Biofunctional Photopolymers for Micro-Stereolithography

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Micro-Stereolithography (μSL) is a computerized fabrication technique that has gained increasing interest in tissue engineering applications because of its ability to produce objects with complex geometry and inner structure, like cellular structures as they appear in natural bone. In these investigations we aim at the development of a new polymer for tissue engineering, which can be formed by μSL and fulfills the requirements from the medical point of view. For that an acrylate-based monomer formulation has been developed by testing several commercially available acrylic compounds concerning photoreactivity, mechanical properties and biocompatibility. By use of a modified gelatin as a photoreactive crosslinker biodegradability can be introduced into the polymer. Further components of such a formulation are appropriate photoinitiators and filler materials to further improve the mechanical properties and to act as Ca source in the body in the case of bone replacement.

Keywords: Tissue engineering, micro-Stereolithography, biodegradable, photopolymer

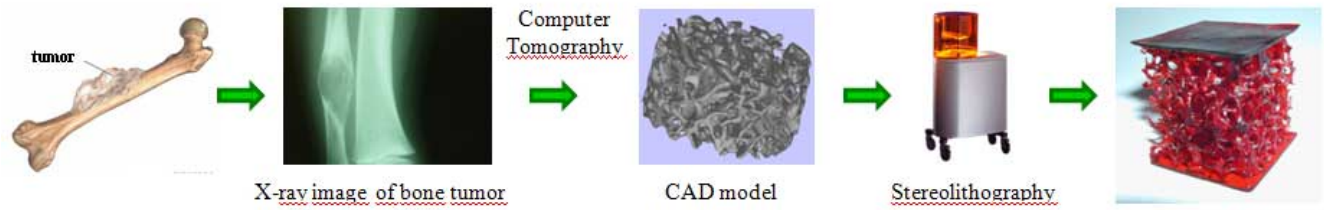


Figure 1: Pathway towards cellular biophotopolymers

1. Introduction

Tissue 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 are under investigation since many years. 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) [1]. These polyesters are suitable for surgical sutures and fixation elements, but their hydrolytic degradation mechanism limits their applicability. Especially in the case of hard tissue replacement e.g. after removal of a bone tumor, they cannot be used, because the autocatalytic degradation 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. For that purpose, proteins with polymerizable moieties, e.g. acrylic groups, can be used. If this acrylate modified protein is mixed with other acrylic compounds as reactive diluents, one gains a liquid monomer formulation that can easily be turned into any desired shape using Stereolithography (SL). Figure 1 shows the overall planned pathway.

In contrast to other Rapid Prototyping Techniques (e.g. Fused Deposition Modeling, Selective Laser Sintering, which are used for the fabrication of polyester based biomaterials) all processing steps take place at room temperature. This enables the incorporation of temperature sensitive materials (e.g. growth factors) into the material. Furthermore, lithographic methods are capable of producing parts with excellent feature resolution and small layer thicknesses. The feature resolution of commercially available SL-systems is around 25μm, research systems are capable of achieving resolutions down to 5μm. By using two-photon-processes feature resolutions <300nm can be achieved [2].

Furthermore photosensitive resins can be tailored fairly easily regarding their biological and mechanical properties. By choosing various base monomers the hydrophility of the surface can be tuned and by changing the degree of

crosslinking the elastic modulus of the final product can be varied over several orders of magnitude.

In these investigations we aim at the development of a monomer formulation for the preparation of biodegradable bone replacement materials by micro-Stereolithography (µSL). This formulation must achieve 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. And in addition also Ca-based fillers should be applied, that can 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.

2. Experimental

2.1 Materials

All reagents, unless otherwise noted, were purchased from Sigma Aldrich and used as received. The monomer 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

Syntheses: Monomers DBA and DPA were prepared as described [3]. As biodegradable basis monomer a gelatin hydrolysate was modified with methacrlyamide 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 lysine were modified with a poly(ethylene glycol) methylether succinate [4] (PEG 1000, 1.5 eq, N_2, room temperature, dry DMSO, 24 h) and subsequently free acidic groups were reacted with glycidylmethacrylate (2 eq, 40°C, water, 16h). Purification was performed by dialysis against deionized water.

