

# Triblock copolymers based on $\epsilon$ -caprolactone and trimethylene carbonate for the 3D printing of tissue engineering scaffolds

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## ABSTRACT

**Background:** Biodegradable PCL-*b*-PTMC-*b*-PCL triblock copolymers based on trimethylene carbonate (TMC) and  $\epsilon$ -caprolactone (CL) were prepared and used in the 3D printing of tissue engineering scaffolds. Triblock copolymers of various molecular weights containing equal amounts of TMC and CL were prepared. These block copolymers combine the low glass transition temperature of amorphous PTMC (approximately -20°C) and the semi-crystallinity of PCL (glass transition approximately -60°C and melting temperature approximately 60°C).

**Methods:** PCL-*b*-PTMC-*b*-PCL triblock copolymers were synthesized by sequential ring opening polymerization (ROP) of TMC and  $\epsilon$ -CL. From these materials, films were prepared by solvent casting and porous structures were prepared by extrusion-based 3D printing.

**Results:** Films prepared from a polymer with a relatively high molecular weight of 62 kg/mol had a melting temperature of 58°C and showed tough and resilient behavior, with values of the elastic modulus, tensile strength and elongation at break of approximately 120 MPa, 16 MPa and 620%, respectively. Porous structures were prepared by 3D printing. Ethylene carbonate was used as a crystallizable and water-extractable solvent to prepare structures with microporous strands. Solutions, containing 25 wt% of the triblock copolymer, were extruded at 50°C then cooled at different temperatures. Slow cooling at room temperature resulted in pores with widths of  $18 \pm 6 \mu\text{m}$  and lengths of  $221 \pm 77 \mu\text{m}$ , rapid cooling with dry ice resulted in pores with widths of  $13 \pm 3 \mu\text{m}$  and lengths of  $58 \pm 12 \mu\text{m}$ . These PCL-*b*-PTMC-*b*-PCL triblock copolymers processed into porous structures at relatively low temperatures may find wide application as designed degradable tissue engineering scaffolds.

**Conclusions:** In this preliminary study we prepared biodegradable triblock copolymers based on 1,3-trimethylene carbonate and  $\epsilon$ -caprolactone and assessed their physical characteristics. Furthermore, we evaluated their potential as melt-processable thermoplastic elastomeric biomaterials in 3D printing of tissue engineering scaffolds.

**Keywords:** 3D printing, Degradable thermoplastic elastomers, Poly( $\epsilon$ -caprolactone), Poly(trimethylene carbonate), Tissue engineering, Triblock copolymers

## Introduction

Extrusion-based additive manufacturing processes (3D printing) are of great interest in preparing designed implants

for biomedical applications such as tissue engineering (1). Recent advances in scaffold fabrication with 3D printing enable the manufacturing of designed porous structures with highly controlled geometries and interconnected pore network architectures into which the cells can migrate and grow. However, the number of biodegradable polymeric materials available for 3D printing of tissue engineering scaffolds and biofabrication is very limited (2, 3)

Poly( $\epsilon$ -caprolactone) (PCL) is the preferred synthetic thermoplastic biomaterial used in 3D printing. It is a semi-crystalline polymer obtained by conventional ring opening polymerization of  $\epsilon$ -caprolactone using stannous octoate as catalyst. The polymer has a low glass transition temperature of approximately -60°C and a melting temperature of approximately 60°C (4, 5). Its low melting temperature and rapid

