degradation behavior of biomaterials is commonly simulated by hydrolysis *in vitro* under physiological conditions at pH 7.4 using phosphate buffered saline at 37 °C. As the degradation time can be up to several years,[16] accelerated hydrolytic study was done under basic conditions in a 1 M NaOH solution.[17] Degradation kinetics were compared to the state-of-the-art thermoplasts PLA and PCL [Fig. 4(a)] and time required to achieve a complete degradation was estimated [Fig. 4(b)].

Accessibility of water to the hydrolysable bond is the first prerequisite for degradation. As can be seen, the

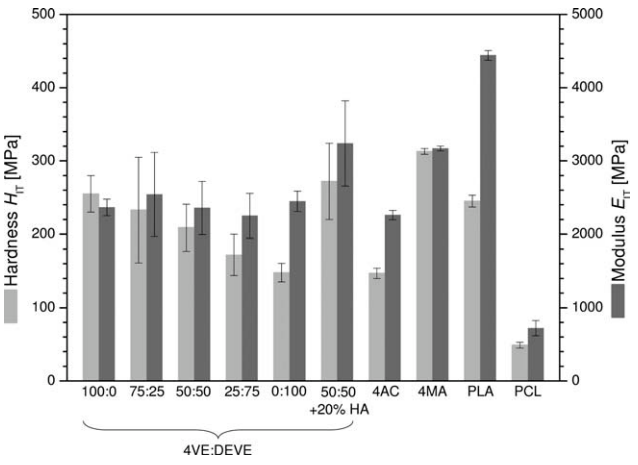


FIGURE 3 Indentation hardness and modulus.

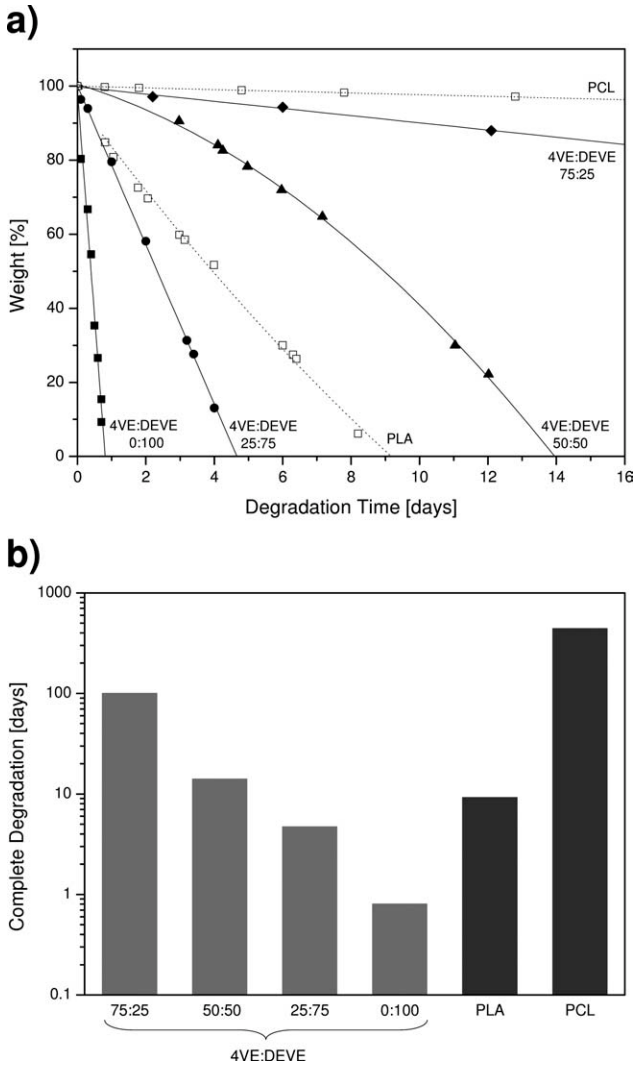


FIGURE 4 Degradation of photopolymers in 1 M NaOH at 37 °C: (a) weight loss with time, (b) time required to achieve a complete degradation.

