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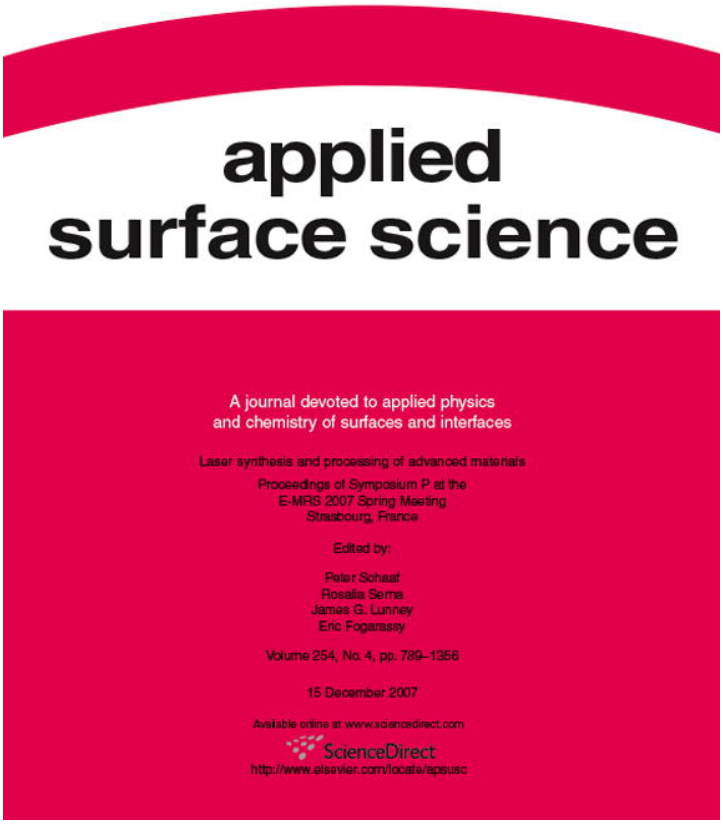

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3D-shaping of biodegradable photopolymers for hard tissue replacement

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Abstract

Continuously increasing life expectancy results in a rising number of bone diseases and fractures. Replacements from natural sources are not only limited in their availability, they also have some serious disadvantages such as possible immunological reactions or transmission of diseases. New synthetic biodegradable materials based on photopolymers could be an alternative solution. In these investigations an acrylate-based monomer formulation has been developed, consisting of a biodegradable basis monomer which is derived from gelatin, different reactive diluents, an appropriate photoinitiator and filler materials. For the three-dimensional shaping process stereolithography is the method of choice because of its capability to produce cellular structures with high resolutions.

Keywords: Photopolymerization; Bone replacement material; Stereolithography; Biodegradable

1. Introduction

The disadvantages of materials that are currently used in orthopeadic surgery have led to the development of new synthetic biodegradable materials. Degradable polymers that are already in clinical use are usually based on polyesters such as poly(ε-caprolactone) or poly(α-hydroxy acids) (e.g. copolymers of lactic and glycolic acid). These polyesters, which are used for surgical sutures and fixation elements cannot be used in the case of larger defects, e.g. after removal of a bone tumor, because of their hydrolytic degradation, which causes quite fast loss in mechanical strength. Moreover, the locally high concentration of free acids can result in tissue necrosis.

To overcome the problem of uncontrolled hydrolytic cleavage of ester containing monomers, biodegradability can also be introduced by amide-based crosslinkers that can be cleaved enzymatically in vivo. Compared to the polyesters, which are only processable by solvent or melt techniques, such liquid acrylamide-based formulations are suitable for fast and UV-controlled curing using stereolithography. By layer-by-layer photopolymerization objects of any conceivable shape can be produced. Fig. 1 shows the overall planned pathway.

In these investigations we aim at the development of a formulation that achieves the requirements from both, the medical and the fabrication point of view. For that, different components are required, such as a biodegradable basis monomer, which is in our case based on gelatin because of its natural origin and enzymatic degradation mechanism. Secondly, different reactive diluents are used to adjust network density and mechanical properties. A third component should be a biocompatible photoinitiator with appropriate absorption behavior. In addition, Ca-based fillers can be applied to act as a Ca source in the body and further improve the mechanical stability of the implant. Therefore reactivity, biocompatibility and mechanical properties of different potential components were investigated.

