Tough Photopolymers Based on Vinyl Esters for Biomedical Applications

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ABSTRACT: Photocurable vinyl esters have recently been introduced as suitable alternatives to (meth)acrylates in biomedical applications. While (meth)acrylates exhibit good mechanical properties, their cytotoxicity and degradation products principally disqualify them from medical use. Vinyl esters exhibit much lower cytotoxicity and give biocompatible degradation products, but their disadvantage are relatively low mechanical properties, particularly brittleness. This study focuses on the identification of suitable functional groups that are capable of introducing enhanced impact strength into the vinyl ester network, for example, cyclic structures or urethane groups. A new pathway for the synthesis of vinyl esters carrying these groups was established and resulting monomers were tested regarding their photoreactivity and cytotoxicity. Mechanical properties and degradation behavior of the new materials were investigated as well. In addition, the thiol-ene reaction was utilized to enhance photoreactivity and tune hydrolytical degradation. The new vinyl esters exhibit excellent biocompatibility and good photoreactivity that can be significantly enhanced with thiols on to the level of highly photoreactive acrylates. Ultimately, the impact strength was improved by a factor of more than ten compared to commercial vinyl esters. © 2016 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. 2016, 54, 1987–1997

KEYWORDS: biocompatibility; mechanical properties; photopolymerization; thiol-ene reaction; vinyl ester

INTRODUCTION Treating injuries of the skeletal system in human bodies is one of the major challenges in tissue engineering. Intervention in a complex and sensitive biological system such as the human body leads to a number of prerequisites that have to be fulfilled to make this intervention successful.1 Biocompatibility, no inflammation reactions or cytotoxic response, is absolutely mandatory, but osteoinductivity and osteoconductivity are desired as well. Degradability is not necessarily a precondition but eligible, whereby degradation products must not be toxic and should be easily transportable inside the metabolic system. Mechanical properties of the biomaterial should match the ones of the replaced tissue initially and during degradation.1,2 Treatment of injured bone utilizing (meth)acrylates for nonresorbable fixation techniques, that is, bone cements, or resorbable polycondensates, for example, poly(lactic acid) (PLA) and poly(ε-caprolactone) (PCL), are currently state-of-the-art.3,4 However, there are limitations present for both types of materials regarding the prerequisites mentioned above.

Methacrylate bone cements were introduced already 1960 by Sir John Charnley.5 They are applied in many surgical applications, mainly used for anchoring cemented arthroplasties helping to transfer loads from the prosthesis to the bone.6–12 Although bone cements are successfully applied for decades now, they have got some serious drawbacks that limit their utility, for example, necrosis of bone tissue due to the high temperature of the exothermic polymerization reaction, chemical necrosis due to the release of unreacted methyl methacrylate (MMA), large volumetric shrinkage, brittleness and weakness compared to the prosthesis and bone.13–18 Furthermore, it is not possible to process them into custom-made scaffolds, for example, by means of additive manufacturing technologies (AMT). However, several alternative concepts are existent that base on different approaches.

Dimethacrylates, which upon polymerization result in thermoset networks rather than thermoplastics, have been
Biopolymers that undergo degradation under physiological conditions are also applied in bone regeneration therapies. Biopolymers can originate from natural sources or be synthetically produced, whereby synthetic biopolymers, for example, PLA or PCL, offer more predictable degradation behavior and mechanical properties as well as a reduced risk of immune response or infections. Unfortunately, PLA and PCL are thermoplastics whereby their utility is inherently limited. Therefore, biopolymers based on PLA or PCL were developed that are produced or further utilized via photopolymerization techniques by equipping them with (meth)acrylate groups. However, photopolymers based on (meth)acrylate chemistry often suffer from being irritant or even cytotoxic, and unfortunately, upon degradation poly[(meth)acrylic acid] is among the degradation products. Together with the predominant autocatalytic bulk erosion mechanism of the biopolymer, this leads to large amounts of free acid that can lead to inflammatory response and ultimately fracture due to implant failure.

Accordingly, in addition to reduced cytotoxicity and irritancy, a different degradation mechanism with nonproblematic degradation products is desirable. Recently, a new class of photocrosslinking monomers has been introduced for the use in tissue engineering applications: low cytotoxic and nonirritant vinyl esters. The degradation product of poly(vinyl ester)s is nontoxic poly(vinyl alcohol), which is already used in biomedical applications and approved by the United States Food and Drug Administration (FDA). Also for poly(vinyl ester)s upon degradation, acid is formed that could ultimately lead to autocatalytic bulk erosion, but as these acid molecules are of very low molecular weight they are easily transported out of the implant, thus reducing the autocatalytic degradation effect. Unfortunately, the mechanical properties of these polymers, in particular their brittleness, need improvement.

The aim of this study is to identify functional groups that are capable of improving the mechanical properties of poly(vinyl ester)s and gain degradable, low cytotoxic materials suitable for the application as bone replacement materials. Therefore, new compounds had to be synthesized using precursors with functional groups or cyclic structures that are anticipated to give good mechanical properties. Unfortunately, standard protocols for the synthesis of vinyl esters could not be utilized for lack of suitable precursors. Accordingly, new synthesis routes had to be developed and established, resulting in new compounds that were tested regarding their cytotoxicity and photoreactivity. Cured specimens produced of these monomers were analyzed for their mechanical properties and degradation behavior. In addition, as it is anticipated that a final formulation of these vinyl esters will contain lower MW compounds, such as hexanedioic acid divinyl ester (divinyl adipate, DVA), to tune viscosity, hydrophobicity and mechanical properties, mixtures of the new monomers with DVA were tested as well. Furthermore, the impact of the thiol-ene reaction on the photoreactivity and biodegradability was studied.