hydrophilicity of the polymers has a very strong impact on the degradation behavior and allows tuning of the degradation rate. The homopolymer of **4VE** is almost nondegradable under test conditions; only a minor weight loss was detected within the testing time. On the other hand, the homopolymer of **DEVE** is completely degraded within 1 day. **4VE**:**DEVE** 0:100 and 25:75 exhibit an even faster degradation than PLA. From the curve progressions, it can be concluded that with increasing content of the hydrophobic **4VE** a comparatively strong increase of the time for complete degradation can be observed.

On *in vivo* degradation, thermoplastic references (PLA and PCL) lose their mechanical properties typically after 1–3 years. Based on the results on photoreactivity, mechanical properties, and degradation rate, formulation **4VE**:**DEVE** 50:50 seems to be an appropriate candidate for bone replacement application.

Digital Light Processing

DLP was chosen as AMT technique for 3D printing of scaffolds due to two main advantages: low material consumption and short production times. Its resolution is good enough to replicate human bone with its inner structure. Based on experiments in preliminary light penetration depth tests,[18] 3 wt % of Irgacure® 819 and 0.15 wt % CGL097 were used as photoinitiator and light absorber, respectively.

Formulation **4VE**:**DEVE** 50:50 was successfully printed [Fig. 5(a)] with a pore size and wall thickness of 500 μm that is within the optimum range. To improve the mechanical properties, formulation **4VE**:**DEVE** 50:50 filled with HA was printed by DLP, too. About 20% HA represents the highest possible concentration while maintaining a stable suspension without sedimentation. These low concentrations of filler already limited the light penetration depth making the use of additional light absorbers unnecessary for these formulations. However, as light scattering occurs at the particles, a decline of the *xy*-resolution was observed [Fig. 5(b)]. Higher concentrations might be possible using dispersing agents but light penetration still remains a challenge.

EXPERIMENTAL

Materials

All reagents, HA, and PCL ($M_n \approx 10^7$ Da) were purchased from Sigma-Aldrich, Fluka and TCI Europe and were used without further purification. PLA samples (BioSorb™ FX 2.0 Plate, 70L/30DL) were obtained from Linvatec Biomaterials. The photoinitiator Irgacure® 819 and light absorber CGL 097 were donated by Ciba SC. The solvents were dried and purified by standard laboratory methods.[19]

Characterization

NMR spectra (200 MHz for ^{1}H and 50 MHz for ^{13}C, respectively) were recorded with a Bruker AC 200 spectrometer, using $CDCl_3$ as a solvent (grade of deuteration of at least 99.5%). The solvent signal was used as a reference. ATR–FTIR measurements were carried out on a Biorad FTS 135 spectrophotometer with a Golden Gate MkII diamond ATR equipment (LOT). GC–MS runs were performed on a Thermo Scientific GC–MS DSQ II using a BGB 5 column (l = 30 m, d = 0.32 mm, 1.0 μm film, achiral) with the following temperature method (injection volume: 1 μL): 2 min at 80 °C, 20 °C min^{-1} until 280 °C, 2 min at 280 °C.

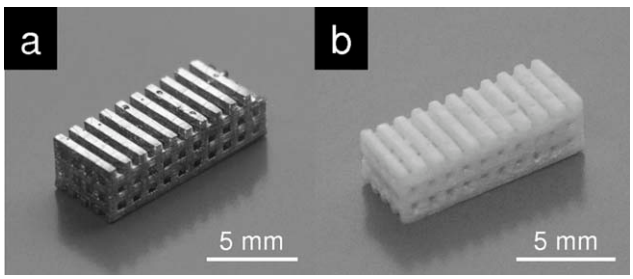


FIGURE 5 3D scaffolds from formulation **4VE**:**DEVE** 50:50 unfilled (a) and filled (b) with HA.

