Tuned polyurethanes for soft tissue regeneration
Jovanovic, Danijela

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Novel biomedical polyurethanes based on oligo(ε-caprolactone-co-γ-butyrolactone) copolymers via chemo-enzymatic pathways

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Abstract

The aim of this study was to design biomedical polyurethanes (PUs) that would hydrolyze faster than poly(ε-caprolactone) (PCL) based PUs. A series of γ-butyrolactone (γ-BL) and ε-caprolactone (ε-CL) co-polyesters with molar weights (MW) between 700 - 2150 g/mol was prepared by 1,4-butanediol initiated ring-opening polymerization (ROP), catalyzed by Candida Antarctica Lipase B (CAL-B; Novozyme 435) at room temperature. A maximum of 26 mol% of γ-BL could be incorporated in the oligodiols with relatively good product yield. Introduction of hydrophilic γ-BL disturbed PCL crystallization and resulted in enhanced hydrolytic degradability of PBCL-PUs. The oligodiols were successfully converted into the PUs by the end-capping with 1,4-butanediisocyanate (BDI) and subsequent chain-extension with 1,4-butanediol (BDO). The thermal and mechanical properties of the PUs are shown to depend on the oligodiol MW. With the increase in oligodiol MW, the PU crystallinity and the degree of the microphase separation decreased, followed by a decrease in the Young’s Modulus. The proposed manufacturing route to obtain oligodiols for the synthesis of biomedical PUs is attractive due to the employment of the low-cost monomers (γ-BL and ε-CL), efficient and easily removable catalyst and low reaction temperature. Furthermore, PBCL-PUs were shown to be biocompatible both in vitro and in vivo, which renders this type of PUs excellent candidates for manufacturing of scaffolds to be used in tissue regeneration.
1. Introduction

A tremendous amount of research has been devoted to the applications of degradable polymers for biomedical devices. Especially the potential of degradable scaffolds for temporary support in tissue regeneration has drawn a lot of attention in scientific research\(^1\textsuperscript{-4}\). Currently the first applications appear in the clinic\(^5\textsuperscript{,}6\). Traditionally, aliphatic polyesters were considered materials of choice for design of scaffolds for medical applications. However, they suffer from poor mechanical properties, as they have low glass transition temperature (\(T_g\)) and mechanical stability can only be achieved by allowing the polyester to crystallize at least partially. The presence of crystals impairs degradability and was seen to cause inflammation upon implantation, such as in case of the resorbtion of polylactic acid bone fixation plates\(^7\textsuperscript{,}8\).

Aliphatic, segmented, thermoplastic polyester urethanes represent a superior class of polymers where properties are easily adapted to the desired requirements. A key feature of segmented polyester urethanes (SPEUs) is the phenomenon of microphase separation in the material between the urethane segments and the polyester segments leading to the physical crosslinking of the molecules, thereby providing the superior mechanical properties of the polymeric materials\(^9\textsuperscript{-12}\). This phenomenon is determined by the thermodynamic incompatibility between the urethane and polyester segments and depends on the chemical constitution and chain length of the blocks\(^9\textsuperscript{,}11\). In SPEUs therefore the microphase separation depends on the type of polyester and urethane and their molar weight and molar weight distribution.

In vitro, SPEUs mainly degrade by bulk hydrolysis of the ester and urethane groups by absorbed water. This can be ascribed to the low value of the equilibrium constant for the ester formation while urethane groups were shown to be far less sensitive for hydrolytic scission\(^13\textsuperscript{-15}\). Degradation results in water soluble and metabolically consumable, \textit{i.e.} resorbable degradation products. The degradation rate is therefore dependent on the rate of random chain scission, and diffusability and solubility of the transient degradation products (short oligoesters and monomers)\(^16\textsuperscript{,}17\).