**EXPERIMENTAL**

**Materials**

Divinyl adipate (DVA; TCI), 2,2,4-trimethyl-1,6-diisocyanatohexane (TMDI; Sigma), tris(2-hydroxyethyl) terephthalate (Aldrich), lipase acrylic resin from *Candida antarctica* (CAL-B; Sigma), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM]BF₄, Aldrich) were used as received. Dianhydro-D-glucitol (Aldrich) was recrystallized from chloroform. NaCl, KCl, Na₂HPO₄ 2H₂O, K₂HPO₄, NaOH and HCl were purchased from Sigma-Aldrich and used as received. Ethylene glycol and all other solvents were redistilled before use. Pentaerythritol tetra-functional monomers, tetra-mercapto propionate (TT) and bis acrylate were kindly donated by Bruno Bock. 2-Hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propyl ketone (Irgacure 2959) was received as a gift from BASF. O,O'- (7,7,9-Trimethyl-4,13-dioxo-3,14-dioxo-5,12-diazahexadecane-1,16-diy) dimethacrylate (urethane dimethacrylate, UDMA), [2-hydroxy-3-[4-[2-hydroxy-3-(2-methylprop-2-enoyloxy)propoxy]phenyl]propan-2-yl]phenoxylpropyl] 2-methylprop-2-enoate (bisphenol A diglycidyl methacrylate, Bis-GMA), and 1,10-decanediol dimethacrylate (D3MA) were kindly supplied by Iovcari Vivadent. A 1:1:1 equimolar formulation of UDMA, Bis-GMA and D3MA was used as reference material referred to as “MA”. Tetra(ethylene glycol) dimethacrylate (TETGDM, ≥90%) was obtained from UCB. Poly(lactic acid) (PLA) with a molar-lactide ratio of 85:15, glass transition temperature (Tg) of 57 °C (thermal DSC), a Mn of 152 kDa and Mw of 232 kDa (Pd = 1.53) was provided by Synthes GmbH (Oberdorf, Switzerland).

**Characterization**

NMR spectra (200 MHz for 1H and 50 MHz for 13C, respectively) were recorded with a Bruker AC 200 spectrometer, using CDCL₃ as a solvent (grade of deuteration of at least 99.5%). The solvent signal was used as a reference. ATR-FT-IR measurements were carried out on a Biorad FT 135 spectrophotometer with a Golden Gate MkII diamond ATR equipment (LOT). Elemental microanalysis was carried out with an EA 1108 CHNS-O analyzer from Carlo Erba at the microanalytical laboratory of the Institute for Physical Chemistry at the University of Vienna.
Synthesis of Monomers

**Synthesis of Urethane Divinyl Adipate**

O,O’-(7,7,9-Trimethyl-4,13-dioxo-3,14-dioxo-5,12-diazahexadecane-1,16-Diy1) Divinyl Diadipate (UDVA)

For the synthesis of UDVA, first the precursor bis(2-hydroxyethyl) (2,2,4-trimethylhexane-1,6-diy1)dicarbanate (UD) was prepared. Freshly distilled ethylene glycol (55.91 g, 900 mmol) was dissolved under argon atmosphere in dry dioxane (345 mL). Tin(II) 2-ethylhexanoate (100 mg) was added as well as pyrogallol (145 mg) were added. The solution was heated to 75 °C. Afterwards, freshly distilled 2,2,4-trimethyl-1,6-dioisocyanatohexane (9.47 g, 45 mmol) was dissolved in dioxane (10 mL) and added to the solution via a dropping funnel. The reaction was heated for 24 h at 75 °C. Afterwards dioxane was evaporated and the excess of ethylene glycol removed by vacuum distillation (6 mbar, 75 °C) to give 14.3 g bis(2-hydroxyethyl) (2,2,4-trimethylhexane-1,6-diy1)dicarbanate (urethane diol, UD) as a dark yellow, highly viscous oil. Yield: 95%. 1H NMR (200 MHz, CDCl3, δ, ppm): 5.64–5.26 (m, 2H, −NH−), 3.70 (m, 4H, −CH2−), 3.59 (br s, 2H, OH), 3.18–2.76 (m, 4H, −CH2−), 1.73–0.74 (m, 14H, −C(CH3)2−CH2−CH2CH3−CH2−).

The urethane precursor (UD) (1.03 g, 3 mmol) was dissolved in DVA (12.21 g, 60 mmol) and lipase acrylic resin (100 mg) as well as pyrogallol (145 mg) were added. The solution was heated to 65 °C for 40 h, filtered, washed with acetone, and concentrated. The excess of DVA was removed by vacuum distillation (0.12 mbar, 85 °C) and the crude product purified by column chromatography (PE:EE = 1:1) to give 1.3 g of O,O’-(7,7,9-Trimethyl-4,13-dioxo-3,14-dioxo-5,12-diazahexadecane-1,16-diy1) divinyl diadipate (urethane divinyl adipate, UDVA) as a highly viscous, slightly orange liquid.