Synthesis of 3,6,9-Trioxaundecanedioic Acid Divinyl Ester (DEVE)

$Pd(OAc)_2$ (0.75 g, 3.34 mmol), KOH (0.38 g, 6.79 mmol), and 500 ppm of hydroquinone as inhibitor were added to a stirred solution of 3,6,9-trioxaundecanedioic acid (15.1 g, 68.7 mmol) in a large excess of vinyl acetate (175 g). The reaction mixture was heated to 60 °C for 48 h under argon atmosphere. The cooled solution was diluted with ethyl acetate and extracted with water. The organic layer was dried over sodium sulfate and concentrated. The product was purified by kugelrohr distillation at 140 °C and 0.3 mbar, yielding 6.2 g of **DEVE** as a colorless liquid.

Yield 33%. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 7.26 (dd, ^3J = 13.9 Hz, ^3J = 6.3 Hz, 2H, 2 × C$\underline{H}$=CH_2), 4.89 (dd, ^3J = 13.9 Hz, ^2J = 1.4 Hz, 2H, 2 × CH=C$\underline{H}$H), 4.60 (dd, ^3J = 6.3 Hz, ^2J = 1.6 Hz, 2H, 2 × CH=CH$\underline{H}$), 4.21 (s, 4H, 2 × CH_2—C=O), 3.80–3.64 (m, 8H, 2 × O—$\underline{CH_2}$—$\underline{CH_2}$—O), ^{13}C NMR (50 MHz, $CDCl_3$, δ, ppm): 167.7 (C=O), 140.5 (C$\underline{H}$=CH_2), 98.5 (CH=$\underline{CH_2}$), 71.0 (CH_2), 70.7 (CH_2), 68.2 (CH_2); IR (ATR, cm^{-1}): 2932, 2882, 1768, 1649, 1240, 1181, 1112, 949, 875; GC–MS (m/z): 253, 207, 191, 129, 87.

Synthesis of 2-(Phenylseleno)ethanol (1)

To a stirred solution of diphenyl diselenide (8.00 g, 25.6 mmol) in anhydrous ethanol (50 mL) cooled to 0 °C, a solution of $NaBH_4$ (2.92 g, 77.3 mmol) in anhydrous ethanol (50 mL) was added in small portions during 10 min under argon. After discoloration of the solution, 2-chloroethanol (1.77 g, 51.6 mmol) was added dropwise at 0 °C during 15 min. Then the mixture was refluxed for 3 h, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL) and extracted with 1 M HCl solution (50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate, 4:1) to afford 8.6 g of compound **1** as a yellowish oil.

Yield 83%. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 7.59–7.46 (m, 2H, H_{arom}), 7.32–7.22 (m, 3H, H_{arom}), 3.76 (t, ^3J = 6.3 Hz, 2H, O—CH_2), 3.08 (t, ^3J = 6.3 Hz, 2H, Se—CH_2), 2.34 (s, 1H, OH).

Synthesis of Hexanedioic Acid 2-(phenylseleno)Ethyl Ester (2)

EDC.HCl (1.77 g, 9.23 mmol), 4-dimethylaminopyridine (0.19 g, 1.57 mmol), and hexanedioic acid (0.50 g, 3.42 mmol) were added consecutively to a stirred solution of **1** (1.62 g, 8.03 mmol) in DMF (15 mL). The reaction mixture was stirred for 16 h at room temperature. Afterward, the solution was diluted with ethyl acetate (50 mL) and washed with 1 M HCl (2 × 25 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, petroleum ether/ethyl acetate, 4:1) to afford 1.1 g of compound **2** as a colorless oil.