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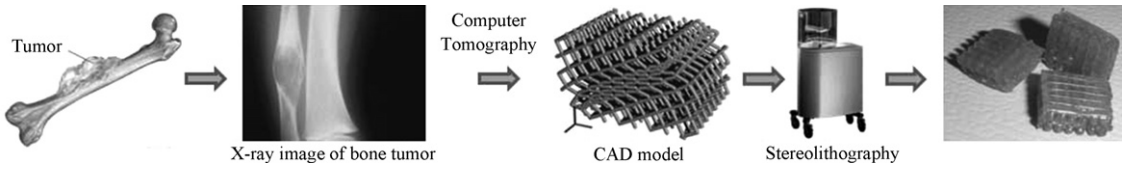

Fig. 1. Pathway towards cellular biophotopolymers.

2. Experimental

2.1. Materials

All reagents, unless otherwise noted, were purchased from Sigma–Aldrich and used as received. The monomer urethane-dimethacrylate (UDMA) was obtained from IVOCLAR VIVADENT AG as a gift. Photoinitiators Irgacure 819 and Irgacure 2959 were obtained from Ciba SC as a gift.

2.2. Methods

2.2.1. Syntheses

The monomer diisobutylacrylamide (DBA) was prepared as described [1]. As biodegradable basis monomer a gelatin hydrolysate was modified with methacrylamide moieties and additional moieties for improved organo-solubility. In a typical procedure 5 g of gelatin were first dialyzed against deionized water (24 h), then the free amino groups of the lysine units were modified with a poly(ethylene glycol) methylether succinate [2] (PEG 1000, 1.5 equiv., N_2, room temperature, dry DMSO, 24 h) and subsequently free acidic groups were reacted with glycidylmethacrylate (2 equiv., 40 °C, water, 16 h). Purification was performed by dialysis against deionized water. NMR spectroscopy was used to verify the degree of substitution.

2.2.2. Reactivity

The reactivity of the monomers and photoinitiators was investigated by differential scanning photocalorimetry (Photo-DSC) using a modified Shimadzu DSC 50 equipped with a aluminium cylinder [3] and a light guide (EXFO Omnicure 2000). Filtered light (320–500 nm) with an intensity of 1000 mW/cm^2 at the tip of the light guide was used.

2.2.3. Mechanical properties

Test specimens (rods, 3 mm × 2 mm × 30 mm) were prepared by photopolymerization in silicon molds and subsequent extraction of any residual monomer. To determine the storage modulus, the beams were placed in a dynamical mechanical analysis machine (TA Instruments DMA 2980, frequency 1.0 Hz, 10–50 °C) with a span-width of 20 mm. The bending strength and failure strain were measured with a universal tensile testing machine (Zwick Z050).

2.2.4. Biocompatibility

Test specimens were prepared, extracted, sterilized and seeded with MG63 osteoblast-like cells. Cell viability and multiplication was measured with EZ4U (Biomedica, Austria).

2.2.5. Stereolithography (SL)

A prefactory SL-machine from Envisiontec working with the digital light processing (DLP) principle was used to build

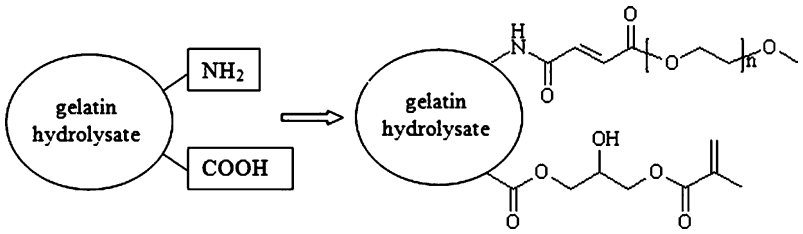

Fig. 2. Biodegradable basis monomer.

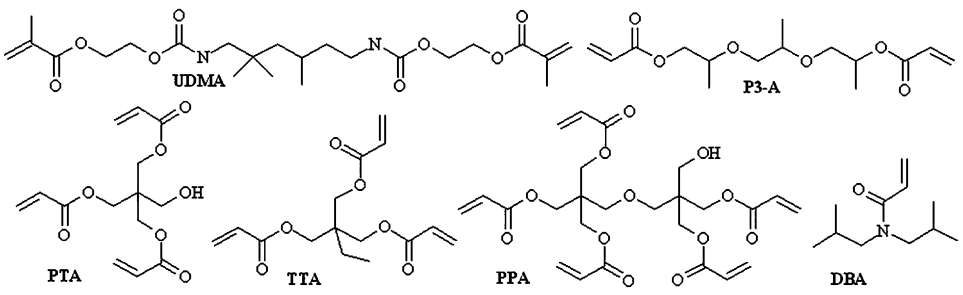

Fig. 3. Reactive diluents.