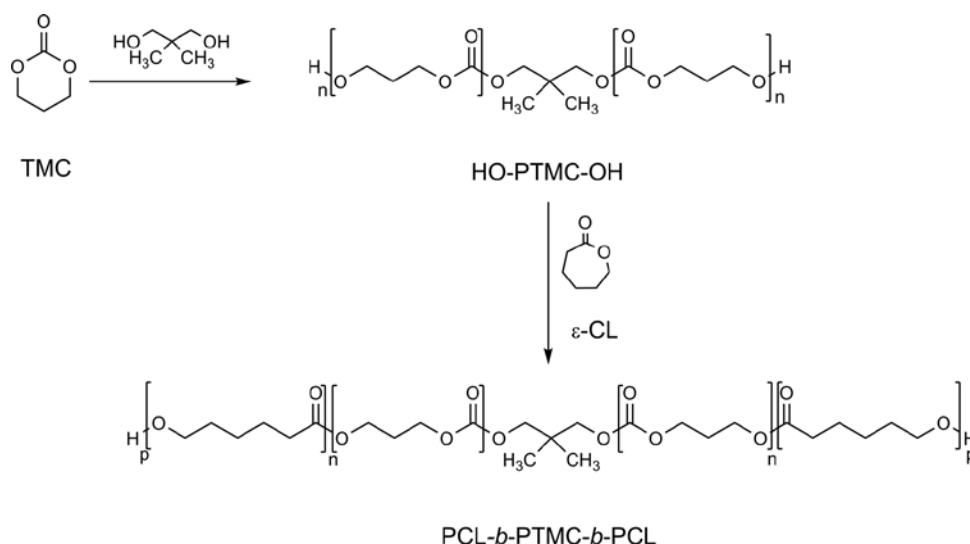
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**Fig. 1** - Synthesis of PCL-*b*-PTMC-*b*-PCL triblock-copolymers by sequential ring opening polymerization of TMC and  $\epsilon$ -CL.

crystallization and solidification rate allow the polymer to be readily processed by extrusion-based, additive manufacturing techniques. Form-stable designed 3D structures can so be prepared in a consistent and reproducible manner (6, 7).

At room temperature, PCL of sufficiently high molecular weight has a tensile modulus of 350 to 450 MPa, a yield strength of approximately 15 MPa, an elongation at yield of approximately 15%, a maximum tensile strength of 15 to 40 MPa and an elongation at break of 500% to 1000% (8, 9). Another characteristic feature of the polymer is that when implanted *in vivo*, it degrades at a very slow rate, and PCL of molecular weights higher than approximately 40 kg/mol barely degrade *in vivo* (9-11). The somewhat rigid nature of the material and its very low rate of degradation *in vivo* may make PCL less suited for the manufacturing of biomedical implants, devices and tissue engineering scaffolds. It would therefore be of great interest to develop more flexible biodegradable materials for use in the 3D printing of designed scaffolding structures.

Particularly interesting in this regard are block copolymers, as their physical properties can readily be tuned by variation of the nature and length of the respective blocks (12). In previous work we have described the preparation and properties of biodegradable thermoplastic elastomers based on trimethylene carbonate (TMC) and L-lactide (LLA) (13). While poly(trimethylene carbonate) (PTMC) is a rubber-like, amorphous polymer with a low glass transition temperature of approximately -17°C, poly(L-lactide) (PLLA) is a semi-crystalline polymer with a relatively high glass transition close to 60°C and a melting point of approximately 180°C. We were able to prepare biodegradable triblock copolymers by sequential polymerization of the monomers. These thermoplastic elastomers combined the elastic properties of rubbers with the thermal processability of semi-crystalline thermoplastic materials.

## Materials and methods

### Materials

1,3-trimethylene carbonate monomer (TMC, obtained from Huizhou ForYou Medical Devices Company), diphenyl

phosphate (DPP, obtained from Tokyo Chemical Industry UK) and 2,2-dimethyl 1,3-propanediol (DMP, obtained from Sigma-Aldrich) were used as received.  $\epsilon$ -caprolactone monomer ( $\epsilon$ -CL, obtained from Acros Organics) was purified by drying over  $\text{CaH}_2$  and distillation under vacuum. Ethylene carbonate and  $\text{CDCl}_3$  were obtained from Sigma-Aldrich and used as received. Dichloromethane and ethanol, respectively obtained from VWR Chemicals and Merck, were of analytical grade and used as received. Poly( $\epsilon$ -caprolactone) with a molecular weight of 80 kg/mol (PCL<sub>80</sub>) was obtained from Sigma-Aldrich.

### Synthesis of PCL-*b*-PTMC-*b*-PCL triblock copolymers

PCL-*b*-PTMC-*b*-PCL triblock copolymers were synthesized by sequential ring opening polymerization (ROP) of TMC and  $\epsilon$ -CL as shown in Figure 1. Using 2,2-dimethyl 1,3-propanediol (DMP) as an initiator and diphenyl phosphate (DPP) as a catalyst (14), a dihydroxy group-terminated PTMC oligomer was first prepared by polymerization in an argon atmosphere for 72 hours at a temperature of 70°C. The molar ratio of DPP catalyst to DMP initiator was 1:1. Subsequently, this PTMC oligomer was used to initiate the ROP of  $\epsilon$ -CL. This reaction was allowed to proceed for another 24 hours under the same conditions, leading to the formation of a PCL-*b*-PTMC-*b*-PCL triblock copolymer. The weight ratio of the TMC:CL monomers used was 1:1. All polymerizations were carried out in bulk in 3-necked 500 mL glass flasks on a 50-g scale, a mechanical stirrer was used to ensure homogeneity of the reaction mixtures.

