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Evaluation of Biocompatible Photopolymers II: Further Reactive Diluents

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Summary. Within these investigations a new monomer formulation – consisting of a biodegradable basis monomer, reactive diluents, fillers, and an appropriate photoinitiator – was developed for the stereolithographic fabrication of bone replacement materials. In the current paper we describe the testing of several acrylate based reactive diluents – having different functional groups – concerning reactivity, biocompatibility, and mechanical properties. Polymers from tripropylene glycol diacrylate, propoxylated glycerol diacrylate and tricyclodecan dimethanol diacrylate showed the most promising results regarding these 3 criteria. Three-dimensional spongiosa-like structures were built by stereolithography using different monomers, fillers, and a suitable photoinitiator.

Keywords. Biocompatibility; Bone Tissue Engineering; Mechanical properties; Photopolymerization; Stereolithography.

Introduction

To enable proper healing and rebuilding of a bone, *e.g.* after removal of a tumor, it is necessary to implant a biodegradable tissue scaffold, which perfectly fits in the hole and gives mechanical support. The most important characteristics of a bone replacement material are biocompatibility, bioresorbability, and the support of attachment and differentiation of osteogenic cells. Existing bone replacement materials include autografts and allografts, which consist of tissue obtained from the same or another subject of the same species. They are still the standard material for tissue repair and substitution, although, they both have some serious disadvantages, such as limited availability and the possibility of donor site morbidity in the case of autografts and complications, such as viral transmission and immunogenicity in the case of allografts. To overcome these drawbacks new synthetic biocompatible and biodegradable materials have been developed. Since these defects differ in size, shape, and location in the body, it is necessary to develop a technique that allows the fabrication of a replacement in any conceivable shape. State of the art are injectable bone cements based on poly(lactic acid) oligomers, that are modified with acrylic moieties to enable *in vivo* curing [1]. But the evolving heat of polymerization can damage the surrounding tissue and the resulting polymer is not porous, which limits or even inhibits the supply with nutrients and cells. Another problem of almost all existing biopolymers is their hydrolytic degradation due to their polyester structure.

A different approach is the fabrication of an implant according to the computer tomography data of

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the injured site prior to operation. Stereolithography is a computerized generative fabrication technique, that offers the possibility to produce objects with highly complex geometry and inner structure out of photopolymerizable formulations [2]. Only few papers were published that focus on photopolymerizable monomers that lead to biocompatible and biodegradable polymers. Substances under investigations are poly(propylene fumarate) that can be photocrosslinked with diethyl fumarate [3–5], a photopolymerizable lysine based monomer [6], and new materials based on (meth)acrylate modified oligopeptides [7]. All these photopolymerizable monomers are solid and therefore alone not useful for stereolithography.

The objective of the current work is the development of an adequate monomer formulation, consisting of a biodegradable basis monomer, different reactive diluents to control network density and processing viscosity, fillers, and photoinitiators. For the biodegradable basis monomer investigations are still on going in the field of modified peptides [8]. Some commercially available acrylic compounds and photoinitiators have already been tested for their applicability in a biocompatible monomer formulation [9]. In these investigations further possible reactive diluents – mono- and multi-acrylated compounds having different functional groups and molecular structures – were tested concerning reactivity, biocompatibility, and mechanical properties.

Results and Discussion

Different commercially available monomers either mono-acrylates (Fig. 1) or multi-substituted monomers (Fig. 2) were selected.