Yield: 65%. Rf = 0.70 (SiO2, PE/EE, 1:1); 1H NMR (200 MHz, CDCl3, δ, ppm): 7.25 (dd, 2H, J1 = 13.9 Hz, J2 = 6.3 Hz, −CH−), 5.14-4.86 (m, 2H, −NH−), 4.87 (dd, 2H, J1 = 13.9 Hz, J2 = 1.6 Hz, −CH2 trans), 4.57 (dd, 2H, J1 = 6.3 Hz, J2 = 1.6 Hz, −CH2 cis), 4.25 (s, 8H, −O−CO−CH2−CH2−CO−O−), 3.24–2.80 (m, 4H, −CH2−), 2.47–2.30 (m, 8H, −CH2−), 1.77–1.60 (m, 8H, −CH2−), 1.60–0.81 (m, 14H, −C(CH3)2−CH2−CH2CH3−CH2−); 13C NMR (50 MHz, CDCl3, δ): 172.9 (−CO−), 170.4 (−CO−), 156.3 (−CO−), 141.0 (−CH−), 97.7 (−CH2), 62.5 (−CH2−), 48.5 (−CH2−), 46.0 (−CH2−), 39.3 (−CH2−), 37.2 (−CH2−), 35.3 (−CH2−), 27.4 (−C(CH3)2−), 27.3 (−CH3), 26.2 (−CH2−), 25.1 (−CH2−CH2−), 24.2 (−CH2−), 23.9 (−CH2−), 22.3 (−CH3−); IR (ATR): ν (cm−1): 3380, 2957, 1723, 1646, 1528, 1460, 1368, 1235, 1137, 949, 876, 775; Anal. calc. for C28H34O12: C: 85.9, H: 7.84, N: 4.36, O: 18.47; found: C: 85.8, H: 7.24, N: 4.23, O: 18.50.

**Synthesis of Terephthal Divinyl Adipate**

O,O’-(Terephthaloylbis(Oxy))Bis(ethane-2,1-Diyl) Divinyl Diadipate (TDVA)

Dianhydro-ω-glucitol (1.06 g, 7 mmol) was dissolved in 25 mL acetone and mixed with DVA (8.63 g, 44 mmol), lipase acrylic resin (100 mg), and pyrogallol (25 mg). The mixture was heated to 65 °C for 40 h, filtered, washed with acetone and concentrated. The excess of DVA was removed by vacuum distillation (3.3 × 10−2 mbar, 65 °C) and the crude product purified by column chromatography (PE:EE = 1:1) to give 2.1 g O,O’-(hexahydrofuro[3,2-b]furan-3,6-diyl) divinyl diadipate (glucitol divinyl adipate, GDVA) as a colorless liquid.

Yield: 62%. TLC (PE:EE = 1:1), Rf = 0.80; 1H NMR (200 MHz, CDCl3, δ): 7.20 (dd, 2H, J1 = 14.0 Hz, J2 = 6.3 Hz, −CH−), 5.17–5.03 (m, 2H, −CH2 trans), 4.81–4.72 (m, 1H, −CH(O)−), 4.50 (dd, 2H, J1 = 14.0 Hz, J2 = 1.6 Hz, −CH2 cis), 4.44–4.28 (m, 1H, −CH(O)−), 3.94–3.68 (m, 4H, −CH2−O−), 2.44–2.23 (m, 8H, −CH2−), 1.74–1.60 (m, 8H, −CH2−); 13C NMR (50 MHz, CDCl3, δ): 172.3 (−CO−), 170.2 (−CO−), 141.1 (−CH−), 97.6 (−CH2), 85.9 (m, 2H, −CH(O)−), 80.7 (−CH−), 73.5 and 70.3 (−CH(O)−), 33.5 (−CH2−), 24.0 (−CH2−); IR (ATR): ν (cm−1): 3093, 2965, 1735, 1720, 1646, 1465, 1381, 1250, 1160, 1101, 942, 882, 795, 728; Anal. calc. for C22H32O12: C: 59.87, H: 6.09, O: 34.13; found: C: 59.58, H: 5.89, O: 34.53.

**Characterization of the Monomers**

**Cell Culture Experiments**

For Alamar Blue assays, 1 M solutions of the new vinyl esters in DMSO (HYBRI-MAX®, Sigma) were prepared. Each solution was diluted with Dulbecco Modified Eagles Medium (DMEM, Sigma), 10% fetal calf serum (FCS, PAA), 100 U mL−1 penicillin (Invitrogen) and 100 μg mL−1 streptomycin (Invitrogen), to acquire solutions with seven different
concentrations of the monomers (10, 5, 2.5, 1.25, 0.63, 0.31, and 0.16 mM). Osteoblasts cells [strain C57Bl/6 of mus musculus (ATCC Catalog No. CRL-2593, MC3T3-E1, Subclone 4)] were cultured in 100 μL DMEM medium supplemented with 10% FCS, 100 U mL⁻¹ penicillin and 100 μg mL⁻¹ streptomycin, in 96-well plates at a density of 6.4 × 10^3 cells per well for 24 h in humidified air (95% relative humidity) with 5% CO₂ at 37 °C. The next day, the cells were treated with 100 μL of the different concentrations of the test substances for 5 days in triplicates. 10 μL of resazurin were added and the cells were incubated for 4 h at 37 °C. The fluorescence intensity was measured for excitation at 570 nm and emission at 585 nm and compared to untreated cells (cells + medium). As control groups, cells treated with 1% DMSO solution, a blank value, and PBS buffer were used. The results represent the mean with standard deviations of triplicate assays (n = 3). The concentration at which more than 50% of cells survived (LC₅₀) was used to compare results.