Reactivity: The reactivity of the monomers and photoinitiators was investigated by Differential Scanning Photocalorimetry (Photo-DSC) using a modified Shimadzu DSC 50 equipped with an aluminium cylinder [5] and a light guide (EXFO Omnicure 2000). Filtered light (320-500nm) with an intensity of 1500mW/cm² at the tip of the light guide was used.

Mechanical Properties: Test specimens (30x4x2mm) 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 dynamic mechanical analysis machine (TA Instruments DMA 2980, frequency 1.0Hz, 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).

Biocompatibility: Test specimens (13mm diameter, 2mm height) were prepared, extracted, sterilized and seeded with MG63 osteoblast like cells. Cell viability and multiplication was measured with EZ4U (Biomedica Austria).

µ-Stereolithography (SL): A Perfactory SL-machine from Envisiontec working with the digital light processing principle was used to build three-dimensional objects. Since the resolution of the utilized DLP-process is limited to 40µm, additional experiments with a UV-laser-based micro-stereolithography system were performed,enabling feature resolutions in the xy-plane down to 5µm.

3. Results and Discussion

Figure 2 shows the *biodegradable basis monomer*, which is derived from gelatin 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 photopolymerization.

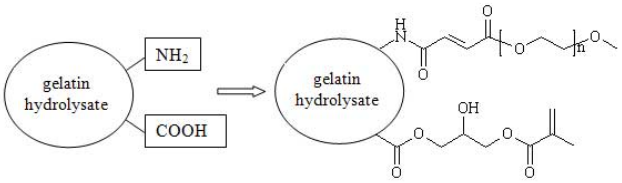


Figure 2: biodegradable basis monomer

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 (Figure 3 shows some examples) were tested concerning reactivity (photo differential scanning calorimetry; Figure 4), biocompatibility (cell seeding experiments with MG63 osteoblast-like cells;Figure 5), and mechanical properties (dynamic mechanical analysis and bending strength test, Figure 6 and Figure 7).[6,7]

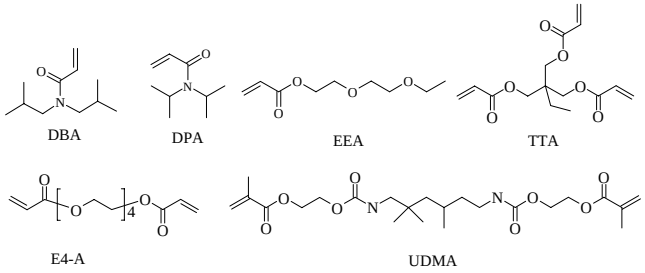


Figure 3: reactive diluents

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) [7]. As expected, acrylamides usually gave higher values for R_P than acrylates. The exceptional low value of diisopropylacrylamide (DPA) can be assigned to the sterically demanding substituents. Tetraethyleneglycol diacrylate (E4A) showed excellent polymerization behavior, which can be addressed to the flexible spacer. All of the shown monomers are suitable from the viewpoint of reactivity.