In this study, we focused on acrylates and methacrylates with hydroxy and ether groups. Especially

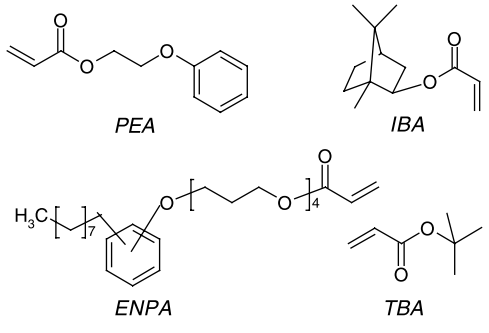

Fig. 1. Mono-acrylated monomers

oligoethyleneglycol units are well known to have only a minor effect on the cell adhesion behaviour [10], which is of significant interest if cell adhesion selective peptides should be applied. Mechanical properties are expected to be dependent on the network density and, therefore, on the number of functional groups and on hydrogen bond formation by hydroxy groups. Biocompatibility and biodegradability cannot be classified in that way, and is more related to the structure of the whole building block and the mechanical properties of the polymer [11]. Difunctional monomers, bisphenol A diglycidylmethacrylate (*BisGMA*) and dodecyl dimethacrylate (*D3MA*) are well known from dental applications.

In contrast to our first study, we were only interested in hydrolytically cleavable monomers. Amide and peptide based monomers will be considered in a subsequent study.

Photoreactivity

Beside biocompatibility and mechanical properties, photoreactivity is an important selection criterion for the monomers because sufficient double bond conversion and short building times for the fabrication by stereolithography are desired. Differential scanning photocalorimetry (Photo-DSC) is a unique method to obtain various important reactivity parameters with one single measurement. The calculation of the time to reach the maximum heat of polymerization (t_{max}/s), the total double bond conversion (DBC/%), and the initial rates of polymerization ($R_p/mol/dm^3\,s$) has been described recently [9].

For the Photo-DSC measurements 1 wt% of an equimolar mixture of camphorquinone (*CQ*) and *N*,*N*-dimethylaminobenzoic acid ethyl ester (*DMAB*) was used as photoinitiator (PI), because this combination is well known from dental applications and has been described to be excellently biocompatible [12]. Due to the high volatility, *t*-butyl acrylate (*TBA*) could not be examined.

Monoacrylates are known to cure slowly due to linear chain formation and the delayed gel effect compared to crosslinked systems. Because of that, higher DBC was achieved mostly. This applies especially to our monomers 2-phenoxyethylacrylate (*PEA*) and ethoxylated (**4**) nonylphenol acrylate (*ENPA*). The exceptional low reactivity of isobornyl acrylate (*IBA*) can be assigned to the sterically demanding substituent.