As hydroxy groups initiate the polymerization of TMC and  $\epsilon$ -CL, polymers with different predetermined molecular weights and block lengths were prepared by varying the initiator to monomer ratio. PTMC oligomers with targeted molecular weights of 20, 30 and 40 kg/mol (PTMC<sub>20</sub>, PTMC<sub>30</sub>, PTMC<sub>40</sub>) were used to prepare PCL-*b*-PTMC-*b*-PCL block copolymers with targeted PCL block lengths of 10, 15 and 20 kg/mol (PCL<sub>10</sub>-PTMC<sub>20</sub>-PCL<sub>10</sub>, PCL<sub>15</sub>-PTMC<sub>30</sub>-PCL<sub>15</sub>, PCL<sub>20</sub>-PTMC<sub>40</sub>-PCL<sub>20</sub>). The reaction products were dissolved in dichloromethane, precipitated in cold ethanol and subsequently dried under vacuum at 40°C.

### Characterization of the synthesized PTMC oligomers and PCL-*b*-PTMC-*b*-PCL triblock copolymers

The conversion of the TMC and  $\epsilon$ -CL monomers, the chemical compositions and the number average molecular weights ( $M_n$ ) of the prepared (triblock co)polymers were determined by nuclear magnetic resonance (NMR) spectroscopy. A 400 MHz  $^1\text{H}$ -NMR apparatus (Bruker Ascend 400) was used to record spectra of the polymer solutions in  $\text{CDCl}_3$  (Sigma). 100 MHz  $^{13}\text{C}$ -NMR spectroscopy (Bruker Ascend 400) was performed to confirm the block structure of the prepared triblock copolymers.

The thermal properties of the purified copolymers were evaluated by differential scanning calorimetry (DSC). Samples (5–10 mg) were placed in aluminum pans and analyzed using a Pyris 1 DSC (Perkin Elmer). The samples were heated from  $-100$  to  $150^\circ\text{C}$  at a rate of  $10^\circ\text{C}/\text{min}$ . After this first scan, the samples were quenched rapidly ( $100^\circ\text{C}/\text{min}$ ) to  $-100^\circ\text{C}$ , after 5 minutes at that temperature a second scan at a rate of  $10^\circ\text{C}/\text{min}$  was recorded. The data presented were determined in the second heating scan.

### Mechanical properties of the synthesized PCL-*b*-PTMC-*b*-PCL triblock copolymers

The triblock copolymers were dissolved in chloroform at a concentration of  $0.3\text{ g/mL}$  and cast on glass plates. After careful drying at ambient conditions for 7 days,  $150$ - to  $200\text{-}\mu\text{m}$  thick films were obtained. Specimens for tensile testing were punched out from these films in accordance with the dimensions of ASTM D882-91.

The mechanical properties of the polymers were determined in 5-fold at room temperature in tensile tests according to ASTM D882-91 specifications. A universal tensile tester (Zwick Z020) equipped with a  $500\text{ N}$  load cell was operated at a crosshead speed of  $50\text{ mm}/\text{min}$ .

The yield stress, maximum tensile strength, elongation at yield and elongation at break were determined from the stress-strain diagrams. The yield stress and elongation at yield were determined from the intersection of tangents to the curves. The Young's modulus was determined from the initial slope of the stress-strain curves; since the elongation of the specimens was determined from the grip-to-grip separation, the values only indicate the stiffness of the materials. The toughness of the specimens (expressed in  $\text{N}/\text{mm}^2$ ) was determined as the area under the stress-strain curves.