**Photoreactivity by Photo-DSC**

Photo-DSC measurements were conducted on a Netzsch Photo-DSC 204 F1 Phoenix. The photoreactivity was analyzed with formulations containing 2 wt % of 2959 as photoinitiator (PI). For each measurement accurately 10 mg of the respective formulation were weighed into an aluminum pan. Samples were purged with nitrogen and thermostated at 25 or 70 °C for 3 min and subsequently irradiated (filtered UV light (280–500 nm), double light guide (OmniCur-eVR 2000), light intensity of 3 W cm⁻²) from a distance of 25 mm twice for 5 min. The corrected photo-DSC curve was subsequently calculated by subtracting the second irradiation segment (irradiation of cured polymer) from the first one (actual photopolymerization). From the area of the peak ΔH₀, [J g⁻¹], the double-bond conversion (DBC) was calculated with ΔH₀,p [J mol⁻¹] being the theoretical heat for 100% conversion and Mw the molecular weight of the monomer. For vinyl esters with divinyl adipate end groups ΔH₀,p values 175.6 kJ mol⁻¹.

\[
DBC = \frac{M_w \cdot \Delta H_0}{\Delta H_{0,p}}
\]

(1)

The polymerization rate R₀ can be calculated from the specific heat flow at maximum (= the height of the peak at the peak maximum h [mW mg⁻¹]), the density (ρ) and the polymerization heat (ΔH₀).

\[
R_0 = \frac{h \cdot \rho}{\Delta H_0}
\]

(2)

**Photoreactivity by Real-Time FT-IR-Spectroscopy**

Real-time (RT-)FT-IR-spectroscopy was performed with formulations containing 2 wt % of 2959 as PI and 0.1 wt % of pyrogallol on a Bruker Vertex 80 spectrometer in transmission mode with a self-made attachment for an Exfo OmniCure™ series 2000 light source. Reactions were conducted in a cell prepared by sandwiching samples between two roughened polyethylene films at a thickness of 50 μm while the IR sample chamber was continuously purged with dry air. The thickness of the layer was calculated from the mass of the drop of the formulation that was used (5 mg). This amount was related to the surface area of the layer between both films (10 × 10 mm²). Filtered UV light (280–500 nm) from an Exfo OmniCure™ series 2000 with a light intensity of 3000 mW cm⁻² at the exit of the light guide was coupled into the IR spectrometer via a light guide at an angle of 45° in a working distance of 20 mm between the tip of the light-guide and the sample. At the surface of the sample, the UV-A light intensity of this setup was 50 mW cm⁻²; all samples were irradiated for 60 s. The kinetics were followed using the “Rapid scan”-method by recording a set of single spectra (in time intervals of 0.07 s) with the aid of a MCT-detector. The spectra were base line-corrected with the software OPUS from Bruker with a so-called “konkave Gummißband Methode” with 512 base line points and 10 iteration steps. The C=C bending peak at 1646 cm⁻¹ and the C=O peak at 1722 cm⁻¹ were integrated for the single spectra and exported into ascii-format using the software OPUS. The DB₅ was determined by computing the area ratios between the double bond signal and the carbonyl signal.

**Characterization of the Polymers**

**Sample Preparation for Testing**

Specimens prepared by bulk photopolymerization were fabricated in silicone molds of appropriate size. As positive, specimens of the desired shape were fabricated with Rapid Prototyping (EnvisionTec Perfactory® SXGA+W/ERM Mini Multi Lens-System with resin formulation R11). The formulations contained 2 wt% 2959 as PI. In the case of formulations containing thIols, 0.1 wt% of pyrogallol as inhibitor was added. For poly-TDVA samples, the monomer/PI mixture was melted, subsequently casted and quickly cured before solidification could occur. For dynamic mechanical thermo analysis (DMTA) and Charpy impact tests prismatic specimens (DMTA: 2 × 5 × 40 mm³, Charpy: 4 × 10 × 80 mm³) and for nanoindentation and degradation studies cylindrical platelets (diameter 5 mm, height 1 mm) were produced. Photopolymerization was carried out in a ventilated UV-chamber (Uvitron UV 1080 Flood Curing System with Uvitron IntellRay 600 halide lamps, irradiation power 600 W, UV-A: 125 mW cm⁻², and Vis: 125 mW cm⁻², distance from light source: 130 mm, intensity at curing position: 120 mW cm⁻²) with an exposure time of 600 s on both sides of the specimens.

**Dynamic Mechanical Thermo Analysis**

Dynamic mechanical thermo analysis (DMTA) was conducted using a TA instruments DMA 2980 Dynamic Mechanical Analyzer in 3-point bending configuration by heating under load with an amplitude of 5.0 μm, a preload force of 0.05 N, 1 Hz, and a load track of 125% at a rate of 3 K per minute from −50 °C up to 80 °C. Initially, the specimen (prismatic test bars sized 2 × 5 × 40 mm³) was cooled to −50 °C and kept isothermal for 10 min to allow for setting of the temperature in the entire specimen; after this the measurement was started.
Charpy Impact Tests

Charpy impact tests were performed on a Frank impact testing machine on unnotched, prismatic specimens sized 4 × 10 × 80 mm³ with a drive hammer energy of 0.5 or 1.0 J, span length of 40 mm and temperature and humidity kept at standard conditions (20 °C, 50% RH). The unnotched Charpy impact strength \( a_{\text{un}} \) [m m⁻²] was corrected for the machine parameter.