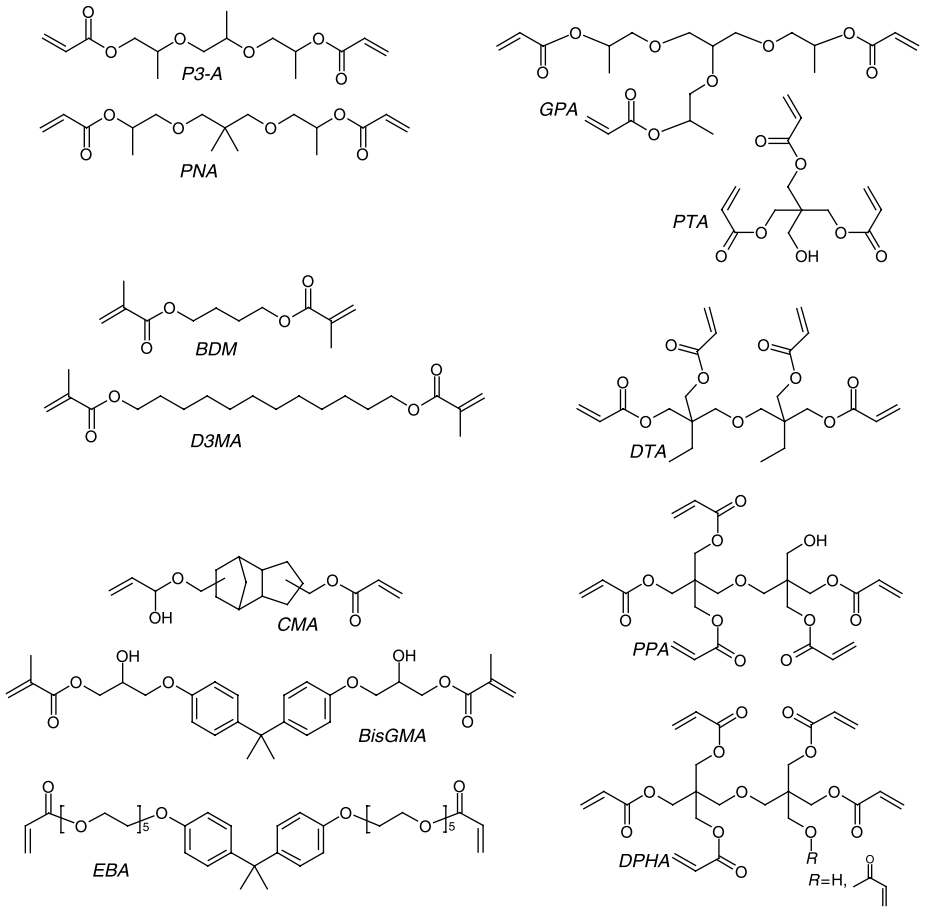

Fig. 2. Multi-acrylated monomers

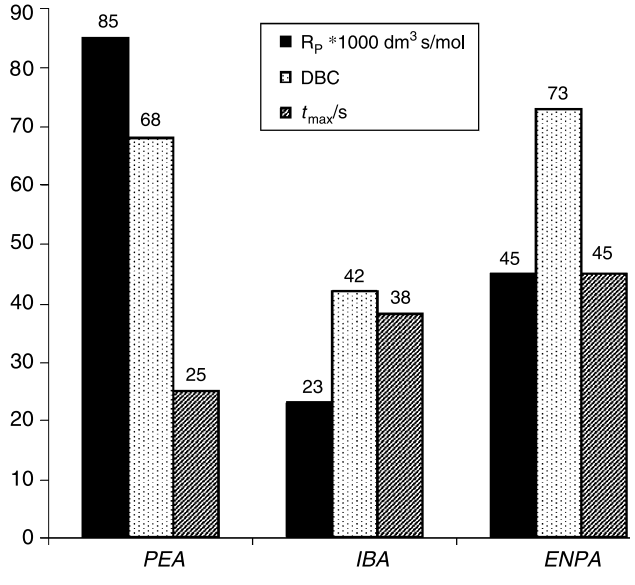

Fig. 3. Photo-DSC data of monofunctional monomers

The diacrylates tripropylene glycol diacrylate (*P3-A*) and propoxylated (**2**) neopentyl diacrylate (*PNA*) are very similar in their structure and showed therefore nearly identical polymerization behaviour. Dimethacrylates with aliphatic spacers (butandiol dimethacrylate (*BDM*) and *D3MA*) showed poor performance and are therefore not suitable from a viewpoint of reactivity. The presence of methacrylic groups is responsible for that fact. Comparably low DBC of *BDM* can be explained by the low molecular weight and less flexibility of the monomer, thus giving rigid and tight networks. If cyclic structures are present in the main chain as in tricyclodecanedimethanol diacrylate (*CMA*), ethoxylated (**10**) bisphenol A diacrylate (*EBA*) or *BisGMA*, highly reactive monomers can be found although in the case of *BisGMA*, a methacrylate is the reactive species. Some kind of preorganization similar to β-hydroxyacrylates might be possible [13]. Due to the flexible spacer in *EBA* a nearly complete DBC of 92% was observed.

In the case of acrylates having 3 reactive groups higher DBC of propoxylated (**3**) glycerol diacrylate (*GPA*) compared to pentaerythritol triacrylate (*PTA*) can be explained by the flexible core of *GPA*. In the