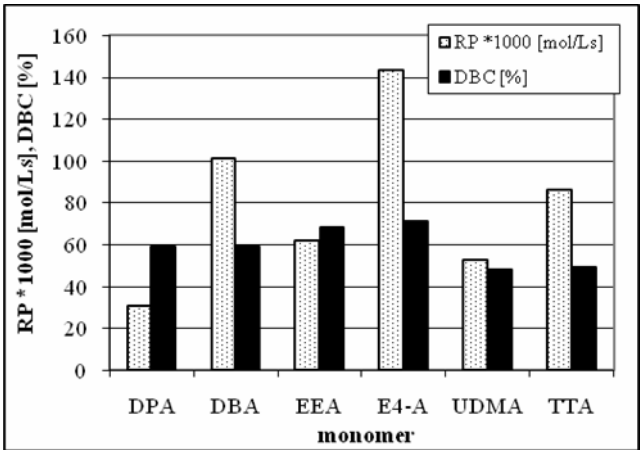


Figure 4: Photoreactivity

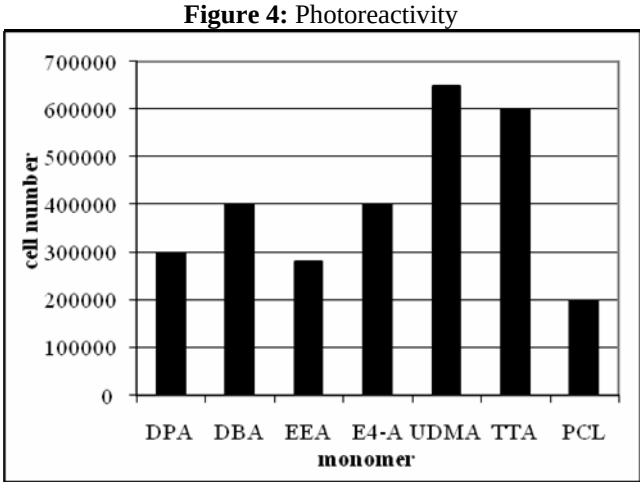


Figure 5: Biocompatibility

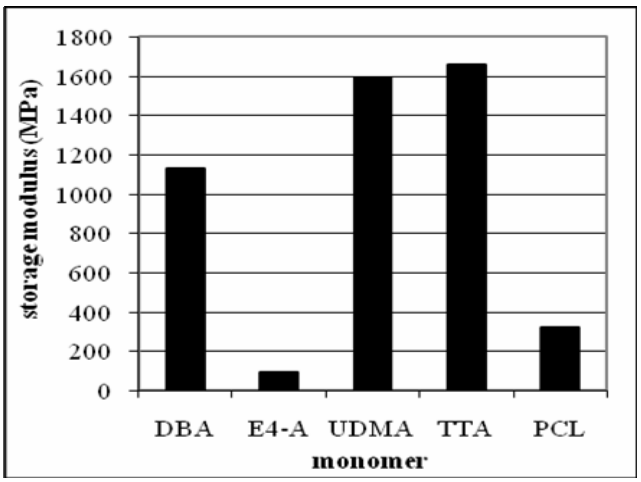


Figure 6: Dynamic mechanical analysis

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 similar or better performance in cell viability than the reference material. The comparably low value of E4A can 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 [8,9] – additionally oligo(ethylene glycol) units are well known to withstand cell adhesion. The mechanical properties of urethane dimethacrylate (UDMA) and trimethylolpropane triacrylate (TTA) may also be the reason for their good performance as substrate for cell adhesion. Generally, acrylamides showed slightly better values for the biocompatibility than acrylates.