1,4-Butane disocyanate(BDI)-based SPEUs have been extensively studied in our laboratory\(^18\textsuperscript{-20}\). Heijkants et al. designed poly(\(\varepsilon\)-caprolactone)-BDI-based segmented PUs for meniscus regeneration\(^20\). The PUs were shown to be biocompatible \textit{in vivo}\(^21\). However, these PCL-PUs were found to degrade very slowly and were still present in the test animals two years after implantation\(^22\). However, due to the relative high hydrophobicity of the monomer\(^23\), degradation is rather slow and biomedical applications therefore include only those devices and scaffolds where prolonged survival of the material is required. For those tissues which regenerate faster this prolonged presence of the scaffold might become an obstacle for full tissue regeneration. Therefore, SPUEs with tunable degradation profiles are needed for further extending the application area of this
remarkable class of biomedical materials. The degradability of these PUs can be enhanced by the incorporation of other more hydrophilic co-monomers in the synthesis of oligodiols. Poly(4-hydroxybutyrate), a polyester produced microbially, was successfully applied in the tissue engineering of small caliber vascular grafts\textsuperscript{24} and trileaflet heart valves\textsuperscript{25}, providing a good substrate for cell attachment and proliferation. However, with or without the help of a catalyst, the monomer \(\gamma\)-butyrolactone (\(\gamma\)-BL) was initially found to be chemically non-polymerizable\textsuperscript{26}. The low reactivity of \(\gamma\)-BL has been ascribed to the low ring strain\textsuperscript{27, 28}. The attempts that followed were mainly focused on the design of novel catalysts and initiators such as organometallics\textsuperscript{29-31}, lanthanides\textsuperscript{32, 33} or molybdenum\textsuperscript{34} compounds. In all cases \(\gamma\)-BL homopolymerization yielded very low molar weight products (\(MW\)) in low yield\textsuperscript{29, 35}. However, the copolymerization of \(\gamma\)-BL with the other lactones was more successful. Even though some chemical pathways delivered relatively high \(MW\) \(\gamma\)-BL-containing polyesters\textsuperscript{29, 33, 36}, the biocompatibility of the applied catalysts represents a highly important issue. Although a catalyst \textit{per se} is not toxic, it is almost impossible to fully predict its fate and an effect they could provoke \textit{in vivo}. The hydrolysability and the (bio)degradation of the copolyesters were observed to increase with the incorporation of the \(\gamma\)-BL units\textsuperscript{36-38}, which made the \(\gamma\)-BL-containing polyesters interesting candidates for biomedical applications. The discovery of enzymes as catalysts that could be employed in the ring-opening polymerization (ROP) of lactones\textsuperscript{39-43} (among vast majority of other reactions) broadened the range of possibilities for obtaining \(\gamma\)-BL copolyesters\textsuperscript{44, 45}. Enzymes are especially attractive for manufacturing of biomaterials since they remain solid during the polymerization and could be as such easily removed from the reaction mixture, rendering the biomaterials free of potentially toxic catalysts.

The aim of this study was to develop novel biodegradable PUs with enhanced degradability as potential biomaterials for scaffold preparation. By varying reaction parameters, we prepared a series of oligodiols based on \(\varepsilon\)-CL and \(\gamma\)-BL utilizing an immobilized lipase, \textit{Candida Antarctica} (CAL-B; Novozyme 435). The oligodiols were further successfully reacted with the BDI and subsequently chain-extended with 1,4-butanediol (BDO) to yield the PU. \textit{In vitro} degradability of the novel PUs was assessed and compared to similar poly(\(\varepsilon\)-caprolactone)-BDI-based PUs.

2. Material and Methods

2.1 Materials

\(\gamma\)-Butyrolactone (\(\gamma\)-BL), 1,4-butanediol (BDO) and toluene, all analytical grade reagents were purchased from Aldrich (Zwijndrecht, The Netherlands). BDO was distilled from 3Å
Novel biomedical polyurethanes based on oligo(ε-CL-co-γ-BL)

mol. sieves. Toluene was freshly distilled over sodium. ε-Caprolactone (ε-CL) was obtained from Union Carbide (Terneuzen, The Netherlands) and was purified by distillation under reduced pressure from calcium hydride (CaH₂). 1,4-Butanediisocyanate (BDI) was purchased from Bayer (Leverkusen, Germany) and it was subsequently distilled under reduced pressure by means of a short-path distillation. Novozyme 435 was a kind gift from Novozymes (Luud, Sweden). Novozyme 435 was stored dry in a refrigerator at 4°C. Chloroform and n-hexane as analytical grade solvents were purchased from Acros (Geel, Belgium) and were used without further purification.

2.2 Synthesis of γ-BL/ε-CL oligodiols

The reactions between γ-BL and ε-CL monomers (molar ratio γ-BL and ε-CL varied: 0/100, 20/80, 40/60, 50/50 and 100/0), and initiator (BDO) (molar ratio ε-CL/BDO varied: 4.5/1, 10/1, 12/1, 15/1 and 20/1) with Novozyme 435 (typically 1 g per 11 g of reaction mixture) as a catalyst were conducted in freshly distilled toluene for 22 h on the shaking platform at ambient temperature. All the reactions were performed in thoroughly cleaned and dried glassware under argon gas. After the polymerization, the resulting reaction mixture was diluted by adding ~ 10 mL of chloroform to the reaction flask. Novozyme 435 was filtered out through a P4 glass filter and 0.1 μm syringe PTFE filter (PURADISC, Whatman). The collected filtrate was subjected to reduced pressure at a Rotavap apparatus in order to remove chloroform and toluene. Unreacted monomers and remaining solvents were removed by short-path distillation (Büchi GKR-51, Switzerland) with gradual temperature increase from ambient temperature to 80°C at the pressure of ~ 0.02 mbar.

2.3 Synthesis of PUs

Polyurethanes were prepared via a two-step procedure according to the method developed by Heijkants et al.²⁰ Briefly, in the first step oligodiol obtained as previously described was end-capped with the 6-fold excess of BDI and reacted for 3.5 h with magnetic stirring at 80°C. Prior to the second step, the excess of the diisocyanate was removed by short-path distillation at 80°C until constant mass was reached. Subsequently, the end-capped oligodiol was chain-extended with a slight excess of BDO (1:1 to 1.2:1) for 16 h at 80°C. The use of catalyst was avoided in both reaction steps. The obtained PU (denoted PBCL-PUs in further text) was released by breaking the glass flask and stored at 4°C until further use.