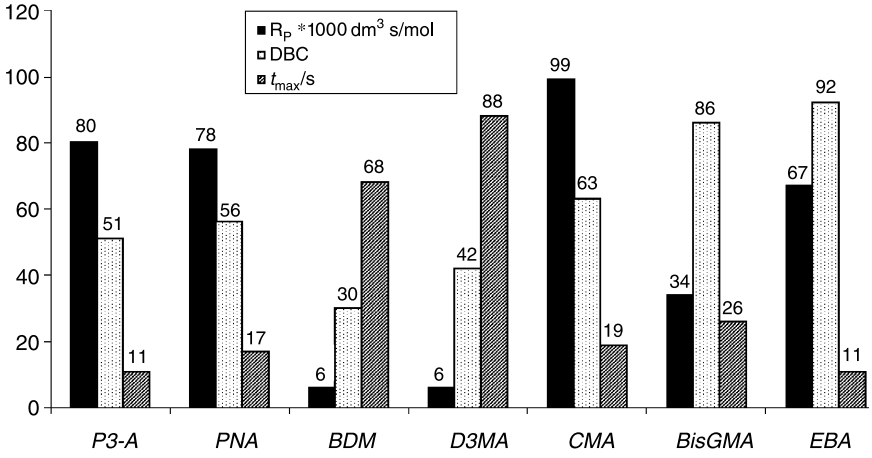

Fig. 4. Photo-DSC data of difunctional monomers

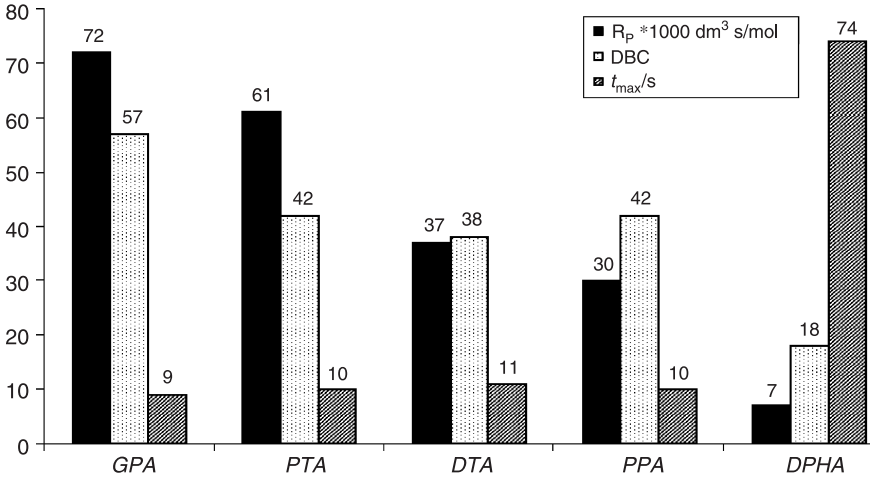

Fig. 5. Photo-DSC data of difunctional monomers

case of *PTA* tight and less flexible networks are formed.

Acrylates with more than 3 reactive groups gave a DBC below 50%, which can be well explained by the highly cross-linked network and therefore poor mobility of the remaining unreacted double bonds. t_{max} does not depend on the number of reactive groups.

Mechanical Properties

Polymers from mono-acrylated monomers were tested with 20 wt% trimethylolpropane triacrylate (*TTA*) as crosslinker – that was necessary to enable extraction of residual monomer and biocompatibility tests in aqueous culture medium.

In comparison with traditional biopolymers some of the polymers described in this work exhibit excellent strength and stiffness values. Several parameters that influence the mechanical properties can be derived from the summarized data in Figs. 6 and 7.

As expected polymers from di- or multi-acrylated monomers, which form dense networks, performed better than polymers from mono-acrylated monomers *PEA*, *IBA*, *TBA*, *ENPA*, that were only slightly crosslinked with an additional crosslinker. The exceptional low values of *ENPA* regarding strength and stiffness can be explained by the long and flexible side chain.

