Development of Biodegradable Photopolymers for Bone Tissue Engineering

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Introduction

Various orthopaedic methods for the fixation of fractured bone or after removal of bone tumours are currently in clinical use. Autografts, tissue obtained from another site in the same subject of the same species, are the gold standard for tissue repair and substitution. However, the use of autografts has some serious disadvantages, such as additional expense and trauma to the patient, possibility of donor site morbidity, and limited availability. In the case of allografts, in addition to limited supply and high costs, other complications such as viral transmission and immunogenicity are of serious concern. Therefore, there is a critical need to develop bone substitute materials approximating the properties of tissue, which should be replaced, but without the drawbacks of autografts or allografts.
Degradable polymers that are already in clinical use usually consist of a copolymer of lactic and glycolic acid (Figure 1). These polyesters, which serve quite well for the fixation of fractured bone, cannot be used in the case of larger defects, e.g. after removal of a bone tumour, 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.

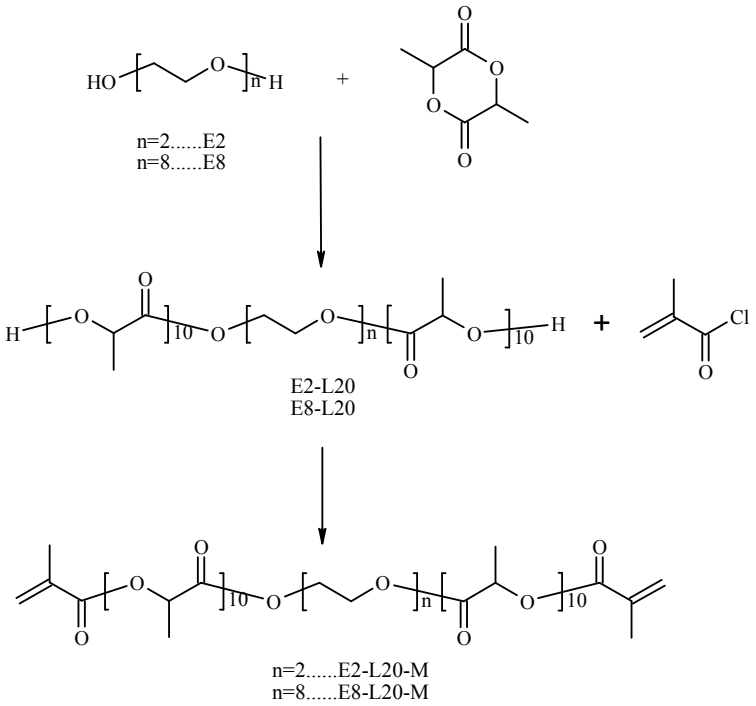
Poly (glycolic acid) Poly (lactid acid)

Figure 1: Biodegradable polyesters

In the present investigations, we aim at the development of a biocompatible monomer formulation for the fabrication of biodegradable cellular structures by Rapid Prototyping, which is a computerized fabrication technique, that offers the possibility to produce highly complex 3-dimensional objects by photopolymerization. Biodegradability is introduced by adding a multifunctional *crosslinker* with amide or ester bonds which degrade in vivo by enzymatic or hydrolytic mechanisms. To adjust the network density monofunctional and mulitfunctional *reactive diluents* are used.[1] Soluble *filler* materials are applied to tune the viscosity for an optimum resolution of the stereolithographic shaping process. Adequate *photoinitiators* as well as fillers for advanced mechanical properties are also required. In this work potential components for the stereolithographic fabrication of cellular implants were investigated concerning reactivity and biocompatibility. First 3-dimensional structures were made by molding techniques.

Crosslinkers

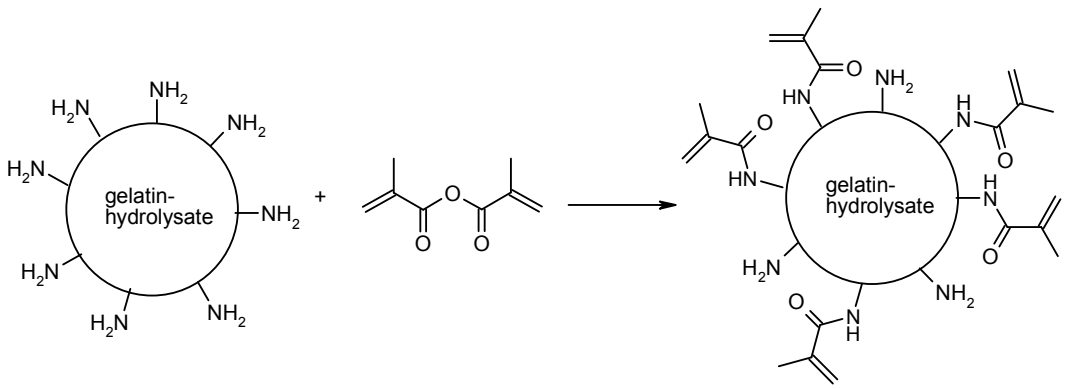
Crosslinkers with ester or amide groups were considered which degrade by hydrolytic or enzymatic mechanisms.
Similar to K.S. Anseth *et. al.*[2] block copolymers of diethylene glycol (E2-L20-M) and polyethylene glycol (E8-L20-M) with lactic acid were prepared by ring opening polymerization and subsequent reaction with methacryloyl chloride.