2.4 ¹H-Nuclear magnetic resonance (¹H-NMR)

The sample preparation consisted of dissolving approximately 15 mg of either oligodiol or PBCL-PU in ~ 0.8 mL of deuterated chloroform (d-chloroform).¹H-NMR spectra were
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recorded at room temperature using a 300 MHz Varian NMR apparatus. $^1$H-NMR was used to determine the $\gamma$-BL/$\varepsilon$-CL mol/mol ratio and oligodiol MW using Eq. 1 and Eq. 2, respectively.

\[
\frac{\gamma - BL}{\varepsilon - CL \text{ ratio}} = \frac{I\Delta\delta (2.1 \text{ ppm} - 1.8 \text{ ppm})}{I\Delta\delta (1.5 \text{ ppm} - 1.3 \text{ ppm})} \tag{Equation 1}
\]

\[
Mn(\text{oligodiol}) = \frac{I\Delta\delta (1.5 \text{ ppm} - 1.3 \text{ ppm})}{I\Delta\delta (3.8 \text{ ppm} - 3.6 \text{ ppm})} \times 2 \times Mn(\varepsilon - CL) + \frac{I\Delta\delta (2.1 \text{ ppm} - 1.8 \text{ ppm})}{I\Delta\delta (3.8 \text{ ppm} - 3.6 \text{ ppm})} \times 2 \times Mn(\gamma - BL) + Mn(BDO) \tag{Equation 2}
\]

2.5 Gel permeation chromatography (GPC)

MWs and polydispersities of oligodiols were determined by a GPC system consisting of a Spectra Physics P1000 pump, Spectra Physics AS 1000 auto-sample injector, Viskotek H-502, Shodex RI-71 detector and a set of two PL gel mixed C columns maintained at 30°C. THF was used as an eluent with the flow rate of 1.0 mL/min. The injected volume was 100 μL (concentration 3 mg/mL). All samples were filtrated over a 0.45 μm filter prior to measurement. Universal calibration with polystyrene (PS) standards was performed. Data analysis was conducted with the Trisec GPC version 3.0 software package.

MWs and molar weight distribution ($D$) of the PBCL-PUs were determined by utilizing DMF with 0.01 M LiBr as an eluent on a Waters 600 Powerline system, equipped with 2 mixed-C PL gel 5 μm columns (Polymer Laboratories) at 70°C. All samples were filtrated over a 0.45 μm filter prior to measurement. The data analysis was done using conventional calibration with PS standards accompanied by in-house software.

2.6 Attenuated Total Reflectance Fourier-Transform Infrared spectroscopy (ATR-FTIR)

Infrared spectroscopy was performed using a Bruker IFS88 equipped with a Golden Gate (Graseby Specac) single reflection ATR accessory, with the spectral resolution of 4 cm$^{-1}$. Fifty scans were taken to obtain each spectrum. OPUS version 4.2 software was used to analyze the data.

2.7 Differential scanning calorimetry (DSC)

Thermal analysis of oligodiols and the PBCL-PUs was assessed by means of DSC (Perkin-Elmer DSC-7). The measurements were performed with the heating rate of 10°C/min under a nitrogen atmosphere. TA software version 4.0 was employed in the analysis of the resulting endotherms.
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2.8 Tensile test
For the tensile test, PBCL-PU films were cast from 1,4-dioxane solution (concentration 2-3 wt%) onto PFA Petri dishes (perfluoroalkoxy polymer resin; Bergof, Florida, USA) at room temperature for at least 48 h. The films were finally dried in vacuum at 40°C for at least 16 h. Tensile tests were performed on rectangular-shaped specimens (40 x 2.2 x 0.1 mm) cut from the PU films 21°C with a 100 N load cell, using an Instron 4301 tensile tester, and an extension rate of 10 mm/min.

2.9 Degradation study
To assess in vitro degradation, one of the PBCL-PU s, PBCL1620-PU (sample: 4 x 4 x 4 mm³; mass: 0.13 ± 0.03 g; n = 1) was subjected to hydrolysis by immersion in phosphate buffer (solution in water, pH = 7.2; Sigma-Aldrich, Zwijndrecht, The Netherlands) supplemented with sodium azide (0.02 wt%) protected from light at 37°C for a period up to 17 weeks. At each analysis point, a sample was withdrawn, blotted, weighed, dried for 24 h at 40°C under vacuum and weighed again. The mass loss and water uptake were determined according to Eq. 3 and Eq. 4, respectively.

\[
\text{Mass loss} = \left( \frac{m_0 - m_d}{m_0} \right) \times 100\% \quad \text{Equation 3}
\]

\[
\text{Water uptake} = \left( \frac{m_w - m_d}{m_0} \right) \times 100\% \quad \text{Equation 4}
\]

with \(m_o\) is the mass before incubation, \(m_d\) is the mass after drying and \(m_w\) is the mass of the wet sample.

MWs of the degraded PBCL1620-PU were determined by GPC. The measurements were done on a GPCmax VE-2001 with model 302 TDA detectors and Omnisec software (Viscotek), using DMF with 0.01M LiBr at 70°C as eluent. The columns (PLGel 5μ 2x30 cm mixed-C from Polymer Laboratories) were calibrated using universal calibration with narrow disperse PMMA standards (Polymer Laboratories). Hydrolytic rate constants were determined by fitting GPC data by linear regression analysis option of the OriginPro 7.5 software.