Although the monomers *P3-A* and *PNA* are very similar in their chemical structure, *P3-A* showed higher values for strength and stiffness. That might be explained by the more flexible aliphatic core of *PNA*. The monomers *BDM* and *D3MA* are characterized by a linear aliphatic spacer with different chain

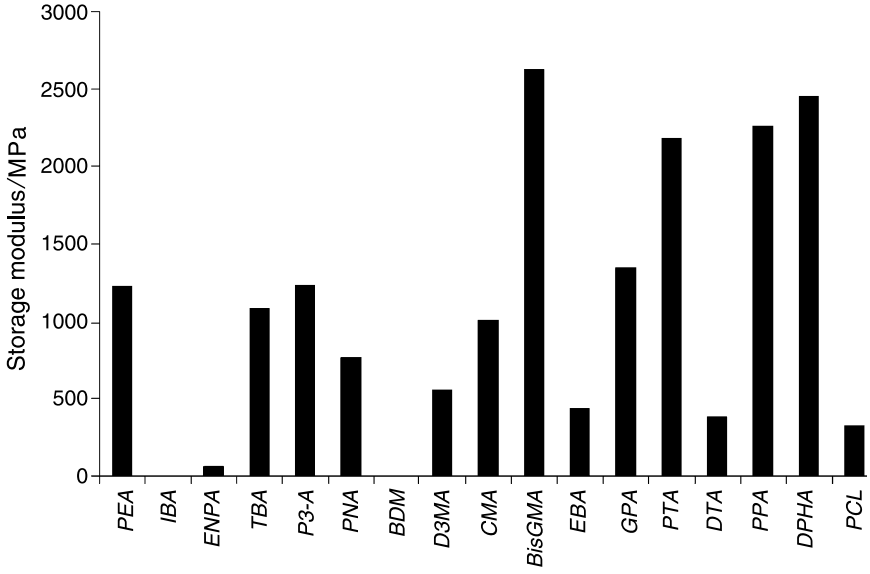

Fig. 6. Dynamical mechanical analysis, empty columns indicate that the material was not strong enough for a valid measurement

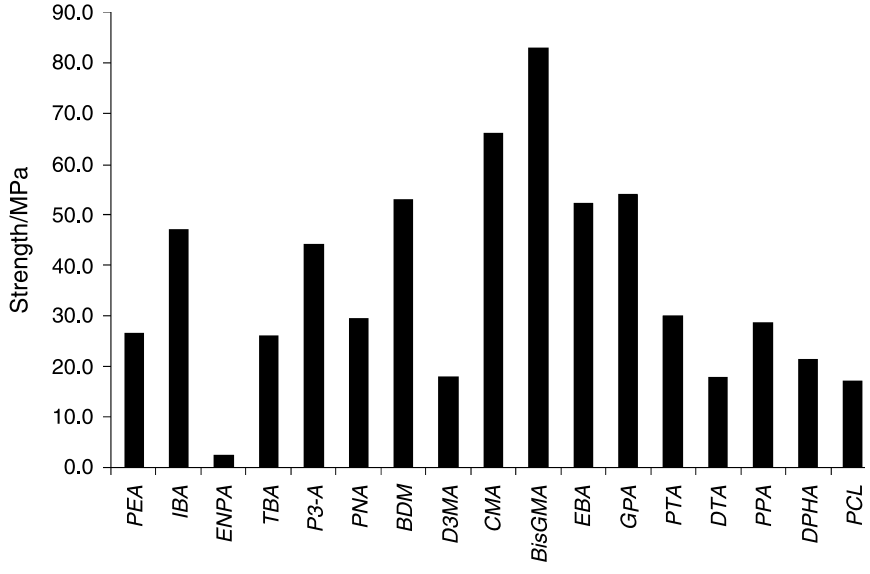

Fig. 7. Three point bending test

lengths. *D3MA* is known as flexibilizing component in dental restorative resins which is well demonstrated by the values obtained in our studies. In the case of the diacrylates *CMA*, *BisGMA*, and *EBA*, the cyclic rings led to excellent values for the strength. The low storage modulus of *CMA* and *EBA* compared to *BisGMA* could be explained by the flexible spacer and the absence of hydrogen bridges.

The triacrylates *GPA* and *TPA* differ in their spacer length, which is reflected in the values for strength and storage modulus. *PTA* gives tight networks resulting in a more brittle material, while *GPA* with its long flexible chains has a softer texture.