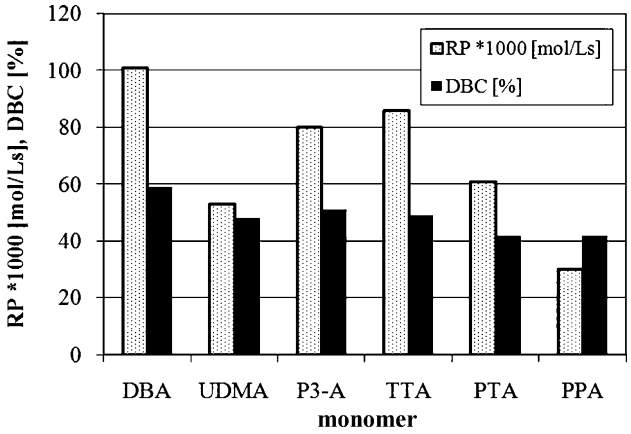

Fig. 4. Photoreactivity: rate of polymerization and double bond conversion of the tested reactive diluents at 320–500 nm.

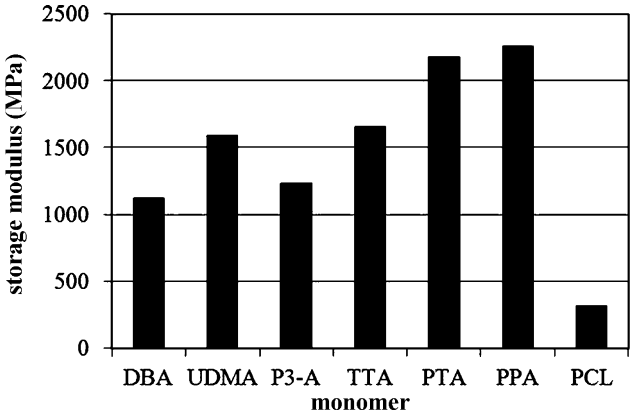

Fig. 6. Dynamical mechanical analysis: measurement of the storage modulus of test specimens.

three-dimensional objects. Since the resolution of the utilized DLP-process is limited to 40 μm, additional experiments with a UV-laser-based micro-stereolithography system were performed, enabling feature resolutions in the xy-plane down to 5 μm.

3. Results and discussion

Modified gelatin (Fig. 2) was used as *biodegradable basis monomer*, which was chosen because of its natural origin and good biocompatibility. Moreover the degradation follows a controllable enzymatic mechanism and gelatin provides a good substrate for bone cell adhesion due to its RGD sequences. The basis monomer was synthesized by modification of gelatin hydrolysate with poly(ethylene glycol) chains for improved organo-solubility and glycidylmethacrylate to enable copolymerization.

Processing properties of the formulation and network density of the polymer can be tuned by *reactive diluents*. A variety of commercially available photocurable monomers, having different functional groups and various numbers of acrylic groups per molecule (Fig. 3 shows some examples) were tested concerning reactivity (photo differential scanning calorimetry; Fig. 4), biocompatibility (cell seeding experiments with MG63 osteoblast-like cells; Fig. 5), and mechanical properties (dynamical mechanical analysis and bending strength test, Figs. 6 and 7).

Photoreactivity is an important selection criterion because double bond conversion (DBC) and short building times with SL are desired. Differential scanning photocalorimetry is a unique method for the fast and accurate evaluation of the photoreactivity of monomers. Various important parameters are obtained with one single measurement. The time to reach the maximum polymerization heat, the overall heat evolved and the height of the peak were measured and used to calculate the DBC and the rate of polymerization (R_P) [4,5]. As expected, the monofunctional acrylamide DBA gave the highest value for R_P, because it has the lowest molecular weight and therefore the highest mobility, and moreover acrylamides generally have a higher R_P than corresponding acrylates. Dipentaerythritol pentaacrylate (PPA), a monomer with five acrylic moieties, shows the lowest R_P and also the lowest DBC, which can be explained by the highly crosslinked network and therefore poor mobility of the remaining unreacted double bonds. By mixing monofunctional and polyfunctional monomers the network density and DBC can be optimized.

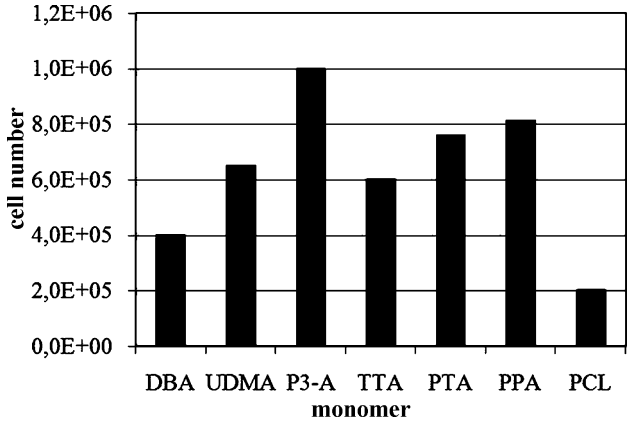

Fig. 5. Biocompatibility: cell adhesion of MG63 osteoblast-like cells on polymer specimens.