3. Results and Discussion

3.1 Synthesis of oligodiols and PUs
In this study we demonstrate that we were able to synthesize a set of novel biodegradable PUs by BDO-initiated, enzyme-catalyzed ROP of ε-CL and γ-BL and
subsequent reaction of the oligodiols with an aliphatic diisocyanate (BDI), and a chain-extender (BDO) (Fig. 1).

**Figure 1.** Enzymatic polymerization of γ-BL with ε-CL and PBCL-PU reaction scheme.

Due to its low ring strain (36.5 KJ/mol as compared to ε-CL ring strain of 44.7 KJ/mol), γ-BL does not polymerize readily via conventional preparative methods. Since the enzymatic catalysis of the polymerization only requires a recognizable reactive centre and does not depend on the ring strain of the lactone, several research groups employed
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various lipases for the ROP of γ-BL and other lactones\textsuperscript{44-46}. The enzyme activity and efficiency in the ROP was proven to be higher if the lipases were immobilized on an inert carrier\textsuperscript{47}. Therefore, an immobilized enzyme, lipase CAL-B (trade name Novozyme 435) was chosen as a catalyst. Preliminary experiments at 60°C and 80°C with stirring of the reaction mixture disrupted the morphology of the Novozyme 435 beads, and could lead to difficulties of fully removing small catalyst particles from the reaction mixture. In addition, when similar experiments were repeated at room temperature with shaking, oligodiol $M_W$s and yields were comparable to the ones obtained at higher temperatures. Bearing in mind that the efficacy of enzymes might decrease in organic solvents at higher temperatures, we chose to conduct the reactions at the milder reaction conditions.

Table 1. Oligodiols with different feed composition. PCL - ε-CL homopolymer; PBL - γ-BL homopolymer; PBCLxx - copolymer of γ-BL and ε-CL with xx mole % feed ratio of ε-CL; PBCL-yyBDO - 50/50 mol% feed ratio of γ-BL and ε-CL with yy ε-CL/BDO mol% ratio. All reactions were performed in toluene with ~ 50 wt% concentration of monomers at room temperature for 22 h.

<table>
<thead>
<tr>
<th>Oligodiol</th>
<th>γ-BL/ε-CL molar ratio</th>
<th>ε-CL/BDO molar ratio</th>
<th>Yield mass%</th>
<th>γ-BL/ε-CL\textsuperscript{a} molar ratio</th>
<th>$M_n$\textsuperscript{a} (g/mol)</th>
<th>$M_n$\textsuperscript{b} (g/mol)</th>
<th>$D$\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL100</td>
<td>0 / 100</td>
<td>4.5 / 1</td>
<td>73</td>
<td>0 / 100</td>
<td>702</td>
<td>1185</td>
<td>1.40</td>
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<tr>
<td>PBCL80</td>
<td>20 / 80</td>
<td>4.5 / 1</td>
<td>68</td>
<td>17 / 83</td>
<td>783</td>
<td>850</td>
<td>1.71</td>
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<tr>
<td>PBCL60</td>
<td>40 / 60</td>
<td>4.5 / 1</td>
<td>65</td>
<td>15 / 85</td>
<td>758</td>
<td>940</td>
<td>1.72</td>
</tr>
<tr>
<td>PBCL50</td>
<td>50 / 50</td>
<td>4.5 / 1</td>
<td>43</td>
<td>26 / 74</td>
<td>772</td>
<td>920</td>
<td>1.65</td>
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<tr>
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<td>50 / 50</td>
<td>4.5 / 1</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PBL100\textsuperscript{d}</td>
<td>100 / 0</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PBL100</td>
<td>100 / 0</td>
<td>-</td>
<td>7</td>
<td>100 / 0</td>
<td>276</td>
<td>410</td>
<td>2.17</td>
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<tr>
<td>PBCL-10BDO</td>
<td>50 / 50</td>
<td>10 / 1</td>
<td>n. m.</td>
<td>24 / 76</td>
<td>1250</td>
<td>1465</td>
<td>2.00</td>
</tr>
<tr>
<td>PBCL-12BDO</td>
<td>50 / 50</td>
<td>12 / 1</td>
<td>63</td>
<td>25 / 75</td>
<td>1620</td>
<td>n. m.</td>
<td>n. m.</td>
</tr>
<tr>
<td>PBCL-15BDO</td>
<td>50 / 50</td>
<td>15 / 1</td>
<td>58</td>
<td>18 / 82</td>
<td>2126</td>
<td>1760</td>
<td>2.25</td>
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<tr>
<td>PBCL-20BDO</td>
<td>50 / 50</td>
<td>20 / 1</td>
<td>54</td>
<td>23 / 77</td>
<td>2134</td>
<td>2200</td>
<td>2.43</td>
</tr>
</tbody>
</table>