The comparably low strength of ditrimethylolpropane tetraacrylate (*DTA*), dipentaerythritol pentaacrylate (*PPA*), and dipentaerythritol penta/hexa-acrylate (*DPHA*) might again be assigned to the high crosslinking density and therefore brittle materials.

Promising materials from a mechanical point of view include polymers from *BisGMA*, well known

Table 1. Mechanical properties of common polymers and biomaterials. The values for *PE*, *PA*, and *POM* were obtained from the Cambridge Engineering selector (Granta Design)

Material	Strength/ MPa	Storage modulus/MPa
Polyamide (*PA*)	90	2800
Polyethylene (*PE*)	25	500
Polylactic acid (*PLA*) [16]	50	3500
Polycaprolactone (*PCL*) [17]	17	318
Polyoxymethylene (*POM*)	90	2900
Compact bone [18]	50–150	11000

from dental materials, but also *P3-A*, *GPA*, and *CMA*. All these materials exhibit strength and stiffness values comparable to or beyond commonly used biopolymers (see Table 1).

Biocompatibility

Test specimens of mono-acrylated monomers *PEA*, *IBA*, *ENPA*, and *TBA* were prepared using 20 wt% of *TTA* as crosslinker. Poly(ε-caprolactone) (*PCL*), a material already in clinical use was used as reference polymer (Fig. 8).

Generally, all polymers investigated showed similar or better performance in cell viability compared to the reference *PCL*. The comparably low values for *D3MA* and *ENPA* could be assigned to the soft and more flexible material – cell viability and proliferation are influenced by the mechanical properties of the material as has recently been shown [11, 14] – and to the presence of aliphatic and polyethylene glycol-like structures which is well known to withstand cell adhesion. The lower value for strength of *D3MA* compared to *BDM*, but more reasonable the long hydrophobic spacer between the ester groups led to a significant decrease in cell viability, although the chemical structure is very similar.

From a structural point of view, excellent results of *IBA* can be assigned to the camphor-like structure which is well known to show good biocompatibility.

The poor cell adhesion of *BisGMA* compared to *CMA* and *EBA* might be assigned to the presence of the hydroxy-groups which have shown recently to give poor values for cell adhesion [9]. The good values for stiffness and strength seem to be overruled by the chemical structure.

In the case of the monomers with 3 or more acrylate groups (*GPA*, *PTA*, *DTA*, *PPA*, *DPHA*), very