Nanoindentation

Nanoindentation experiments were carried out on a Nanoindenter XP, MTS Systems Inc. For the measurements, platelets (diameter = 5 mm, thickness = 1 mm) were fabricated as described above. The specimens were glued on to an aluminum cylinder with an epoxy-based adhesive and the surface was grinded and polished. The specimens were indented with a rate of 20 nm s⁻¹ until an indentation depth of 2 μm was reached. At least seven measurement points were analyzed. The indentation modulus \( E_i \) was calculated starting from the slope of the unloading curve’s tangent at the maximum force, with \( v_i \) being the Poisson’s ratio of the sample, \( v_i = 0.35 \), \( v_i \) the Poisson’s ratio of the indentor tip (for diamond \( v_i = 0.07 \)), \( E_r \) [MPa] the reduced Young’s modulus of the indentation contact (\( E_r = 1140 \) GPa for diamond), \( S \) [N m⁻¹] the contact strength (defined as the resistance of two particles against their mutual displacement), and \( A_p \) [m²] the projected area of the imprint.

\[
E_i = \frac{1 - (v_i)^2}{E_r} + \frac{1 - (v_i)^2}{E_r} \tag{3}
\]

\[
E_r = \frac{\sqrt{\pi} \cdot S}{2 \cdot \sqrt{A_p}} \tag{4}
\]

The indentation hardness \( H_i \) was calculated from the maximum force \( F_{\text{max}} \):

\[
H_i = \frac{F_{\text{max}}}{24.5 \cdot h_c^2} \tag{5}
\]

\[
h_c = h_{\text{max}} - \varepsilon \cdot (h_{\text{max}} - h_r) \tag{6}
\]

with \( F_{\text{max}} \) [N] being the maximum force, \( h_{\text{max}} \) [m] the penetration depth at maximum force, \( h_r \) the intersection of the tangent of the unloading curve at maximum load with the x axis [m] and \( \varepsilon \) an indentor constant.

In Vitro Degradation Studies

In vitro degradation studies were performed on platelets with a diameter of 5 mm and a thickness of 1 mm. Platelets were subjected to an extraction process to remove residual monomer: 2 × in ethanol for 24 h and 1 × in deionized water for additional 48 h. After this the platelets were thoroughly dried in vacuo and weighed to determine their starting weight. Following that, the platelets were placed in vials with 5 mL of PBS buffer (phosphate buffered saline: 8.01 g NaCl 0.2 g KCl, 1.78 g Na₂HPO₄ 2 H₂O, and 0.27 g KH₂PO₄ in 1 L of distilled water, pH value 7.4) and put into a climate chamber at 37 °C. After 7 and 19 weeks, the buffer solution was removed and the platelets thoroughly washed with deionized water and dried in vacuo at 45 °C for 15 h. Afterwards they were weighed again and fresh PBS solution was added.

RESULTS AND DISCUSSION

Functional groups such as urethane groups or ring systems are anticipated to be responsible for good mechanical properties, in particular high impact strength, as found in dimethacrylates for dental or bone replacement applications. As vinyl esters have been demonstrated to be a versatile alternative to methacrylates in those applications, but suffer from limited mechanical properties, it appeared worthwhile to introduce these functional groups into the network of poly(vinyl ester)s as well. Three different divinyl esters, a urethane divinyl adipate (UDVA), a terephthal divinyl adipate (TDVA) and a glucitol divinyl adipate (GDVA), were envisioned.

Synthesis of the Monomers

As conventional synthesis of vinyl esters, utilizing vinyl acetate and a (di)acid via a Pd(OAc)₂ catalyzed transesterification reaction, could not be performed due to lack of suitable precursors, a new route had to be established. This was based on the reaction of a difunctional alcohol with hexanedioic acid divinyl ester (divinyl adipate, DVA), catalyzed by a lipase (Scheme 1). The starting point of this was the well-known reaction of carbohydrates with a divinyl ester to give the acylation product thereof. For the transesterification of corresponding diols, DVA was used in large excess to prevent transesterification of both vinyl ester groups. The reaction was catalyzed by lipase from C. Antarctica (CAL-B). The stability and activity of CAL-B could be enhanced by addition of an ionic liquid [BMIM]BF₄ in case of TDVA, for GDVA no enhancement was observed.

Corresponding urethane diol (UD) for the synthesis of UDVA was synthesized from 2,2,4-trimethyl-1,6-diisocyanatohexane (TMDI) and ethylene glycol in the presence of a catalytic amount of tin(II) 2-ethylhexanoate [Scheme 1(a)]. UD was subsequently dissolved in DVA and reacted in the presence of CAL-B. For the synthesis of a terephthal divinyl adipate (TDVA) [Scheme 1(b)], bis(2-hydroxyethyl) terephthalate (HET) and to obtain a glucitol divinyl adipate (GDVA) [Scheme 1(c)], dihydroxy-α-glucitol (DAG) were reacted with DVA in the presence of CAL-B and for TDVA the ionic liquid [BMIM]BF₄, respectively. After removal of CAL-B and excessive DVA, crude divinyl esters were purified by column chromatography and obtained in good yields (62–79%).

Cytotoxicity

Photopolymers utilized for biomedical applications potentially release unreacted monomers into their vicinities. Thus, it is important to gain information about possible influences of these compounds onto bone cell proliferation and differentiation. To access these parameters cell viability measurements were conducted. For this purpose, mouse cells (MC3T3-E1)
were incubated with varying concentrations of the monomers from 0.16 to 10 mmol L\(^{-1}\). The concentration at which 50% of the cells survived after five days (LC\(_{50}\)) was used to grade the newly synthesized monomers (Table 1).