\textsuperscript{a} - Determined by $^1$H-NMR
\textsuperscript{b} - Determined by GPC
\textsuperscript{c} - Reaction conducted without the catalyst
\textsuperscript{d} - Homopolymerization of γ-BL in bulk
n.m. - not measured.
Table 1 summarizes the results of the synthesis and characterization of a set of γ-BL copolymers and ε-CL with variable feed ratios of monomers and the initiator (BDO). The γ-BL homopolymer could not be obtained in the bulk. Conducting the reaction in toluene delivered a product with an unsatisfactory yield and MW. Similar observations have been reported by Nobes et al.\textsuperscript{44}. They polymerized γ-BL in the presence of porcine pancreatic lipase or Psudomonas cepacia at 60°C for almost 8 days only to obtain 900 g/mol homopolymer. A negative control, the reaction between γ-BL and ε-CL performed without the employment of the Novozyme 435, did not yield any product, confirming that the rest of the synthesized oligodiols were indeed enzyme-catalyzed. The polymerization yield of copolymers was between 43 wt% and 68 wt%, which is comparable to the maximal 62 wt% yield of copolymers of γ-BL and ε-CL obtained by He et al. also utilizing Novozyme 435 as the catalyst\textsuperscript{45}. In cases where other, non-immobilized lipases were used, the copolymer yields were generally lower\textsuperscript{44, 46}.

The chemical compositions, the MWs and the polydispersity of the oligodiols were determined with \textsuperscript{1}H-NMR spectroscopy (Fig. 2A) and GPC (Table 1). Experimentally determined γ-BL/ε-CL ratios revealed that the percentage of build-in γ-BL did not depend on the initial feed ratio. A maximum of 26 mol% γ-BL was incorporated in the oligodiol. Similar results have been observed by various research groups. Regardless of the chosen synthetic pathway, chemo-synthesis\textsuperscript{30, 34, 36, 48} or enzymatic catalysis\textsuperscript{45}, a maximum of 18 mol% of γ-BL\textsuperscript{48} incorporation was found. Low incorporation of γ-BL in the chemo-synthesis of γ-BL copolymers could be explained by already mentioned low reactivity of the γ-BL ring due to the low ring strain\textsuperscript{28}. The enzymatic catalysis proceeds via a different mechanism and is not influenced by the lactone ring stability. It is believed that the enzymatically catalyzed ROP proceeds more readily with hydrophobic substrates\textsuperscript{49}. Therefore, the lower hydrophobicity of γ-BL compared to the ε-CL, renders the lactone less prone to the lipase approach.

As expected, decreasing the amount of BDO resulted in the higher MWs of the oligodiols (Table 1). The obtained oligodiol MWs were in the range of 700 g/mol to 2130 g/mol. The polydisperisities of oligodiols (D; Table 1) increased with the increase of the oligodiol MW. The oligodiols were designed to be further used in the synthesis of the PBCL-PU copolymers similar to the polyester urethanes developed by Heijkants et al\textsuperscript{50}. They shown that higher oligodiol polydispersity decreases the microphase separation of PUs and consequently leads to poorer mechanical properties. However, as shown later in this paper, the microphase separation and the mechanical properties of PBCL-PU were not affected.
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3.2 Thermal analysis

In addition to chemical composition of γ-BL/ε-CL oligodiols, their thermal properties were also studied (Fig. 2). The melting temperatures of all the copolymers were lower than the melting temperature of the PCL homopolymer. Furthermore, the increase in the γ-BL content (for the same oligodiol MW) led to the decrease of the melt enthalpies (Fig. 2A). An explanation for this behavior can be related to the disturbance of PCL crystallization by the insertion of the γ-BL units. This has also been observed by He et al.\textsuperscript{45} and

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Figure 2. Thermal properties of oligodiols. $T_{m1}$ - 1\textsuperscript{st} melting temperature; $T_{m2}$ - 2\textsuperscript{nd} melting temperature; $\Delta H_m$ - enthalpy of melting. The results are extracted from the 2\textsuperscript{nd} heating scan.

A) Dependence of thermal properties of oligodiols in respect to the γ-BL mole content. The graph represents thermal properties of oligodiols with the MW of 770 g/mol on average.

B) Dependence of thermal properties of oligodiols with respect to the oligodiol MW. The graph represents thermal properties of oligodiols with the 23 mol% of γ-BL on average.
Nakayama et al.\textsuperscript{36}. In all the oligodiols, including the PCL homopolymer (all enzymatically-catalyzed) a double melting peak was detected (Fig. 2A). A possible cause for the additional melting peak could be a crystallization of cyclic oligodiols which were possibly formed\textsuperscript{51-53}. Two different melting peaks indicate a presence of the two different crystalline structures. However, only one peak was observed in GPC chromatographs indicating that either that cyclic oligodiol structures were removed by filtration during GPC sample preparation, or that their concentration was very low, hence, not detectible. With the variation of the oligodiol \textit{MW} with similar \(\gamma\)-BL contents, the melting temperatures and the corresponding enthalpies of melting increased (Fig. 2B). This can be related to the thermal properties observed for the series of PCL homopolymers of comparable \textit{MWs}\textsuperscript{20}.

