

# Biodegradable stereolithography resins with defined mechanical properties

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Solid Freeform Fabrication methods based on photopolymerization (e.g. stereolithography - SLA, digital light processing DLP, two photon absorption TPA) offer the possibility to shape complex structures with high feature resolution and excellent surface quality. If these methods are to be used for applications in regenerative medicine, new photopolymers with defined functional and mechanical properties have to be developed.

For applications in the field of bone replacement materials scaffolds with defined external and internal geometry are required. The ideal pore size is between 200 and 500 $\mu\text{m}$  which is an achievable minimum feature resolution for lithographic methods. Further requirements are non-toxicity of the scaffolds which requires proper selection of monomers and photoinitiators. Using acrylate modified gelatine as crosslinker, the final photopolymer can be made biodegradable by enzymatic mechanisms. To further increase the elastic modulus, hydroxyapatite can be used as filler material.

Using these polymers cellular scaffolds have been built and osteoblast like cells adhered and proliferated excellently. The obtained elastic moduli can range from 0,1 MPa to 8.000MPa.

## 1 INTRODUCTION

Rapid Prototyping (RP) methods have gained increasing interest as manufacturing method for the fabrication and characterization of cellular biomaterials. RP can be used to deliver experimental data for the finite element simulations on the mechanical properties of cellular structures (Luxner et al. 2005), (Luxner et al. 2007). RP is also used widely to fabricate cellular scaffolds for tissue engineering applications (Manjubala et al. 2005), (Woesz et al. 2005). Various RP techniques have been applied to shape the required materials. Special emphasis has been put on methods based on Fused Deposition Modeling (Hutmacher 2001) and 3D-printing (Leukers et al. 2005) (Lam et al. 2002). Due to a lack of available resins, lithographic methods have been used only to a small extent up to now.

## 2 FABRICATION

RP methods based on photopolymerization (SLA, DLP) offer several advantages compared to other RP-methods regarding the fabrication of cellular structures for biomedical applications. In contrast to other RP-techniques (e.g. Fused Deposition Modeling, Selective Laser Sintering) all processing steps take place at room temperature. This enables the incorporation of temperature sensitive materials (e.g. growth factors) into the build material. Furthermore, lithographic methods are capable of producing parts with excellent feature resolution and small layer thicknesses. The feature resolution of commercially available SLA- and DLP-systems is around 25 $\mu\text{m}$ , research systems are capable of achieving resolutions down to 5 $\mu\text{m}$ . By using two-photon-processes feature resolutions <300nm can be achieved (Serbin et al. 2003), (Heller et al. 2007).

Furthermore photosensitive resins can be tailored fairly easily regarding their biological and mechanical properties. By choosing various base monomers the biocompatibility and biodegradability can be tuned and by changing the degree of crosslinking the elastic modulus and the strength of the final product can be varied over several orders of magnitude.

Issues with photoreactive resins are mostly related to their toxicity and cell compatibility. A careful choice of the utilized monomers together with appropriate post-processing methods can help to solve these problems.

### 3 MATERIALS

In this work we aim at the development of acrylate-based formulations for cellular structures, which can be photopolymerized directly by stereolithography. To tune the material properties regarding processability, biocompatibility as well as mechanical and degradation properties several components such as crosslinkers, reactive diluents, fillers and initiators have been considered. To overcome the problem of uncontrolled hydrolytic cleavage of ester containing monomers, biodegradability is introduced by multi-acrylated crosslinkers based on gelatin (Schuster et al. 2005) that can be cleaved enzymatically *in vivo*. Processing properties of the formulation and the network density of the polymer can be tuned by reactive diluents. Soluble filler materials are applied to tune the viscosity for an optimum resolution of the stereolithographic shaping process. Adequate photoinitiators are required as well as ceramic fillers which can increase the elastic modulus of the material significantly.

