Bone regeneration scaffolds prepared from crosslinkable biodegradable polymers


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Introduction
Osteous tumors, trauma, disease and birth defects can create a need to fill bone tissue deficiencies in the skeletal system. We have developed in situ crosslinkable, biodegradable, methacrylate–endcapped polymers based on glycolide, D,L-lactide, trimethylene carbonate (TMC) and ε-caprolactone as well as poly(ortho-esters) for bone tissue regeneration. Crosslinkage can be initiated by redox type or photoinitiators. By adding controlled amounts of gelatine powders of selected size, scaffolds can be obtained with controlled porosity, pore size and pore connectivity. In addition calcium phosphates or other osteoconductive materials like bioplastic HTR or demineralised bone can be added.

Experimental
Materials. Linear and branched low molecular weight copolymers with viscous-liquid properties were prepared using ring opening polymerization of cyclic monomers, zinc acetate as catalyst and different types of alcohol initiators. In a second step the hydroxyl moieties were derivatized into methacrylate endgroups by methacryloyl chloride [1].

1st step
R –OH m + α / 2 mn ZnOAc2 n
2nd step
R (O)m O m
methacryloyl chloride
3rd step
D,L-camphorquinone
N-phenylglycine

Poly(ortho esters) bismethacrylates were prepared in a one-pot synthesis as shown in Figure 1.

The diols and HEMA were dissolved in dry tetrahydrofuran (THF). The mixture is stirred until all solids are dissolved; then 3,9-di-ethylidene-2,4,8,10-tetraoxaspiro[5,5]-undecane (DETOSU) is added. The polymerization is initiated by the addition of a trace of a 10 mg/ml solution of p-toluene sulfonic acid (pTSA) in THF. The reaction temperature rapidly rises to the boiling point of THF and than gradually decreases. After 60 min the polymers were precipitated in MeOH and triethylamine is added as stabilizer. To complete precipitation the mixture was kept overnight in a freezer at –22°C. Afterwards the methanol was removed and the polymers were dried in a vacuum oven during two days to remove traces of solvents.

The methacrylate–endcapped copolymers were converted into a solid, 3-D polymer network by visible-light irradiation in the presence of D,L-camphorquinone/N-phenylglycine initiator system [2]. By mixing, before irradiation, the liquid-viscous oligomers with different types of additives such as gelatin with defined particles size, α- or β-tricalcium phosphate salts or HTRTM–24 (Biophant®) materials with pore forming and osteoconductive properties can be obtained [3].

Results and discussion
Polyester-based scaffolds. Using monomer/initiator ratio 20/1 and polymer composition: 50:50(mol%) D,L-lactide/ε-caprolactone (trimethylene carbonate) and 30:70(mol%) glycolide/ε-caprolactone (trimethylene carbonate) we are able to obtain viscous-liquid methacrylate-endcapped oligomers which can be mixed manually with different types of additives. To improve the viscous properties and decrease the Tg of the copolymers based on glycolide or D,L-lactide and TMC, triacetin was used as a plasticizer.

Mechanical properties of the crosslinked polymer network can be controlled by using different types of alcohol initiators such as 1,6-hexanediol [HXD], trimethylolpropane [TMP], pentaerythritol [PENT] and dipentaerythritol [DPENT].

Table I. Molecular weight measured by GPC (PS standards, eluent – THF), thermal properties (DSC, 2nd run) and compressive modulus of the copolymers based on D,L-lactide-ε-caprolactone and different types of the alcohol initiators.

<table>
<thead>
<tr>
<th>Type of the initiator</th>
<th>Mw [g/mol]</th>
<th>Mn/Mw</th>
<th>Tg [°C]</th>
<th>E [MPa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>HXD</td>
<td>2800</td>
<td>1.5</td>
<td>-26.4</td>
<td>27</td>
</tr>
<tr>
<td>TMP</td>
<td>3000</td>
<td>1.5</td>
<td>-21.9</td>
<td>43</td>
</tr>
<tr>
<td>PENT</td>
<td>3300</td>
<td>1.4</td>
<td>-21.9</td>
<td>46</td>
</tr>
<tr>
<td>DPENT</td>
<td>2900</td>
<td>1.4</td>
<td>-21.3</td>
<td>91</td>
</tr>
</tbody>
</table>

Additionally the mechanical properties of the composites can be significantly improved using different types of additives. The gelatin particles are leached out leaving open cells with a pore size and morphology defined by the gelatin particles and providing the osteoconductive properties of the composites. Finally, by changing the polymer composition, crosslinking density, type and amount of additives we are able to control the degradation rate of the composite.

Polyorthoester-based scaffolds. The molecular weight of the polymers could not be controlled by varying the stoichiometry of the monomer. Therefore, a monofunctional monomer, HEMA, is added in the beginning of the reaction.

Figure 2 shows that an increase in HEMA results in a decreased molecular weight. In the absence of HEMA the molecular weight is more than double as we expected.

![Figure 1](image1.png)

**Figure 1:** Synthesis of low molecular weight poly(ortho esters).

![Figure 2](image2.png)

**Figure 2:** Control of the molecular weight by adding an increasing amount of HEMA the reaction.
HEMA addition allows to control the molecular weight and the endgroup functionality in the polymer chain. The physical properties, such as glass transition, hydrophobicity and viscosity, can be controlled using different diol monomers. Polymers were characterized by NMR, GPC, DSC and FT-IR. The glass transition temperatures of the polymers can be controlled by using different diols (Figure 3).

**Figure 3**: Glass transition temperatures for different homopolymers using diols with different chain flexibility.

The glass transition temperature decreases when the chain flexibility of the polymers increases. To obtain viscous polymers flexible diols have to be used. The prepolymer were converted into a biodegradable network, after addition of a photo initiator, by irradiation with a dental lamp. Conversion profiles of the bismethacrylate prepolymer were determined by real-time Fourier transform infrared spectroscopy.

**Biological evaluation.** In vitro biocompatibility studies, using fibroblast or osteoblast cell lines and the MTT-method as a test for cell viability indicated that the various crosslinked polymers and composites were well tolerated. Studies with osteoblast cell lines clearly showed that the ingrowth of cells is strongly related to the degree of porosity and the pore size. A porosity of minimal 70 vol% is preferred. Preliminary implant studies in sheep thibia indicated that the composite materials gave no severe inflammation reactions. The materials degraded and bone formation occurred. In a separate study, the osteogenic property was investigated in vivo using cranial critical-sized bone defects (CSD) on beagle dogs as in vivo model. Demineralised Bone Powder was selected as bioactive additive. The implants proved to be compatible. Bone formation occurred from the periphery but the amount of newly formed bone was less than the control defect (autologous bone). The implant site was well revascularized.

More details on the biological studies will be presented and discussed.

**Conclusions**

Low molecular weight bismethacrylate endcapped polyesters and poly(ortho-esters) are synthesized. In case of the polyesters, the molecular weight and chemical composition can be easily controlled by the selection of comonomers and the monomer tot initiator ratio. Mechanical properties can be varied by the functionality of the initiating diol. In case of the poly(ortho-esters), the molecular weight could be controlled using HEMA as a chain stopper in the reaction. Material properties were controlled by the choice of diols. These bismacromonomer-type prepolymer can easily be converted into a 3-D biodegradable network using a photo initiator and irradiation with blue light.

The presented injectable, in situ polymerizable, biodegradable composites may serve as scaffolds for guided bone regeneration and may provide an alternative for irregular bone defect treatment.

**References**


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