3.3 \textit{ATR-FTIR} analysis

Oligodiols were further reacted into PUs. The conversion of oligodiols into PUs was proven by the \textit{ATR-FTIR} analysis (Fig. 3A). From the \textit{ATR-FTIR} measurements (Fig. 3A), three distinctive vibration bands have been detected in the PUs that were not present in the oligodiol spectra: at 3300 cm\(^{-1}\), 1680 cm\(^{-1}\) and 1535 cm\(^{-1}\), originating from N-H stretching vibration, carbonyl stretching vibration (amide I) and NH deformation vibration (Fig. 3A). As can be seen from the Fig. 3B, the ratio between the ester (oligodiol) carbonyl stretching vibration (at 1725 cm\(^{-1}\)) and the carbonyl amide I changed with the soft segment (oligodiol) length. It is clear that the amide I peak decreased with the increase in oligodiol length. The latter is the result of the decrease of the hard segment content and lower degree of microphase separation.

\textbf{Figure 3.} \textit{ATR-FTIR} analysis. A) Conversion of oligodiol (PBCL2130) into the PU (PBCL2130-PU). \(\nu\)(N-H) at 3319 cm\(^{-1}\), \(\nu\)\(_{s}\)(CH\(_2\)) at 2960 cm\(^{-1}\), \(\nu\)\(_{s}\)(CH\(_2\)) at 2935 cm\(^{-1}\), \(\nu\)(C=O) ester at 1721 cm\(^{-1}\), \(\nu\)(C=O) urethane at 1682 cm\(^{-1}\) (amide I), \(\delta\)(N-H) and \(\nu\)(CO-N) (amide II) at 1533 cm\(^{-1}\).
B) Influence of the oligodiol $MW$ on the ratio between ester carbonyl vibration $\nu(C=O)$ and the urethane carbonyl vibration $\nu(C=O)$ (amide I).

### 3.4 Characterization of the PBCL-PUs

The $MW$s of the PBCL-PUs were in the range of 43,000 g/mol to 65,000 g/mol (Table 2). The polydispersity increased with the increase in the oligodiol $MW$, which could be related to the higher polydispersity of the longer oligodiols (Table 1).

**Table 2.** Polyurethane characterization. $M_n$ - number average molar weight, $M_w$ - weight average molar weight, $D$ - polydispersity; $T_g$ - glass transition temperature, $T_{m,h}$ - hard segment melting temperature, $\Delta H_m$ - enthalpy of melting; $HSC_{TH}$ - hard segment content calculated as in reference$^{54}$, $CHS_{CA}$ - crystalline hard segment calculated based on the crystalline hard segment assumption; $CHS_{AA}$ - crystalline hard segment calculated based on the amorphous hard segment assumption.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$ (g/mol)</th>
<th>$M_w$ (g/mol)</th>
<th>$D$</th>
<th>$T_g$ (°C)</th>
<th>$T_{m,h}$ (°C)</th>
<th>$\Delta H_m$ (J/g)</th>
<th>$HSC_{TH}$ (%)</th>
<th>$CHS_{CA}$ (%)</th>
<th>$CHS_{AA}$ (%)</th>
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</thead>
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<td>PCL700-PU</td>
<td>48200</td>
<td>82400</td>
<td>1.71</td>
<td>-41.5</td>
<td>131.3</td>
<td>42.7</td>
<td>0.34</td>
<td>64</td>
<td>59</td>
</tr>
<tr>
<td>PBCL760-PU</td>
<td>42700</td>
<td>67850</td>
<td>1.59</td>
<td>-45.4</td>
<td>129.2</td>
<td>43.0</td>
<td>0.33</td>
<td>67</td>
<td>61</td>
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<tr>
<td>PBCL1250-PU</td>
<td>44450</td>
<td>74600</td>
<td>1.68</td>
<td>-58.1</td>
<td>103.1</td>
<td>23.0</td>
<td>0.24</td>
<td>49</td>
<td>45</td>
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<tr>
<td>PBCL1620-PU</td>
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<td>151600</td>
<td>2.33</td>
<td>-47.2</td>
<td>100.5</td>
<td>22.8</td>
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<tr>
<td>PBCL2130-PU</td>
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<td>141850</td>
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<td>-60.9</td>
<td>71.9</td>
<td>6.3</td>
<td>0.14</td>
<td>23</td>
<td>21</td>
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</tbody>
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As depicted in Fig. 4, an increase of the oligodiol $MW$ resulted in the decrease in the hard segment melting temperature ($T_{m,h}$) and the enthalpy of melting ($\Delta H_{m,h}$). Based on the measured $\Delta H_m$ and a value for the melting enthalpy of a theoretically 100% crystalline hard segment, the crystallinity of the PBCL-PUs was calculated according to already described method$^{20}$. From the Table 2, it was clear that the hard segment crystallinity decreased with the increase of the soft segment portion in the PU. For instance, only 23% of the hard segment in PBCL2130-PU was crystalline, indicating that hardly any microphase separation had taken place. The glass transition temperature ($T_g$) of the PBCL-PUs decreased 15°C when the oligodiol molar weight was increased from 760 g/mol to 2130 g/mol (Table 2), which was expected since the mobility of the chains increased.
Figure 4. Thermal properties of the polyurethanes. $T_{m,h}$ - hard segment melting temperature; $T_g$ - glass transition temperature; $\Delta H_{m,h}$ - hard segment enthalpy of melting. The results are extracted from the 2nd heating scan. The graph represents the relation between the thermal properties of the PBCL-PUs and the oligodiol $MW$. The graph represents thermal properties of oligodiols with the 23 mol% of γ-BL on average.