LC\(_{50}\) values for the three new monomers were in the range of 2.5 to >10 mM, similar to DVA which has already proven to exhibit excellent biocompatibility in \textit{in vivo} tests.\(^{33}\) For standard methacrylates, such as TTEGDMA, this value is usually well below 1 mM or even worse (<0.2 mM) for acrylates.\(^{30-33}\) However, special dental monomers (\textit{Bis}-GMA, UDMA), which are known for excellent biocompatibility\(^{26-28}\) and were the blueprints for the new vinyl esters, are in the same range as the new vinyl esters. Thus, the new types of vinyl esters exhibit excellent biocompatibility as well.

**Photoreactivity**

The reactivity of monomers is a crucial parameter for the success of the envisioned application. Particularly, as curing by UV light is anticipated to be the polymerization method of choice, the photoreactivity had to be analyzed for the new monomers. Thereby three parameters are usually utilized for the quantification of the photoreactivity: the double bond conversion (\(\text{DBC}\)), the polymerization rate (\(\text{Rp}\)) and the time to reach the maximum of the polymerization exotherm (\(t_{\text{max}}\)). \(\text{DBC}\) is a key factor for practical applications, as only high values of reacted double bonds ensure minimization of potential leachables. Additionally, poor mechanical properties are to be expected at low \(\text{DBC}\).

**Photo-DSC**

To analyze the photoreactivity, photodifferential scanning calorimetry (DSC) measurements were performed, resulting in values for \(t_{\text{max}}\), \(\text{Rp}\), and \(\text{DBC}\). As photoinitiator (PI), Irgacure 2959 was employed since this compound exhibits high photoreactivity and additionally this PI does not lead to adverse effects in cell culture experiments of photopolymerized test specimens.\(^{2,57}\) As reference monomer, DVA was used for this compound was shown to exhibit highest photoreactivity among vinyl esters due to lack of additional functionality resulting in low viscosity and a high share of vinyl ester functionality.\(^{32,33}\)

Since it is anticipated that a final formulation of the new vinyl esters will contain lower \(M_w\) compounds, for example, DVA, to tune viscosity, hydrophobicity and mechanical properties, mixtures (1:1 molar) of the new monomers with DVA were tested as well (Fig. 1). The aromatic compound (TDVA) is a solid at 25 °C and thus had to be analyzed at 70 °C. A 1:1:1 equimolar formulation of UDMA, \textit{Bis}-GMA, and D3MA was used as reference material referred to as “MA”.

All three new monomers exhibit similar photoreactivity as the reference compound DVA. TDVA exhibits the highest \(\text{DBC}\) (90%) which is due to the size of this monomer: comprising of long, stiff molecules enables this high conversion resulting in a tight network. UDVA (73%) and GDVA (70%) exhibit similar \(\text{DBC}\) as DVA (73%). The \(t_{\text{max}}\) of TDVA (8.4 s) is a bit higher compared to DVA (4.9 s), GDVA (5.6 s), and UDVA.

**TABLE 1 Cytotoxicity (LC\(_{50}\) Values): New Vinyl Esters and DVA compared to Methacrylates.**

<table>
<thead>
<tr>
<th>Substance</th>
<th>LC(_{50}) (mM)</th>
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<tbody>
<tr>
<td>DVA</td>
<td>&gt;10</td>
</tr>
<tr>
<td>TDVA</td>
<td>&gt;10</td>
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<tr>
<td>GDVA</td>
<td>5</td>
</tr>
<tr>
<td>UDVA</td>
<td>2.5</td>
</tr>
<tr>
<td>TTEGDMA</td>
<td>&lt;0.16</td>
</tr>
<tr>
<td>\textit{Bis}-GMA</td>
<td>2.5</td>
</tr>
<tr>
<td>UDMA</td>
<td>5</td>
</tr>
</tbody>
</table>
of the new monomers with DVA exhibit photoreactivities right in between those of DVA and the new monomers. Compared to the methacrylate reference formulation ($t_{\text{max}} = 6.6$ s, $R_p = 0.17$ mol L$^{-1}$ s$^{-1}$, DBC = 57%), the new monomers exhibit comparable or often even better $t_{\text{max}}$ but significantly increased DBC and $R_p$. This demonstrates that they can easily compete with state-of-the-art methacrylate systems in terms of photoreactivity; improved DBC and low cytotoxicity qualify these new monomers to be particularly suitable as alternative to methacrylates in bone replacement applications.

Recently, thiols have been identified as suitable polymerization accelerator for vinyl esters via the thiol-ene reaction. This is based on a mixed step and chain growth polymerization regime. The effect of thiols onto the photoreactivity was also analyzed for the new vinyl esters (Fig. 2). In addition to pure monomers, formulations containing 20 and 40% based on functional groups of the tetrathiol TT were tested. Again, for TDVA (mp = 53 °C), measurements were performed at 70 °C.

As expected, for all of the three compounds, the maximum of the polymerization exotherm is shifted to shorter times indicating increased photoreactivity. For TDVA, the addition of 40% TT based on functional groups resulted in an increase of the reactivity by almost a factor of 3 from 8.25 to 3.10 s. The two other compounds’ photoreactivity could be significantly increased, too: $t_{\text{max}}$ decreased by a factor $>2$; for UDVA from 8.30 to 3.35 s and for GDVA from 5.95 to 2.60 s. In the end their photoreactivity, expressed by $t_{\text{max}}$, is in the same range as those of highly reactive acrylates, which is below 3 s.