### 3D printing of PCL-*b*-PTMC-*b*-PCL triblock copolymers

Scaffolding structures were prepared by extrusion of solutions of the triblock copolymers in ethylene carbonate. This benign, inert and water-soluble solvent has a very high boiling point of  $243^\circ\text{C}$  and a low melting temperature of  $37^\circ\text{C}$ . The triblock copolymers were dissolved in ethylene carbonate at  $50^\circ\text{C}$  or  $60^\circ\text{C}$  at a concentration of  $20$  or  $25\text{ wt}\%$  and printed using a BioScaffolder dispensing system (SYS + ENG) onto a glass surface in a  $0^\circ/90^\circ$  lay-down pattern where fibers were deposited at  $90^\circ$  angles between successive layers. The diameter of the nozzles was  $100$  or  $234\text{ }\mu\text{m}$ . The extruded strands and the scaffold being built were cooled in air or with

dry ice. The printed PCL-*b*-PTMC-*b*-PCL triblock copolymer scaffolds were then extracted with water and dried at ambient conditions. The morphology of the fabricated structures was assessed by scanning electron microscopy (HR-SEM; Zeiss 1550) after sputter-coating with gold.

## Results and discussion

### Synthesis of PCL-*b*-PTMC-*b*-PCL triblock copolymers

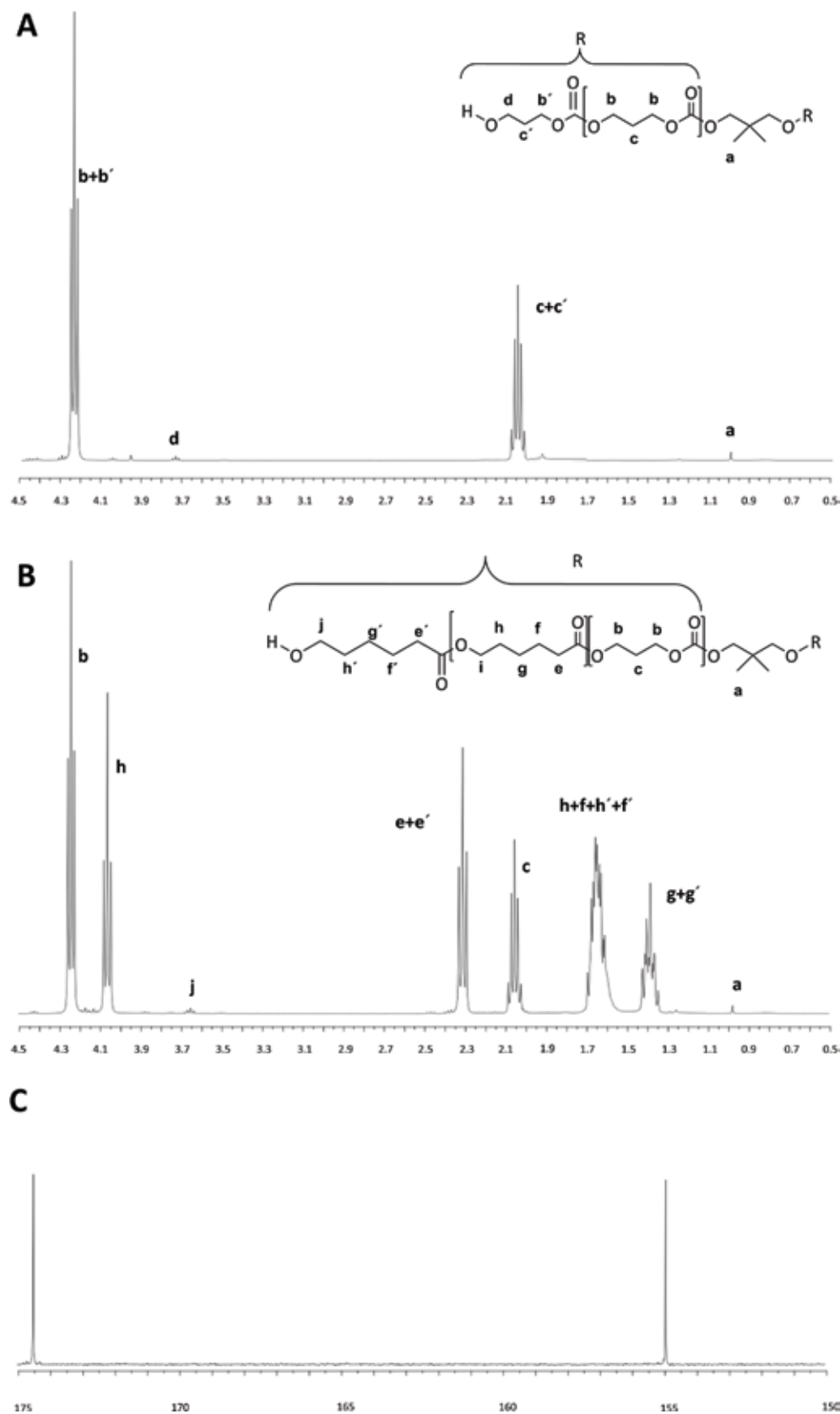
PCL-*b*-PTMC-*b*-PCL triblock copolymers were synthesized by sequential ROP of TMC and  $\epsilon$ -CL at  $70^\circ\text{C}$ . In the first step, dihydroxy group-terminated PTMC oligomers with targeted  $M_n$  values of  $20$ ,  $30$  and  $40\text{ kg}/\text{mol}$  were prepared using DMP as initiator and DPP as catalyst. These PTMC oligomers were then used to initiate the ROP of an equal amount by weight of CL. This is schematically illustrated in Figure 1.