Yield 63%. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 7.59–7.41 (m, 4H, H_{arom}), 7.33–7.18 (m, 6H, H_{arom}), 4.29 (t, ^3J = 7.2 Hz, 4H, 2 × O—CH_2), 3.07 (t, ^3J = 7.1 Hz, 4H, 2 × Se—CH_2), 2.33–2.19 (m, 4H, 2 × CH_2—C=O), 1.67–1.55 (m, 4H, 2 × CH_2); ^{13}C NMR (50 MHz, $CDCl_3$, δ, ppm): 173.0 (C=O), 132.9 (C_{arom}), 129.2 (C_{arom}), 129.1 (C_{ipso}), 127.3 (C_{arom}), 63.7 (O—$\underline{CH_2}$), 33.7 ($\underline{CH_2}$—C=O), 25.4 (CH_2), 24.2 (CH_2).

Synthesis of Hexanedioic Acid Divinyl Ester (4VE)

To a stirred solution of **2** (0.65 g, 1.27 mmol) in THF (5 mL) cooled in an ice bath, a 30% aqueous solution of H_2O_2 (0.45 g, 13.2 mmol) was added dropwise during 5 min keeping the temperature at 0 °C for additional 30 min. Then, the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with $CHCl_3$ (20 mL) and washed with water (15 mL) and brine (15 mL). The organic layer was dried over sodium sulfate and concentrated. The crude product was dissolved in $CHCl_3$ (15 mL), diisopropyl amine (0.50 g, 4.94 mmol) was added to the solution, and the reaction mixture was refluxed for 12 h. The cooled reaction mixture was concentrated, and the residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate, 5:1) to afford 0.15 g of **4VE** as a colorless liquid.

Yield 60%. ^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 7.26 (dd, ^3J = 13.9 Hz, ^3J = 6.3 Hz, 2H, 2 × —C$\underline{H}$=CH_2), 4.86 (dd, ^3J = 14.1 Hz; ^2J = 1.4 Hz, 2H, 2 × —CH=CH$\underline{H}$), 4.55 (dd, ^3J = 6.3 Hz, ^2J = 1.6 Hz, 2H, 2 × —CH=CH$\underline{H}$), 2.48–2.33 (m, 4H, 2 × —$\underline{CH_2}$—C=O), 1.79–1.62 (m, 4H, 2 × CH_2); ^{13}C NMR (50 MHz, $CDCl_3$, δ, ppm): 170.1 (C=O), 141.0 (—C$\underline{H}$=CH_2), 97.6 (—CH=$\underline{CH_2}$), 33.3 (—$\underline{CH_2}$—C=O), 23.8 (CH_2).

Photo-Differential Scanning Calorimetry

Photo-DSC experiments were conducted on a modified Netzsch DSC 204 F1 Phoenix. The measurements were carried out using 5 wt % of Irgacure® 819 as photoinitiator at 25 °C under nitrogen atmosphere. Photoreactivity of the monomers was tested by weighing about 10 mg of the sample with an accuracy of ±0.1 mg into an aluminum DSC pan, which was subsequently placed in the DSC chamber. The samples were purged with nitrogen for 5 min and irradiated with a filtered UV light (280–500 nm) from a double light guide (OmniCure® 2000) attached to the top of the DSC unit with a light intensity of 3 W cm^{-2} at the exit of the light guide. The samples were exposed to the light for at least 10 min, and the heat flow was recorded as a function of time. The double bond conversion (DBC) was calculated according to eq 1.

$$\mathrm{DBC} = \frac{M}{2}\frac{\Delta H_\mathrm{P}}{\Delta H_{0,\mathrm{P}}} \quad (1)$$

where M is the molecular weight of the monomer in g mol^{-1}, ΔH_P is the heat of polymerization expressed by peak area in J g^{-1}, and $\Delta H_{0,P}$ is the theoretical heat of polymerization set to 87.8 kJ mol^{-1} for vinyl esters,[10] 80 kJ mol^{-1} for acrylates,[20] and 60 kJ mol^{-1} for methacrylates.[20] The factor of 2 in the denominator is due to two double bonds per molecule.