**Real-Time FT-IR Spectroscopy**

Photo-DSC measurements of thiol-vinyl ester formulations do not provide information about $R_p$ and DBC as there is a mixed mechanism of chain and step growth polymerization, which exhibit different reaction enthalpies, taking place during the thiol-ene reaction. Thus, in order to obtain results for the DBC of the new monomers in combination with the thiol TT, RT-FT-IR measurements were performed. Exemplarily, a RT-FT-IR curve is displayed in Figure 3 for pure UDVA without thiol and
UDVA containing 20 and 40%, respectively, TT based on functional groups. To track the polymerization process of UDVA the bending peak of the $C\equiv C$ double bond at 1646 cm$^{-1}$ was observed and related to the $C\equiv O$ peak at 1722 cm$^{-1}$.

The results of the RT-FT-IR measurements of UDVA resemble in principle those that were found in a previous study for DVA.$^{10}$ The final $DBC$ of UDVA as established by RT-FT-IR is slightly lower as compared to the value measured by photo-DSC (65 compared to 71%). The formulations containing TT exhibit significantly higher values (70% and 81% for 20% TT and 40% TT, respectively), as expected. The different polymerization regimes are again traceable if a closer look is taken at the initiation phase (Fig. 3, inlay). For pure UDVA the typical trend for chain growth polymerization is displayed, whereas in the case of UDVA containing 20 and 40% TT, respectively, almost immediately after initiation a certain fraction of double bonds, approximately as many as there were thiol-groups present, reacted followed by relatively slow chain growth polymerization.

Mechanical Properties

Dynamic Mechanical Thermo Analysis

Mechanical properties of cured specimens from new vinyl esters were analyzed by dynamic mechanical thermo analysis (DMTA). Polymers derived from pure monomer formulations as well as mixtures with DVA were tested. In Figure 4 (top), the progression of the storage modulus and tan $\delta$ with increasing temperature is depicted for poly-UDVA, poly-GDVA, and poly-TDVA.

The typical behavior of crosslinked polymers can be perceived. Poly-UDVA exhibits its glass transition at 40 °C and for poly-TDVA the glass transition temperature ($T_g$) values 70 °C. The $T_g$ of poly-GDVA could not be observed within the temperature range tested. The lower $T_g$ for poly-UDVA can be explained by the length of the monomer and thus the spacer in between crosslinks leading to a low crosslink density resulting in a low $T_g$. For poly-GDVA, being the shortest molecule, this also explains their $T_g$, which is higher than 80 °C.

All of the new materials exhibited storage moduli at 20 °C between 1670 (poly-GDVA) and 1880 MPa (poly-UDVA), poly-TDVA being located in between at 1730 MPa. Thus these materials were slightly less stiff than pure poly-DVA (2040 MPa). Compared to the methacrylate formulation the new poly(vinyl ester)s were less stiff, too. This can be explained by the dimensions of the network. Even though the new vinyl esters exhibit significantly improved $DBC$, due to their length the network density is decreased resulting in reduced stiffness. At 37 °C the picture is approximately the same, just on a lower level (around 1500 MPa). Polymeric specimens produced from formulations of mixtures of the new materials with DVA (1:1 molar) exhibit the same principal trend (Fig. 4, bottom). For pure poly-UDVA, at 37 °C the modulus has already declined to 320 MPa, due to a $T_g$ of 40 °C, whereby this material would have to be mixed with a low $M_w$ vinyl ester, for example, DVA, to exhibit sufficient stiffness also at 37 °C.

Nanoindentation

Nanoindentation was performed to study the influence of TT onto the modulus and the hardness of the new vinyl esters (Fig. 5) and compared to poly-DVA and poly-MA.

For the smallest compound GDVA the corresponding polymer exhibits the highest network density, resulting in high modulus and hardness. Upon addition of 20% TT, the modulus remains unchanged and the hardness decreases only to a small extent. This can be explained by a good match of the size of the thiol and the relatively small GDVA. At 20% TT hardly any change of the network density and thus reduction of the glass transition temperature takes place resulting in unchanged mechanical properties. At 40%, TT both hardness and modulus are significantly reduced as one would expect. For poly-GDVA, also formulations with TT would be directly suitable for the application as tissue engineering scaffold.

Their hardness and modulus are comparable to poly-DVA but, as for vinyl esters in general, lower as poly-MA due to their inherent higher crosslink density they exhibit because
of their shorter spacer. In the case of TDVA and UDVA, addition of TT leads to a significant decline of hardness and modulus at 20 and 40% TT. Both poly-TDVA and poly-UDVA exhibit relatively low network density and low T_g (see above), which is due to the large size of these monomers. Addition of thiols lowers the glass transition temperature of polymers leading to reduced modulus and hardness. In the case of poly-TDVA and poly-UDVA it can be anticipated that T_g is reduced to values below 20 °C resulting in rubbery materials at room temperature. Accordingly, formulations of TDVA and UDVA, respectively, with thiols would not be of sufficient stiffness to act as tissue engineering scaffold on their own. However, in optimized formulations they are anticipated to be a potential tool contributing to the toughness required.

Charpy Impact Tests
Introduction of functional groups into the vinyl ester network to enhance impact properties was the genuine idea behind this study. It is anticipated that functionalities like ring systems or urethane groups lead to increased intramolecular interactions that decrease brittleness. To demonstrate enhanced impact strength, Charpy impact tests were performed. Tests were performed with specimens produced from pure new vinyl esters as well as from equimolar mixtures of DVA and the respective new vinyl esters and compared to poly-DVA (Fig. 6) and the methacrylate reference system MA. The addition of the low-viscosity compound DVA was performed to reflect the situation in reality as it is strongly anticipated that a final formulation will have to contain a viscosity modifier such as DVA to lower the viscosity of the highly viscous monomers.