Scheme 1: Synthesis of crosslinkers based on lactic acid

The ring-opening polymerization of D,L-lactide (10 eq) with diethylene glycol (1 eq) or polyethylene glycol 400 as starter and stannous 2-ethylhexanoate (0.01 eq) as catalyst was performed at 140°C and vacuum. After 6 h the mixture was cooled to room temperature and diluted with CH_2Cl_2. Subsequently, triethylamine (1.5 eq) and methacryloly chloride (1.5 eq) were added at 0°C. After 36 h the reaction was complete, salts were filtered off and CH_2Cl_2 was evaporated. The viscous liquid was redissolved in an minimum amount of toluene, filtered again and precipitated into a 10-fold excess of cold petroleum ether. The precipitate was re-dissolved in a minimum amount of CH_2Cl_2 and the precipitation cycle was repeated. ^{1}H-NMR analysis indicated the presence of 14 to 20 lactic acid units depending on the starting glycol. The methacrylation efficiency was 100%.

Multifunctional macromers with amide linkages (GHM) were prepared from gelatin hydrolysate, by modification of lysine groups (0.38 mmol/g) with methacrylic anhydride.[3]



Scheme 2: Synthesis of GHM

Gelatin hydrolysate was dissolved in phosphate buffered saline (18.3 g $Na_2HPO_4*2H_2O$; 2 g KH_2PO_4; 2 g KCl; 80 g NaCl; 1000 ml H_2O) at 40°C, methacrylic anhydride was added and the reaction mixture was stirred for 4 h. The resulting product was purified by dialysis (molecular weight cut off 3500) against distilled water. An average degree of derivatization of 50% (1H-NMR) based on lysine units was obtained.

For the determination of the biocompatibility test specimens were made from the synthesized monomers with 1 wt% of an equimolar mixture of camphorquinone (CQ) and N,N-dimethylaminobenzoic acid ethyl ester (DMAB), which is known to be a biocompatible photoinitiating system.[4] In the case of GHM, which is solid, test specimens were made with 80% of a reactive diluent (N,N'-Diethyl-1,3-propylenebisacrylamide[1](EPA)). Photocuring was performed with a high-pressure mercury lamp (1000 W, distance 15 cm) under nitrogen atmosphere for several minutes. After extraction and sterilization of the test specimens, osteoblast like cells (MC3T3-E1) were seeded on the polymers. If the cells could adhere and survive, the material was stated to be biocompatible.

The results of the biocompatibility tests are shown in Table 1.

Table 1: Biocompatibility of crosslinkers

Crosslinker	Biocompatibility.
E2-L20-M	1
E8-L20-M	2
GHM	1

1….good biocompatibility and cell adhesion
2….good biocompatiblity, less cell adhesion
3….no biocompatibility

All tested crosslinkers showed quite good biocompatibility, although on E8-L20-M less cells could adhere. This relies on the longer PEG chain that is known to be a bad substrate for the adhesion of cells.[5]
Polymers with amide linkages tend to degrade more slowly and therefore provide longer mechanical support for the healing bone, and are thus preferred as crosslinker in a biodegradable bone replacement material.

Photoinitiators

The photoinitiating system consisting of CQ and DMAB was selected for the preparation of test specimens because of its known biocompatibility and the suitability for the curing of thick layers due to the photobleaching effect.[6] For an application in Rapid Prototyping the polymerization rate of this Type II photoinitiator might be too low. Therefore, two α-cleavable Type I photoinitiators (Figure 2) were tested for their applicability in a biodegradable tissue scaffold.