The rate of polymerization R_p was determined from eq 2.

$$R_\mathrm{P} = \frac{h \cdot \rho}{\Delta H_{0,\mathrm{P}}} \quad (2)$$

where h is the specific heat flow at maximum in mW mg^{-1} and ρ is a density of the resin in g L^{-1}.

Cell Culture for Cytotoxicity

MC3T3-E1 cells (donated by Dr. Kumegawa, Meikai University, Department of Oral Anatomy, Sakado, Saitama, 35002 Japan) were cultured in alpha MEM (Biochrom, Austria) supplemented with 4.5 g L^{-1} glucose, 5% fetal calf serum (FCS, Biochrom, Austria), and 10 μg mL^{-1} gentamycin. The cells were kept in humidified air under 5% CO_2 at 37 °C. They were subcultured twice a week using 0.001% pronase E and 0.02% EDTA in phosphate-buffered saline (PBS).

For experiments cells were seeded at a density of 20,000 cells cm^{-2} and cultured without the monomers or with increasing concentrations (0.63, 1.25, 2.5, 5, and 10 mmol L^{-1}) for 5 days. For the determination of the cell number (DNA amount), cell layers were washed with PBS and frozen with 1 mmol L^{-1} Tris–HCl buffer (pH 8.0) containing 0.1 mmol L^{-1} EDTA. During thawing, Hoechst dye (Poly-sciences, Warrington, PA) was added (1 μg mL^{-1} in 150 mmol L^{-1} NaCl) and, after an incubation of 15 min at room temperature, the fluorescence of the DNA was measured (excitation 360, emission 465 nm). Calf thymus DNA was used to prepare a standard curve.

For the calculation of the LC_{50}, Prism 4.0 (GraphPad) with a sigmoidal dose response, nonlinear regression was used. The calculation was performed with a constant lower constraint using the fluorescence value of the dye incubation without cells and as constant upper constraint the fluorescence value of the dye incubation of the cell-culture without the monomers.

Viscosity Measurements

Viscosity measurements of the monomers were performed on a Physica MCR 300 (Anton Paar, Austria) with plate-cone geometry. The plate diameter was 25 mm, the cone angle was 1°, and the gap between the plate and cone was 0.05 mm. Tests were carried out at 25 °C at a shear rate of 100 s^{-1}.

Nanoindentation

Cylindrical specimens with 5 mm in diameter and a thickness of 1 mm were prepared in silicon molds using 0.5 wt % of Irgacure® 819 followed by photocuring for up to 30 min using a 600 W metal halide type lamp (IntelliRay 600, Uvitron International) that evenly distributes UVA light (320–390 nm) with the intensity of 100 mW cm^{-2}. Samples were then glued onto an aluminum cylinder with an epoxy-based adhesive and the surface was grinded and polished.

Nanoindentation experiments were carried out on a Nanoindenter XP, MTS Systems. The specimens were indented with a velocity of 20 nm s^{-1} until an indentation depth of 2 μm was reached. From the recorded load versus displacement data, indentation hardness H_{IT} and indentation modulus E_{IT} can be determined.[21,22] H_{IT} was calculated starting from the maximum force F_{max} by applying the eqs 3 and 4.

$$H_{IT} = \frac{F_{max}}{24.5\ h_c^2} \tag{3}$$

$$h_c = h_{max} - \varepsilon(h_{max} - h_r) \tag{4}$$

where F_{max} is the maximum force in N, h_{max} is the penetration depth at maximum force in m, h_r is the intersection of the tangent of the unloading curve at maximum load with the x–axis in m, and ε is an indenter constant.

E_{IT} was calculated starting from the slope of the unloading curve's tangent at the maximum load as shown in the eqs 5 and 6.

$$E_{IT} = \frac{1 - \nu_s^2}{\frac{1}{E_r} - \frac{1-\nu_i^2}{E_i}} \tag{5}$$

$$E_r = \frac{S}{2}\sqrt{\frac{\pi}{A_p}} \tag{6}$$

where ν_s is Poisson's ratio of the sample ($\nu_s = 0.35$), ν_i is Poisson's ratio of the indenter tip ($\nu_i = 0.07$ for diamond), E_r is the reduced modulus of the indentation contact in MPa, E_i is the modulus of the indenter tip in MPa ($E_i = 1.14 \times 10^6$ MPa for diamond), S is the contact strength in N m^{-1} (defined as the resistance of two particles against their mutual displacement) and A_p is the projected area in m^2. At least seven measure points were taken for each sample.