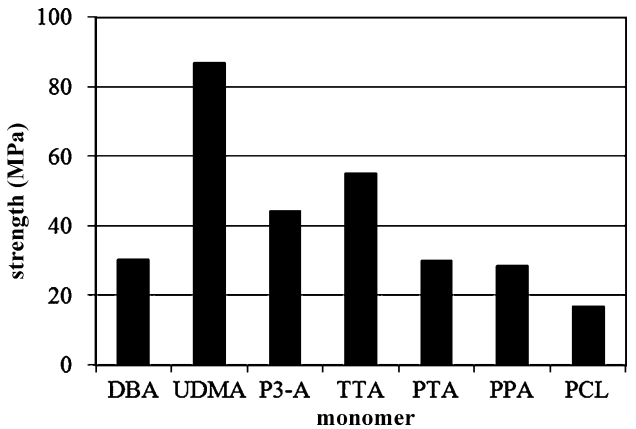

Fig. 7. Bending strength test: measurement of the strength of test specimens.

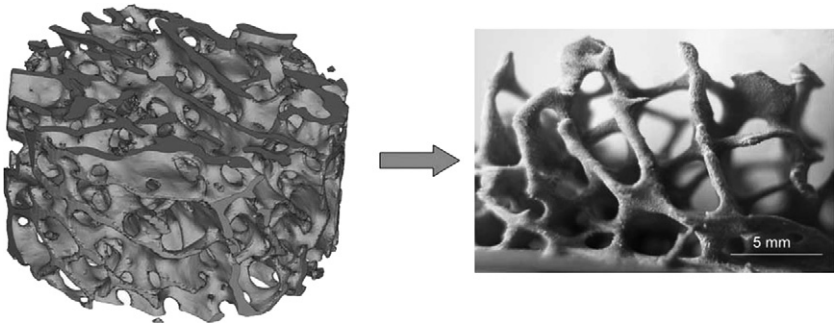

Fig. 8. Spongiosa-like structure prepared by stereolithography.

To gain an idea about the *mechanical properties* of the selected polymers, dynamical mechanical analysis and bending strength tests were performed. PPA and pentaerythritol triacrylate (PTA) give tight networks which results in a material with high storage modulus but also with certain brittleness. UDMA is able to build hydrogen bonds which causes the high strength. By use of 60 wt% of hydroxy appatite as *filler* the storage modulus of a trimethylolpropane triacrylate (TTA) based typical resin rises by a factor of 4 and reaches 3/4 of the storage modulus of bone, which lies at 11,000 MPa. The strength of UDMA (87 MPa) comes close to that of natural bone (100–150 MPa), even without filler.

Poly(ε-caprolactone) (PCL), a biodegradable polymer already in clinical use was used as a reference material for cell seeding experiments (*biocompatibility*). Generally, all polymers investigated showed better performance in cell viability than the reference material. It has recently been shown [6,7] that cell viability and proliferation are influenced by the mechanical properties of the material. MC3T3-E1 osteoblast-like cells, which are a model system of differentiating osteoblasts, increase their proliferation rate dramatically when the stiffness of the substrate increases. We found that PPA and PTA which have a comparably high storage modulus are good substrates for bone cells. But the mechanical properties are not the only influencing factor, since tripropylene glycol diacrylate (P3A) which has a lower storage modulus shows the highest cell number. Based on the available data and the chemical structure this effect cannot be explained clearly.

Possible *photoinitiators* with different absorption characteristics—namely camphorquinone with *N*,*N*-dimethylamino ethylbenzoate as coinitiator, Irgacure 819 and Irgacure 2959—were also tested for reactivity and biocompatibility [4]. All of them can be used for the desired application.

A *monomer formulation* was created according to the above shown results. Beside the modified gelatin it contained UDMA and DBA as reactive diluents, 3 wt% Irgacure 819 and 30 wt% hydroxy appatite. Fig. 8 shows a spongiosa-like structure that was built with a SL-machine using the digital light processing principal. Here a CAD model of the desired object is sliced and the image of every slice is transferred into the bottom of a resin tank where layer-by-layer photopolymerization takes place according to the images. Feature resolutions of 30–50 μm are possible with this machine.

4. Conclusions

In the present work, the evaluation of different acrylic compounds for the fabrication of cellular bone replacement materials by SL is presented. Potential components were tested concerning reactivity, mechanical properties and biocompatibility. A modified gelatin serves as biodegradable basis monomer. All the shown reactive diluents are promising candidates for the desired application due to their good results in all criteria compared to the performance of the usually applied thermoplast PCL.

Acknowledgments

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