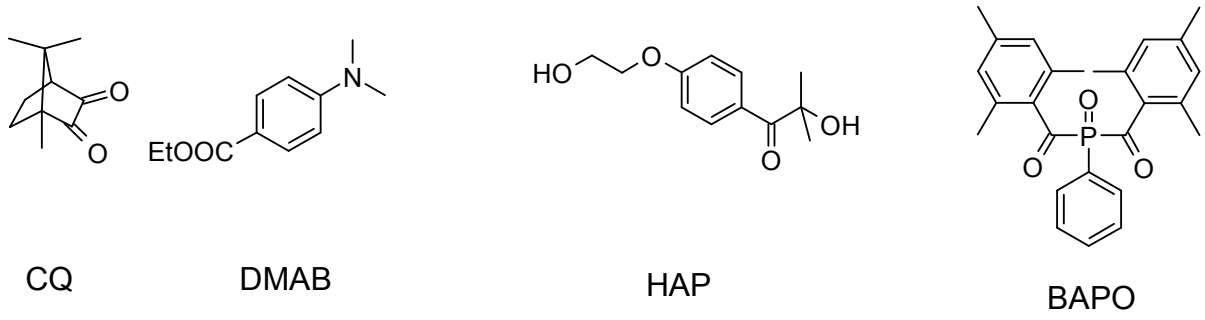


Figure 2: Photoinitiators

The bisacylphosphine oxide bis(2,4,6-trimethylbenzoyl) phenylphosphineoxide (BAPO) is a very promising candidate for rapid prototyping due to its high reactivity and its absorption tailing out in the visible region. This photoinitiator is ideally suitable for the Rapid Prototyping process using the DLP

principle with light emission only in the visible region. It has already found widespread application in dental materials,[7] but not yet in biodegradable systems. The hydroxyalkylphenone 4-(2-Hydroxyethoxy)phenyl-(2-hydroxy-2-propyl)ketone (HAP) has often been used for the photocuring of biopolymers.[8] Because of its absorption below 400 nm, the application is limited to RP machines with appropriate lasers.

Photo DSC was used to compare the efficiency of the photoinitiators. Therefore, 0.5% (w/w) of BAPO and HAP, respectively, and 1% (w/w) of an equimolar mixture of CQ/DMAB were dissolved in EPA and measured with filtered light (365 nm, 1500 mW/cm^2). Results are shown in Figure 3 and Table 2.

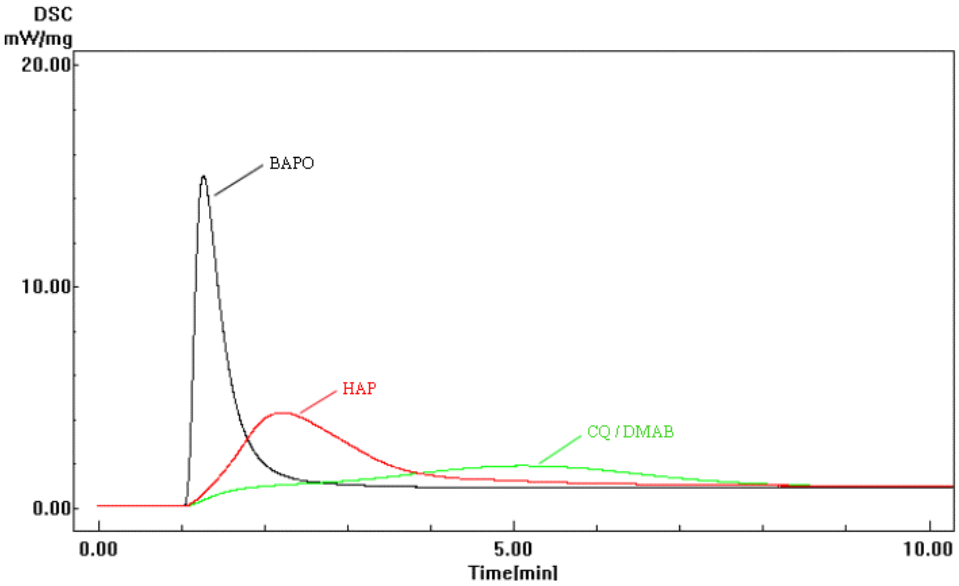


Figure 3: Reactivity of photoinitiators

Using this method of analysis, the advantages of curing with BAPO as photoinitiator are clearly visible. The polymerization was completed within 1 min, whereas with HAP the threefold time was needed. For entire curing with CQ/DMAB a time of up to 8 min was required. Additionally, highest DBC was obtained with the bisacylphosphine oxide, which is of significant importance for low migration systems. Biocompatibility was tested by preparing test specimens of a biocompatible monomer (EPA) with the selected photoinitiators and testing them with osteoblast like cells as described above. Additionally a thermal initiator, benzoylperoxide (BPO), was tested, which is needed for preparing 3-dimensional structures by molding techniques. Results are also shown in Table 2.

Table 2: Reactivity and biocompatibility of photoinitiators

Initiator	**t_{max}** [s]	**DBC** [%]	**$R_P * 10^3$** [mol l^{-1} s^{-1}]	**Biocompatibility**
BAPO	16.2	74	120	1
HAP	73.2	67	28	1
CQ/DMAB	247.2	56	9	1
BPO/DMAB	-	-	-	2

1….good biocompatibility and cell adhesion
2….good biocompatiblity, less cell adhesion
3….no biocompatibility

All tested initiators are perfectly biocompatible and can be used in a monomer formulation for bone replacement materials. Depending on the shaping process the ideal initiator can be chosen by its reactivity under the desired conditions.