Tensile tests showed that with the increase in oligodiol $MW$, PBCL-PUs turned from relatively inelastic (brittle) to ductile materials (Fig. 5).

Figure 5. Mechanical properties of PBCL-PUs.

As expected, the increase in the oligodiol $MW$, i.e. decrease in the hard segment crystallinity and the degree of microphase separation, the Young’s Modulus decreased.
The Young’s Modulus of PBCL1620-PU was slightly higher than the Young’s Modulus of PCL-PU with the same oligodiol $MW$ (85 MPa in comparison to 64.6 MPa)$^{54}$. Although PBCL1620 oligodiol polydispersity was higher than the polydispersity of the corresponding PCL oligodiol, the mechanical properties were not affected.

### 3.5 Degradation of PBCL-PU

To evaluate if novel PBCL-PUs degrade faster than PCL-PUs, PBCL1620-PU was exposed to hydrolytic degradation for a period of 17 weeks (119 days). As can be observed from Fig. 6, approximately 1 wt% mass loss was detected during a course of the degradation study. Even though this mass loss is not high, Heijkants et al. did not observe any mass loss when PCL-PUs were incubated for more than a year$^{54}$. Linear fit of the mass loss data (Fig. 6) reveal the mass loss rate for PBCL1620-PU of 0.007 wt% per day, which would lead to mass loss of $\sim 4$ wt% after 2 years. This percentage is 10 times as high as mass loss observed when PCL and copolymer of trimethylene carbonate (TMC) and $\varepsilon$-CL were subjected to in vitro hydrolysis$^{55}$. The observed mass loss indicated that PBCL1620-PU degraded at least partially by surface erosion mechanism$^{17}$.

Water uptake of PBCL1620-PU was almost constant during the course of degradation with an average value of 0.6 wt%. This finding corresponds well with results of Heijkants et al.$^{54}$ for water uptake of non-degraded PCL-PU films of 1.0 wt%. Although this percentage appears low, it should be taken into account that the corresponding molar percentage of water is higher. Initial number of ester groups per PBCL1620-PU chain was can be determined by using the Eq. 5.

$$ N = \frac{M_n^0 (PBCL1620 – PU)}{M_n (PBCL) + MW (BDI) + MW (BDO)} \times \frac{M_n (PBCL)}{MW_{av} (MW (\varepsilon - CL) + MW (\gamma - BL))} $$

Equation 5

where $N$ is number of ester groups per PBCL1620-PU chain of initial $M_n$ of 65,000 g/mol ($M_n^0$) and $M_n (PBCL)$ is PBCL oligodiol $MW$ (1620 g/mol). Number of moles of ester groups per PBCL1620-PU chain can be calculated for the initial amount of PBCL1620-PU, which yields $1.14 \times 10^{-4}$ moles for a sample of 0.13 g. This finally leads to the molar ratio of water/(ester groups) of $\sim 4/1$, which represent considerable amount of water available to hydrolyze the PBCL1620-PU sample.
Figure 6. Mass loss and water uptake during hydrolytic degradation of PBCL1620-PU.

As expected for typically bulk degrading polymers, such as higher $MW$ PCL and PCL-PU, number average and weight average $MW$s of PBCL1620-PU and the polydispersity decreased during the course of degradation (Fig. 7). $M_n$ data were further used to determine the rate of the hydrolytic degradation of PBCL1620-PU.

Figure 7. Number average molar weight and polydispersity of PBCL1620-PU during hydrolytic degradation.

In vitro degradation of polyester urethanes is primarily ascribed to hydrolysis of the ester functionality of the oligodiol. If we treat PBCL-PUs as aliphatic polyester, the hydrolysis rate can be determined assuming two mechanisms: autocatalytic and non-catalytic\textsuperscript{56, 57}.
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Poly(α-hydroxyesters) (such as PCL), are known to be bulk eroding polymers. Due to the build up of the carboxylic acid formed during hydrolytic degradation, ester hydrolysis is acid catalyzed. Pitt et al. derived the following equation to describe the autocatalytic ester hydrolysis process as a pseudo-first order kinetics:

\[
\ln(M_n) = \ln(M_n^0) - k \times t
\]

Equation 6

where \(M_n\) [gmol\(^{-1}\)] is number average molar weight of polymer at any time point, \(M_n^0\) is initial number average molar weight [gmol\(^{-1}\)], \(k\) is hydrolysis rate constant [day\(^{-1}\)] of and \(t\) is degradation time [day]. Eq. 6 is based on the following assumptions: 1) extent of chain scission is small, 2) only ester bonds hydrolyze and 3) there is no mass loss. By linearly fitting the \(M_n\) degradation data of PBCL1620-PU according to the Eq. 6 (Fig. 8), \(k\) value of \(3.45 \times 10^{-3}\) day\(^{-1}\) was obtained, which was two times as high as \(k\) of comparable PU based on PCL oligodiol soft segment of approximately the same \(M_n\). Although the hydrolysis rate values for pure PCL polyester determined by different research groups assuming autocatalytic degradation mechanism differ significantly (Pitt et al.: 1.8 x 10\(^{-3}\) day\(^{-1}\); Pêgo et al.: 3.1 x 10\(^{-3}\) day\(^{-1}\), Anthunis et al.: 0.42 x 10\(^{-3}\) mol\(^{-1}\)day\(^{-1}\)), we showed that PBCL1620-PU degrade faster.