Degradation

Cylindrical specimens with 5 mm in diameter and a thickness of 1 mm were prepared in silicon molds using 0.5 wt % of Irgacure® 819 followed by photocuring for up to 30 min using a broadband UV lamp (IntelliRay 600) and extracted with ethanol for 24 h to remove residual monomers. For the degradation test, each specimen was placed into a stirred 1 M NaOH solution (2 mL) kept at 37 °C. After certain periods of time, the samples were removed from the alkaline solution, washed with water, dried using a paper towel, and weighed until the complete degradation occurred. The time required for a complete degradation was determined by an extrapolation to x–axis.

Digital Light Processing

With the parameters optimized by penetration tests,[18] 3D scaffolds were printed using the formulation **4VE**:**DEVE** 50:50 containing 3 wt % of Irgacure® 819 and 0.15 wt % CGL097 (with or without 20% HA filler). 3D printing was performed using an EnvisionTec Perfactory® SXGA$^+$ Standard equipped with the lens $f = 75$ mm with a PTFE vat in a resolution of 1400 × 1050.[10,23] It allows shaping parts with a xy-resolution of 42 μm and a minimum layer thickness of 25 μm. Scaffolds were built with a layer thickness of 100 μm and an exposure time of 90 s per layer (180 s for the first three layers) at a lamp power of 800 mW dm^{-2}. The printed scaffolds were cuboidal lattice structures with the $L \times W \times H$ dimensions of 10 × 4 × 2 mm, wall size of 500 μm, and pore size of 450 μm. After completion of the structuring process, the prototype was rinsed with ethanol, followed by postcuring for 10 min under the UV lamp (IntelliRay 600) and subsequent extraction with ethanol for 24 h.

CONCLUSIONS

As state of the art poly(lactic acid) cannot be processed by photopolymerization-based AMT techniques, it has so far been necessary to use (meth)acrylate-based photopolymers. Unfortunately, (meth)acrylates possess serious disadvantages such as considerable cytotoxicity and skin irritancy. Also poly(meth)acrylates degrade to high molecular poly(meth)acrylic acids that cannot be transported within the human body and might cause inflammation reactions. To circumvent the use of (meth)acrylate-based photopolymers, new monomers based on vinyl esters were developed in this study. Cytoxicity tests proved that they were at least one to two orders of magnitude less toxic than (meth)acrylates. In order to adjust the degradation behavior while keeping all other material properties satisfactory, a pair of vinyl ester comonomers was designed: one bearing hydrophobic spacer (**4VE**) and the other hydrophilic spacer (**DEVE**). While the photoreactivity of **4VE** is between those of acrylate and methacrylate, **DEVE** is less photoreactive due to hydrogen abstraction reactions. Nevertheless, the double bond conversion of all formulations is practically not influenced by the content of **DEVE**. Low viscosity of the resin allowed adding HA as filler and both unfilled and filled scaffolds were successfully printed by DLP. Although a highly crosslinked network is formed, we were able to show that the degradation behavior can be easily tuned by the hydrophilicity of the material. To investigate the mechanical properties of the synthesized polymers, nanoindentation was carried out. All polymers were significantly stiffer than PCL. Photopolymer from formulation filled with HA was almost as stiff as PLA.

Photoinitiators from Ciba SC, financial support by the Austrian Science Fund under contract No. P19387, the support of EXFO by providing an OmniCure® 2000 unit, and Ernst Mach Scholarship (OeAD) granted to B. Husár are gratefully acknowledged.

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