Fillers

For the improvement of the mechanical and osteoconductive properties of a bone replacement material hydroxyapatite ($Ca_5(PO_4)_3(OH)$) and β-tricalciumphosphate ($Ca_3(PO_4)_2$) are suitable components.[9] For a direct shaping process with Rapid Prototyping a certain viscosity is required for optimum processing properties and for an optimum resolution. Therefore, several usual soluble filler materials (see Figure 4) were tested concerning biocompatibility.

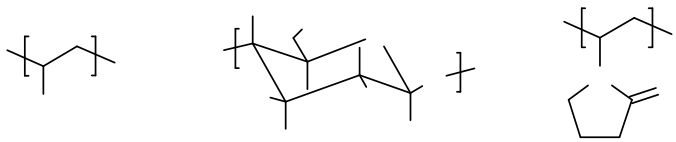


Figure 4: Fillers for tuning the viscosity

These filler materials were chosen, because they have already found application in the medical field. Poly (vinyl pyrrolidone) (PVP) with low molecular weight (polyvidon) for example is used as plasma surrogate and film former in eye drops.[10] Crosslinked PVP, also known as Crospovidon is widely used as tablet disintegrant. Poly (vinyl alcohol) (PVA) is a common thickening agent in pharmaceutic and cosmetic products and also a component of artificial tear fluids. Cellulose acetate butyrate (Cell-A-B) has not yet been investigated a lot concerning biocompatibility, but it has already been used as a compound of mucoadhesive drug delivery systems.[11]
Test specimens were made from EPA with 5 wt% of each filler and tested as described above. Results are shown in Table 3.

Table 3: Biocompatibility of filler materials

Filler	Biocompatibility
PVA	2
Cell-A-B	2
PVP	3

1….good biocompatibility and cell adhesion
2….good biocompatiblity, less cell adhesion
3….no biocompatibility

PVA and Cell-A-B seem to be biocompatible but do not support cell growth. PVP, although it is used in other medical applications, might be cytotoxic for that specific cell line.

3D Molding

First 3-dimensional cellular structures were prepared by molding (Figure 5). An organo-soluble sacrificial mold[12] was made by Rapid Prototyping (1-3) and filled (4) with a biocompatible monomer formulation consisting of 20 wt% crosslinker, 80 wt% reactive diluent and 1 wt% thermal initiator (benzoylperoxide and DMAB). Curing was carried out at 65°C. The same was done with a formulation consisting of 50 wt% hydroxyapatite. The sacrificial mold was then removed (5) by dissolving in THF/n-butylamine. The resulting cellular structures are shown in Figure 6.

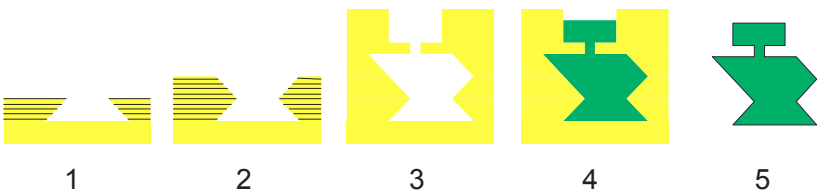


Figure 5: Rapid prototyping of mold material and molding technique

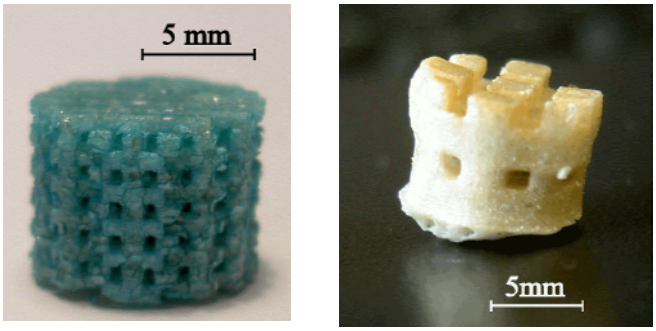


Figure 6: Cellular structures prepared by molding

Summary
Different components for a biocompatible monomer formulation for the preparation of bone replacement materials have been investigated. Biodegradable crosslinkers with amide and ester bonds were synthesized and tested for their biocompatibility. In most cases, good cell adhesion has been proven. Polyethyleneglycol units are not cytotoxic, but gave poor cell adhesion. In addition, several photoinitiators and filler materials were analyzed. All PIs were excellently biocompatible, whereas most fillers show poor cell adhesion. A formulation consisting of the tested components was used to build first 3-dimensional structures by molding techniques.

Acknowledgment:
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