#### 3.1 Biocompatible photopolymers

In order to study the suitability of commercially available monomers for use as biocompatible resins, a large number of materials was investigated in previous work (Schuster, Turecek, Kaiser, Stampfl, Liska, and Varga 2007), (Schuster, Turecek, Mateos, Stampfl, Liska, and Varga 2007). Materials, which turned out to be useful as substrate for the cultivation of osteoblast like cells (see Sec. 4) included the following monomers: N,N-Diethyl-1,3-propylenbisacrylamide (EPA), methacrylic acid 2-hydroxy-ethyl ester (HEMA), N,N-dimethyl-acrylamide (DMA), acrylic acid (AA), 2-methyl-acrylic acid 2,2,2,4-trimethyl-6-[2-(2-methyl-acryloyloxy)-ethoxycarbonylamino]-hexylcarbamoyloxy-ethyl ester (UDMA), ethoxylated trimethylolpropane triacrylate (TTA), methacrylic acid (MA), dipentaerythritol pentaacrylate (PPA) and N,N-diisobutyl-acrylamide (DBA).

The chemical structure of the utilized monomers is depicted in Fig. 1. As photoinitiators Irgacure 819 (Bis(2,4,6-trimethylbenzoyl)phenylphosphine oxide) and Irgacure 2959 (2-Hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone) were used.

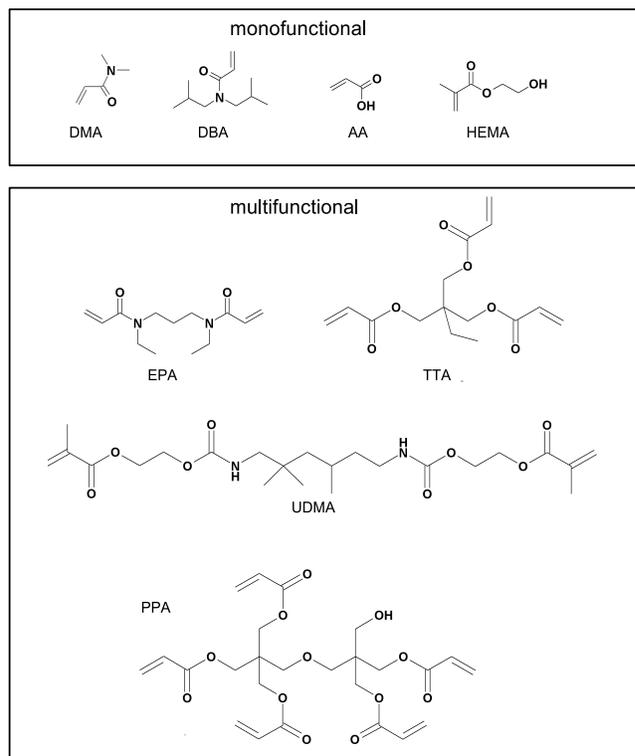


Figure 1: Chemical structure of the utilized monomers.

#### 3.2 Biodegradable photopolymers

A formulation for 3D-photoshaping of bioresorbable bone replacements must consist of several components to tune material and degradation properties. The basis substance should be a biocompatible crosslinker with bonds that can be cleaved *in vivo*. There are two possible ways to achieve biodegradation: by introduction of ester or amide bonds.

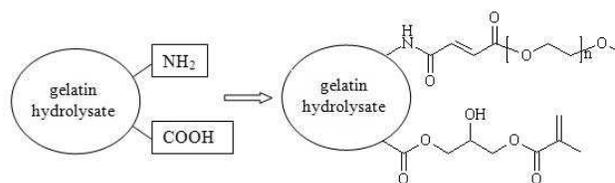


Figure 2: Chemical structure of photopolymerizable gelatine.

Most known biodegradable polymers are based on polyesters like poly(lactic acid), which have the disadvantage that they follow a hydrolytic, autocatalytic

Table 1: Mechanical properties measured in 3-point bending of various photopolymers. The biocompatibility of the respective monomer was measured by the number of cells on the samples which was counted using a standard protocol. The values of PCL are for reference.

Material	Young's modulus $E$ MPa	Bending strength $\sigma_b$ MPa	Cell number
DMA + 20wt%EPA	2.900	55	350.000
DBA + 20wt%EPA	1.810	51	160.000
AA + 20wt%EPA	4.920	93	–
EPA	3.250	42	520.000
UDMA	2.880	87	350.000
TTA	2.010	54	280.000
PPA	2.200	30	800.000
PCL	318	17	400.000

bulk erosion mechanism, which results in quite fast loss in mechanical stability and causes a high amount of free acid, which can damage the surrounding tissue. The other possibility is to use crosslinkers with amide bonds, which degrade by a controlled enzymatic mechanism. From results of preliminary work, methacrylated gelatin hydrolysate (MG) derivatives will be selected which offer also better cell adhesion and yields predominantly natural degradation products. The basis monomer was synthesized by modification of gelatin hydrolysate with poly(ethylene glycol) chains for improved organo-solubility and glycidylmethacrylate to enable photopolymerization (see Fig. 2).