All of the new vinyl esters were capable of significantly improving the impact strength compared to poly-DVA. Impact strengths of 26.3 mJ mm^{-2} (poly-UDVA), 20.2 mJ mm^{-2} (poly-TDVA), and 18.0 mJ mm^{-2} (poly-GDVA) could be achieved, thus outperforming pure poly-DVA (1.5 mJ mm^{-2}) by a factor of more than ten. In addition, the methacrylate formulation (7.9 mJ mm^{-2}) could be also outperformed by a factor of more than two. Furthermore, the pure new vinyl esters were on or even significantly above the level of PLA which had a Charpy impact strength of 19.1 mJ mm^{-2}. The augmentation of the impact strength was explained by the introduction of urethane groups into UDVA and cyclic structures into GDVA and TDVA. Those are responsible for enhanced intramolecular interactions resulting in improved impact strength. The better impact strength compared to the methacrylate formulation can be explained by the absence of a viscosity modifier in pure new vinyl esters. Formulations of the new vinyl esters with DVA as viscosity modifier had impact strengths of 6.2 mJ mm^{-2} [poly-(UDVA-DVA)], 6.5 mJ mm^{-2} [poly-(TDVA-DVA)] and 8.0 mJ mm^{-2} [poly-(GDVA-DVA)], respectively, thus being on the same level as the methacrylate formulation. These results demonstrated that the new set of vinyl esters containing functional groups such as cyclic structures or urethane groups are a vital candidate for the replacement of methacrylates in bone replacement applications.

In Vitro Degradation Studies
The most significant difference between (meth)acrylate systems and vinyl esters and hence the biggest advantage of vinyl esters is their biodegradability. In vitro hydrolytical degradation tests were performed to study whether the new types of vinyl esters are prone to degradation under physiological conditions (37 °C, PBS buffer). Materials formulated of mixtures of DVA and the new vinyl esters were tested as well (Fig. 7).

Poly-UDVA, containing the longest spacer, exhibits significantly higher degradation velocity as compared to poly-DVA, which showed hardly any degradation during the test period at all due to a dense, hydrophobic network formed by a short, aliphatic spacer. Poly-GDVA and poly-TDVA, containing shorter spacers as poly-UDVA exhibited degradation as well, but at significantly reduced speed. After four months, 91.1%
of the initial mass was left for poly-UDVA; for poly-GDVA and poly-TDVA the according values were 98.9 and 97.1%, respectively. The rapid degradation of poly-UDVA is based on its size and the highest polarity it exhibits. Poly-GDVA on the other hand exhibits the densest network among the three new vinyl esters leading to the slowest degradation. For all three poly(vinyl ester)s steady degradation took place, opposed to PLA, which undergoes autocatalytic bulk degradation with no signs of degradation within 4 months of testing. However, PLA rapidly degrades completely after a certain incubation time, depending on the composition around 6 months, whereas vinyl esters undergo steady, continuous degradation. Addition of DVA led to a reduction of the degradation speed for poly-UDVA, due to the increased crosslink density. For poly-TDVA, and poly-GDVA, the addition of DVA resulted in a slight increase in degradation velocity, which might be explained by a slightly reduced DBC that led to a small decrease in the overall crosslink density.

As expected, the addition of 40% TT approximately doubled the degradation speed (e.g., 91.1% residual mass after 4 months for poly-UDVA compared to 78.9% for poly-UDVA + 40% TT), 20% TT led to a degradation behavior that lay in between pure poly-UDVA and poly-UDVA + 40% TT (Fig. 8). The same behavior could be found for poly-TDVA (97.1, 93.9, and 91.4% residual mass for 0, 20, and 40% TT, respectively) and poly-GDVA (98.9, 97.4, and 96.8% residual mass for 0, 20, and 40% TT, respectively) as well.

CONCLUSIONS

Polymers based on vinyl esters for bone replacement applications have recently been successfully introduced but exhibit the drawback of limited impact strength compared to state-of-the-art materials based on (meth)acrylate chemistry. To fully exploit the advantages of vinyl esters compared to conventional (meth)acrylates, that is, lower cytotoxicity and skin irritancy as well as biodegradability, new compounds exhibiting improved impact strength are desirable. This study aimed at the development of vinyl esters bearing functionalities that are capable of augmenting mechanical properties, in particular impact strength. Structures such as urethane groups or cyclic compounds were envisioned and three new monomers been synthesized. Therefore, a new synthesis protocol was established since for state-of-the-art vinyl ester synthesis routes no suitable precursors were available. Cytotoxicity tests proved that the new monomers were on the same level of biocompatibility as commercial vinyl esters and thus one or two orders of magnitude less toxic than conventional (meth)acrylates. The photoreactivity of these monomers was similar to conventional vinyl esters and could be further improved through the addition of thiols via the thiol-ene reaction. Degradation studies unveiled a steady degradation mechanism that could be tuned with the aid of thiols, too. While the Young’s modulus of the new poly(vinyl ester)s was in the range of conventional poly(vinyl ester)s, the impact strength was improved by a factor of more than ten thus outperforming state-of-the-art polymethacrylates demonstrating suitability of these new monomers in bone replacement applications.

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REFERENCES AND NOTES