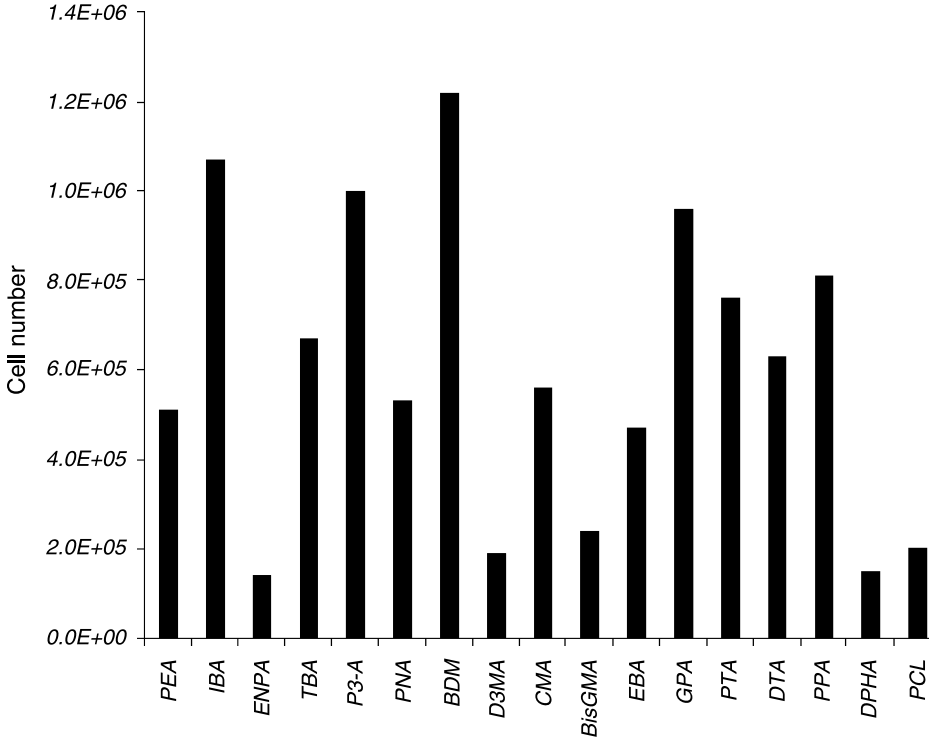

Fig. 8. Cell number on polymers

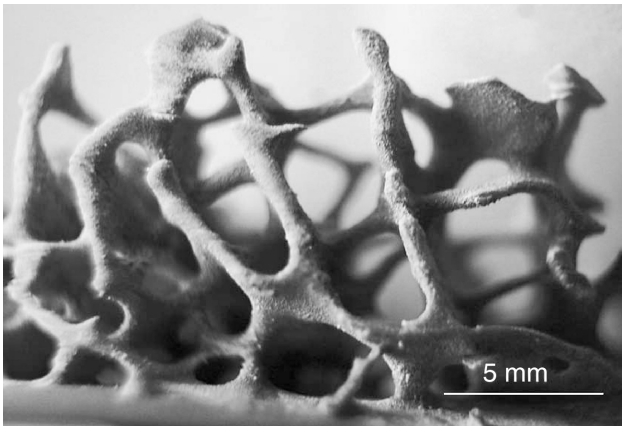

Fig. 9. Spongiosa-like structure prepared by stereolithography

similar cell numbers were observed except in the case of *DPHA*. Since mechanical properties could not explain this behavior, the high value of residual acrylate groups, as can be seen from the DBC, seems to be responsible.

3D-Scaffolds

The monomers presented in this work afford the opportunity of being shaped by stereolithography. Any monomer formulation can be used by adding 3 wt% of Irgacure 819 as biocompatible photoinitiator [9] and cellulose acetate butyrate to adjust the viscosity for the stereolithography process. The spongiosa-like structure shown in Fig. 9 was built using 30 wt% of hydroxy apatite as osteoconductive filler, to enhance the mechanical properties, to further improve the biocompatibility and to act as a calcium source in the body [15].

Materials and Methods

All reagents, unless otherwise noted, were purchased from Sigma-Aldrich and were used without further purification. The monomers *IBA*, *TBA*, and *PTA* were also obtained from Sigma-Aldrich. *BDM* was received from Merck. Further monomers – obtained as a gift – are: *D3MA* (Ivoclar Vivadent), *BisGMA* (Ivoclar Vivadent), *P3-A* (Miramer M220, Rahn AG), *GPA* (Miramer M320, Rahn AG), *TTA* (Genomer 1330, Rahn AG), *PPA* (Sartomer 399, Cray Valley), *PNA* (Sartomer 9003, Cray Valley), *DTA* (Sartomer 355, Cray Valley), *PEA* (Sartomer 339C, Cray Valley), *ENPA* (Sartomer 504, Cray Valley), *CMA* (Sartomer 833S, Cray Valley), *EBA* (Ebecryl 150, Cytec), and *DPHA* (85–90% hexa-acrylate, Cytec).

Differential Scanning Photocalorimetry

Differential scanning photocalorimetry (Photo-DSC) was conducted with a modified Shimadzu DSC 50 equipped with a home-made aluminium cylinder (height 6.8 cm). The mass of the samples was 5 ± 0.2 mg. The measurements were carried out in an isothermal mode at room temperature under air atmosphere for at least 5 min. Filtered light (320–500 nm) was applied by a light guide (EXFO Omnicure 2000) attached to the top of the aluminium cylinder. A light intensity of $1000\,mW/cm^2$ at the tip of the light guide was used. The time to reach the maximum heat of polymerization (t_{max}/s), the total double bond conversion (DBC/%) and the initial rates of polymerization ($R_p/mol/dm^3\,s$) were calculated.