#### 4 BIOCOMPATIBILITY

Test specimens were made from previously selected monomers to verify their biocompatibility. In the case of mono-acrylates 20 wt% crosslinker EPA was added. In all cases 1 wt% of an equimolar mixture of camphorquinone (CQ) and N,N-dimethylaminobenzoic acid ethyl ester (DMAB) was used as initiating system. The mixture was filled into a silicone mold (0.9 cm diameter, 0.15 cm height) and photocured. Afterwards, the test specimens were extracted with different organic solvents, phosphate buffered saline (PBS) and water 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 MG63 osteoblast-like cells were used. Cells were kept in humidified air under 5% CO<sub>2</sub> at 37°C. Cells were seeded at a density of 50.000 cells/cm<sup>2</sup> and cultured for three days. Finally, fresh culture medium was added together with the assay mixture. After a further 3 hours culture period the colour of the medium was measured in a microplate reader at 492 nm against 620nm. The measured ex-

inction was converted to cell number by a calibration curve performed in separate experiments. The measured cell numbers are presented in Tab. 1. High cell numbers indicate good biocompatibility. For comparison, poly- $\epsilon$ -caprolactone (PCL) was used as reference material. Details about the used procedures can be found in (Schuster et al. 2007).

#### 5 MECHANICAL PROPERTIES

Using the materials described in Sec. 3 beams were fabricated by filling a silicone mold with the liquid resin and polymerizing the resin using UV-light. The obtained beams were tested on a universal testing machine in three-point bending in order to obtain bending strength  $\sigma_b$  and elastic modulus  $E$  of the tested material. The obtained results for monofunctional as well as multi-functional materials are listed in Tab. 1. Values for a commercially used biopolymer, polycaprolactone, are listed for reference. Strength and modulus values between the various polymers differ by a factor of 2 and are consistent with values known from other thermosetting polymers (e.g. stereolithography resins based on epoxy or acrylate-chemistry).

Mainly responsible for the measured  $E$ -values is the degree of crosslinking. In order to lower the elastic modulus, the degree of crosslinking can be reduced (e.g. by using photo-polymerizable hydrogels) and elastomer-like behaviour is achieved. Mechanical properties of such a hydrogel based on 50wt% cyanoethylacrylate are listed in Tab. 2. In order to increase the stiffness of the final part, particle reinforced photopolymers can be used. A composite consisting of a TTA/HEMA-matrix with 60wt% hydroxyapatite yields an elastic modulus of 8.000 MPa and a bending strength of 70 MPa, which is close to the values of natural bone, see Tab. 2. When particle filled resins are debinded and sintered, fully dense ceramic parts can be fabricated using stereolithography. Thus the

range of elastic moduli of SLA-shapeable materials covers a range of more than six orders of magnitude.