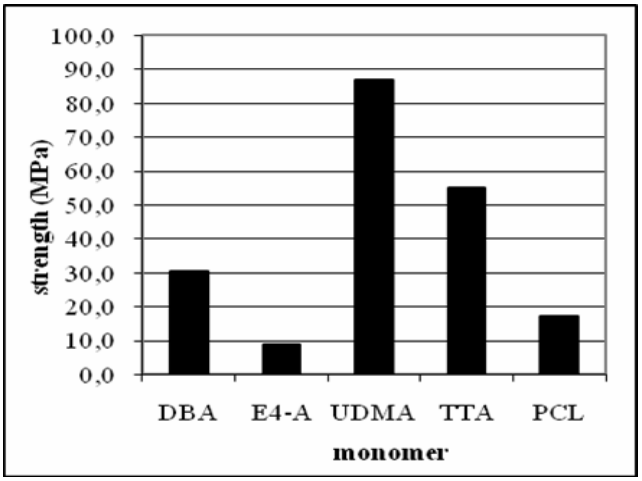


Figure 7: Bending strength test

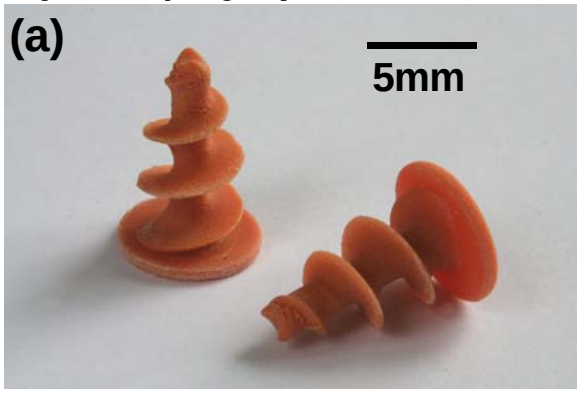
To evaluate the *mechanical properties* of the selected polymers, dynamic mechanical analysis and bending strength tests were performed. As expected, the flexible core of E4A resulted in quite low values for both, storage modulus and strength. EEA and DPA were too soft to be measured by these methods. UDMA and TTA showed quite promising mechanical stability. By use of 60 wt% of hydroxy apatite as *filler* the storage modulus of a TTA based typical resin rises by the factor 4 and reaches 75% of the storage modulus of bone, which is in the order of 11000 MPa. The strength of UDMA (87 MPa) comes close to that of natural bone (100-150 MPa), even without filler.

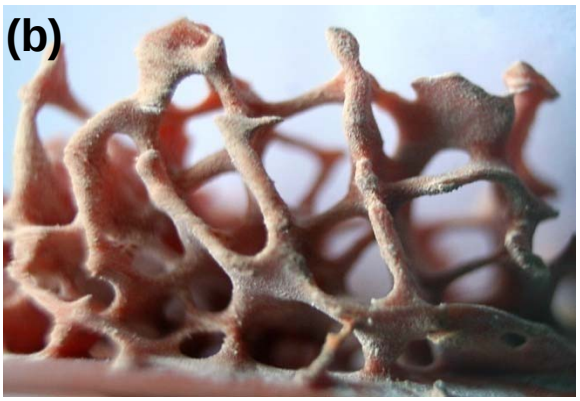
Possible *photoinitiators* with different absorption characteristics – namely camphorquinone with N,N-dimethylamino ethylbenzoate as coinitiator, Irgacure 819 and Irgacure 2959 – were also tested for reactivity and biocompatibility. All of them can be used for the desired application.

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 apatite.

4. Fabrication

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 structure depicted in Figure 8 (a) and (b). The Perfactory allows to shape parts with a xy-resolution of 45 µm and a minimum layer thickness of 25 µm. 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.





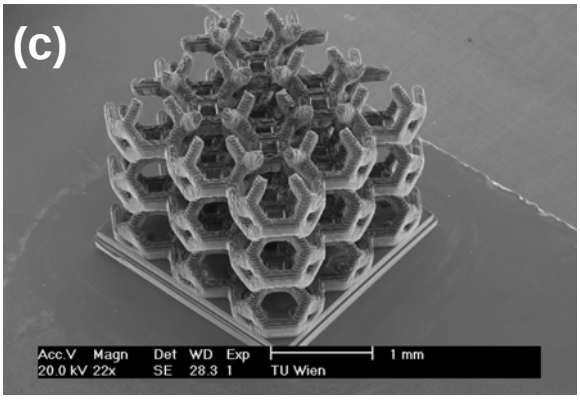


Figure 8: Objects prepared by DLP (a,b) and µ-**s**tereolithography (c)

Alternatively, a micro-stereolithography system based on a UV-laser was utilized. The details of the setup are described by Neumeister *et al.* [10]. The xy-resolution for this system is around 5 µm and the minimum layer thickness is 10 µm. A cellular structure fabricated with this process is depicted in Figure 8 (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 s 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.

5. 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. The reactive diluents DBA, TTA and UDMA seem to be quite promising candidates due to their good results in all criteria compared to the performance of the usually applied thermoplast PCL. Stereolithography is a suitable fabrication technique for shaping complex parts with high feature resolutions.

Acknowledgement

Photoinitiator samples from Ciba SC and monomer samples from IVOCLAR VIVADENT AG, and financial support by the “Austrian Nano Initiative” under contract no. N-703 and FWF under contract no. P 19387 and the DOC-fFORTE fellowship of the Austrian Academy of Sciences are kindly acknowledged. This project was financially supported by the Vienna University of Technology (Innovative Project “3D-Microfab”).

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