Mechanical Testing

To investigate the mechanical properties of the selected polymers, dynamical mechanical analysis and 3-point bending tests were carried out. For this purpose test specimens (beams with 20 mm length, 3 mm width, and 3 mm height) were made from the monomers with 1 wt% of an equimolar mixture of *CQ* and *DMAB* as initiator. Photocuring was performed with a high pressure mercury lamp (1000 W, distance 15 cm) under N_2 within 3–10 min depending on the type of monomer. Afterwards, the test specimens were extracted with different organic solvents ($CHCl_3$, *Me*OH, *Et*OH), phosphate buffered saline (*PBS*), and H_2O in an ultrasonic bath to remove residual monomer similar to the tests of biocompatibility. Mono-acrylates were cured with 20 wt% of *TTA* as crosslinker, because the linear homopolymers would be destroyed in the extraction step. These copolymers were also used for biocompatibility tests to avoid swelling and dissolution in the cell culture.

To determine the elastic modulus, the beams were placed in a dynamic mechanical analysis machine (TA Instruments DMA 2980) with a span-width of 20 mm. An extra initial load was applied in order to assure the direct contact between the sample and the clamp. The beams were tested with a frequency of 1.0 Hz in the range between 10 and 50°C.

The bending strength and the failure strain were measured with a universal tensile testing machine (Zwick Z050, Zwick/Roell). The maximal strain applicable in the middle of the beam was determined. A preload of 0.5 N was used and the velocity of the crosshead was 5 and 10 mm/min after 0.25% strain.

Biocompatibility

Test specimens were made from previously selected monomers to verify their biocompatibility. In the case of mono-acrylates 20 wt% crosslinker *TTA* was added. In all cases 1 wt% of an equimolar mixture of *CQ* and *DMAB* was used as initiating system. The mixture was filled into a silicon mold (0.9 cm diameter, 0.15 cm height) and photocured with a high-pressure mercury lamp (1000 W, distance 15 cm) under N_2. Depending on the type of monomer, the curing time was between 3 and 10 min. Afterwards, the test specimens were extracted with different organic solvents ($CHCl_3$, *Me*OH, *Et*OH), phosphate buffered saline (*PBS*), and H_2O in an ultrasonic bath to remove residual monomer.

To estimate whether osteoblasts accept the new polymers as growth support (biocompatibility) measurements of cell viability and multiplication of cells of the osteosarcoma cell line MG63 with the cell proliferation assay EZ4U (Biomedica, Austria) were used. This assay is based on the conversion of an uncoloured tetrazolium salt into a formazan dye by the mitochondria of living cells.

Cells were kept in a humidified air under 5% CO_2 at 37°C. Culture medium was αMEM (minimum essential medium) supplemented with 5% FBS (fetal bovine serum), 4.5 g/dm^3 glucose and 30 $\mu g/cm^3$ gentamicin. For propagation cells were sub-cultured twice a week using 0.001% pronase E (Roche) and 0.02% *EDTA* in Ca^{2+} and Mg^{2+}-free phosphate-buffered saline (PBS) before reaching confluence.

The test specimens were placed into a multiwell plate and sterilized for 30 min in a distance of 14 cm with a 15 W UV lamp (Sylvana) on both sides. Thereafter, the space between the test specimen and the wall of the well were closed with agarose, cells were seeded at a density of 50000 cells/cm^2 and cultured for three days. Then, a change to fresh culture medium was performed and after 1 h the assay mixture was added. After a further 3 h culture period the colour of the medium was measured in a microplate reader at 492 nm against 620 nm. The measured extinction was converted to cell number by a calibration curve performed in separate experiments. Statistical analyzes were performed by ANOVA with *Scheffe*'s post hoc test using StatView 4.5 (Abacus Concepts, Inc. CA) and $P > 0.05$ was considered to be significant.

Acknowledgement

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