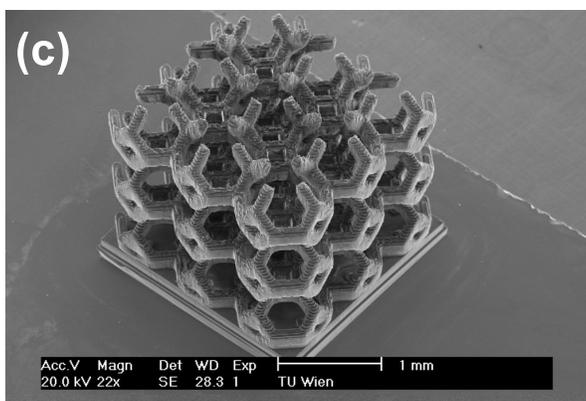
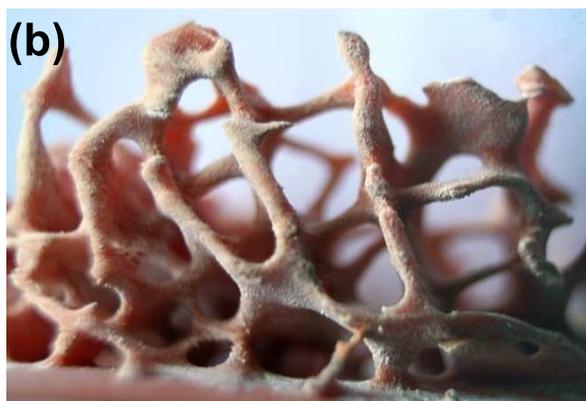
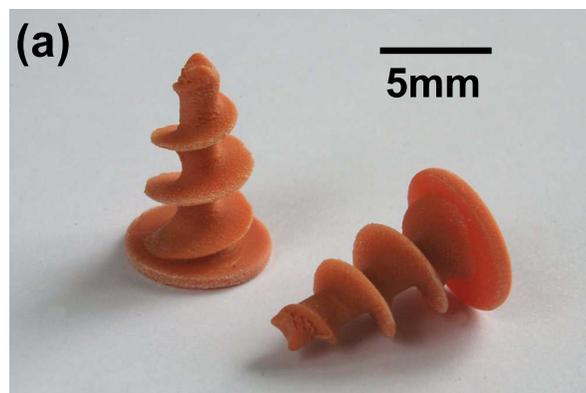


Figure 3: Parts made by structuring biophotopolymers using DLP (a, b) and microstereolithography (c).

## 6 EXAMPLE PARTS

Using the materials described above three-dimensional parts were fabricated by two different methods: A commercial system based on digital light processing (Perfactory Mini, Envisiontec GmbH) was utilized to fabricate the screw- and spongiosa-like structures depicted in Fig. 3 (a) and (b). The Perfactory allows to shape parts with a xy-resolution

of  $45 \mu\text{m}$  and a minimum layer thickness of  $25 \mu\text{m}$ . The resin is exposed from underneath through a silicone vat. Since each individual layer is exposed in one shot, the build speed (approximately 5-15 vertical mm/hr) is fairly high. The vat is only coated with a thin layer of resin, thus the required amount of initial resin is quite low (50-100ml). Drawbacks of the method are mostly related to the attachment of the polymerized resin to the transparent silicone layer. Especially monomers with low molecular weight tend to migrate into the silicone. In this case the silicone layer can be destroyed after a couple of exposures, requiring a replacement of the vat.

Alternatively, a microstereolithography system (micro-SLA) based on a UV-laser was utilized. The details of the setup are described in (Neumeister et al. 2007). The xy-resolution for this system is around  $5 \mu\text{m}$  and the minimum layer thickness is  $10 \mu\text{m}$ . A cellular structure fabricated with this process is depicted in Fig. 3 (c). In contrast to the DLP-systems there are no issues with swelling of the silicone vat since the micro-SLA uses a traditional approach where the resin is solidified at its upper surface. Care has to be taken to use resins with low viscosity, otherwise a consistently thin layers are hard to achieve. Since the resin surface is in contact with air, oxygen inhibition might play a role with some monomers.

## 7 CONCLUSIONS

RP methods based on photopolymerization are a suitable technique for shaping complex parts with high feature resolution. Using appropriate photopolymers and initiators, excellent mechanical (strength, stiffness) and biological functionality could be achieved. By varying the degree of crosslinking, the used base monomers and cross-linkers and the amount of particulate fillers, the functional and structural properties of the final product can be tailored to a large extent.

## 8 ACKNOWLEDGEMENT

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Table 2: Mechanical properties of ceramic ( $\text{Al}_2\text{O}_3$ ), composite (TTA/HEMA filled with hydroxyapatite particles), elastomer-like and biodegradable (gelatin-HEMA) materials that can be shaped with stereolithography. Values for natural bone are listed for comparison.

Material	Young's modulus $E$	bending strength $\sigma_b$
	MPa	MPa
photo-elastomer	0,1	-
TTA/HEMA with 30 weight% HAP	8.000	70
gelatin-HEMA- polyethylenglykoldiacrylat (dry)	1.300	30
alumina	380.000	300
natural bone	10.000-15.000	100-150

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