To explain non-catalyzed polyester hydrolysis, Pitt et al. derived Eq. 7 to describe the degradation as a first order kinetic process.
Equation 7

\[ \frac{1}{M_n} = \frac{1}{M_n^0} + k_2 \times t \]

Fitting \( M_n \) degradation data for PBCL1620-PU according to the Eq. 7 (Fig. 9) resulted in \( k_2 \) of \( 1.73 \times 10^{-7} \text{ mol g}^{-1} \text{ day}^{-1} \).

This model does not take into account concentration of ester groups nor water. Lyu et al. proposed another model to describe the non-catalytic polyester hydrolysis as a second order kinetic process from which the following first order equation was derived\(^5\):  

\[ \frac{1}{N(t)} = k_3 \times C_s \times (t - t_i) \]  

Equation 8

where \( N(t) \) is the number of ester groups that remained in the chain at time \( t \), \( k_3 \) is the rate constant \([\text{L mol}^{-1} \text{ day}^{-1}]\), \( C_s \) \([\text{mol L}^{-1}]\) is water concentration at the moment polyester is saturated with water, \( t \) \([\text{day}]\) is the degradation time and \( t_i \) is the time when polyester is saturated with water.

If \( N(t) \) is calculated according to Eq. 5 and data fitted according to the Eq. 8 (Fig. 9), for an average water content of 0.6 wt% that is absorbed by PBCL1620-PU sample (4 x 4 x 4 mm\(^3\)) weighing 0.13 g on average the rate constant, \( k_3 \), was calculated to be \( 5.88 \times 10^{-7} \text{ L mol}^{-1} \text{ day}^{-1} \). This value is in the same order of magnitude as the rate constant calculated using non-catalytic model of Pitt et al.\(^5\). Disadvantage of both models is that they neglect auto-catalytic effect of the degradation products.

![Figure 9](image_url)

**Figure 9.** Linear fit of PBCL1620-PU \( M_n \) measured during in vitro degradation assuming non-catalytic hydrolysis mechanism (according to Pitt et al.$^{58}$ \( R^2 = 0.938 \), \( p < 0.0001 \)) and Lyu et al.$^{56}$ \( R^2 = 0.821 \), \( p < 0.0001 \)).
Recently, a new model has been proposed to describe hydrolytic degradation of high $MW$ polyesters. Although this model corrects for crystalline domains present in semicrystalline polymers, it requires for the initial acid concentration to be larger than zero, which is not the case with PBCL-PUs.

All mentioned degradation models cannot adequately describe hydrolytic degradation of polyester urethanes, such as PBCL-PUs of this study. They do not account for hydrolytic degradation of urethane hard segment in amorphous state and neglect the effect of the extent of microphase separation. However, they can be used to compare degradation rate of PBCL-PUs with those of relevant degradable polymers. This analysis revealed that PBCL1620-PU degrade faster \textit{in vitro} than PCL-PU of the same oligodiol $MW$ and high $MW$ PCL. Since both hard segment chemistry and extent of microphase separation were almost identical, the enhanced hydrolysability of PBCL1620 can only originate from the introduction of a hydrophilic $\gamma$-BL monomer. Furthermore, $\gamma$-BL monomer decreased the PCL oligodiol crystallinity, which might also have influenced accessibility of ester bonds to hydrolysis.

Recently, we assessed $\gamma$-BL/$\varepsilon$-CL-PU manufactured in the same manner as described in this study for its biological behavior\textsuperscript{62}. This PU was non-cytotoxic and haemocompatible \textit{in vitro} and exhibited good biocompatibility \textit{in vivo}.

4. Conclusions

The goal of this study was to develop biodegradable PUs with enhanced degradability that could be used as biomedical scaffolds for tissue regeneration. We successfully synthesized a range of different molar weight oligodiols via enzymatically-catalyzed, 1,4-butanediol initiated ROP of $\gamma$-BL and $\varepsilon$-CL in a relatively good yield. The oligodiols were converted into PUs of satisfactory $MW$s and good mechanical properties. With varying the oligodiol $MW$ in the PU synthesis it is possible to tailor the materials with the specific physical and mechanical properties. The percentage of incorporated hydrophilic $\gamma$-BL in oligodiols varied from 15 - 26 mol\%, which led to the substantial decrease the PCL crystallinity and improvement of the PU degradation. Employment of the enzymatic catalyst (Novozyme 435) that could be easily removed from the reaction mixture, a relatively low-cost $\gamma$-BL monomer and conducting the polymerizations at room temperature make this process attractive for industrial manufacturing of oligodiols involved in the synthesis of biomedical PUs. Furthermore, PBCL-PUs were shown to be biocompatible both \textit{in vitro} and \textit{in vivo}, which renders this type of PUs excellent candidates for manufacturing of scaffolds to be used in tissue regeneration.
Chapter 4

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References

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