The NMR analyses of specimens taken at different time points of the triblock copolymer synthesis preparation allowed us to characterize the polymerization process and the polymers that were synthesized. Using NMR, the conversion of the monomers in the reaction mixture, we were able to determine the compositions and the number of average molecular weights ( $M_n$ ) of the polymers obtained after precipitation and drying. In Figure 2, characteristic spectra are presented.

Figure 2A shows a  $^1\text{H}$ -NMR spectrum of a PTMC oligomer synthesized in the first polymerization step. The TMC monomer conversion in the first step was determined from the integral values of the characteristic  $-\text{CO}-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{O}-$  peaks of TMC at  $4.44$ – $4.48\text{ ppm}$  centered around  $4.45\text{ ppm}$  and the integral value of the corresponding peaks ( $b+b'$ ) of PTMC at  $4.18$  to  $4.32\text{ ppm}$ . The TMC conversions were found to be in excess of  $99\%$ . The  $M_n$  of the precursor polymer was determined by comparison of the integral value of the methyl H atom of the DMP residue at  $0.94\text{ ppm}$  with that of the ( $b+b'$ ) PTMC peaks at  $4.18$  to  $4.32\text{ ppm}$ .

Upon addition of  $\epsilon$ -CL and subsequent polymerization of the monomer, a triblock copolymer is obtained. NMR analysis showed that under the applied reaction conditions, the conversion of  $\epsilon$ -CL was  $99\%$  or higher (characteristic  $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CO}-\text{O}-$  peaks of  $\epsilon$ -CL monomer at  $2.52$  to  $2.67\text{ ppm}$  can be distinguished from the corresponding polymeric peaks of PCL at  $2.27$  to  $2.34\text{ ppm}$ , spectra not shown). After precipitation and drying, a triblock copolymer is obtained; Figure 2B presents a  $^1\text{H}$ -NMR spectrum of it. Besides the peaks corresponding to the PTMC segments at  $4.18$  to  $4.32\text{ ppm}$  and  $2.01$  to  $2.09\text{ ppm}$ , additional peaks corresponding to PCL can be discerned in the spectra. These peaks can be assigned as follows: the peaks at  $1.35$  to  $1.45\text{ ppm}$  to protons  $g+g'$  in the accompanying structural formula, at  $1.55$  to  $1.70\text{ ppm}$  to protons  $h+f+h'+f'$ , at  $1.95$  to  $2.10\text{ ppm}$  to protons  $c$ , at  $2.26$  to  $2.33\text{ ppm}$  to protons  $e+e'$ , at  $3.60$  to  $3.70\text{ ppm}$  to protons  $j$ , at  $3.95$  to  $4.03\text{ ppm}$  to  $-\text{OCH}_2-$   $h$  and at  $4.00$  to  $4.10\text{ ppm}$  to protons  $b$ .

The composition of the purified triblock copolymers can be calculated from the ratios of the integral values of the peak signals at  $2.01$  to  $2.09\text{ ppm}$  or at  $4.18$  to  $4.32\text{ ppm}$  (corresponding to the PTMC block) and at  $2.26$  to  $2.33\text{ ppm}$  (corresponding to the PCL blocks). Knowing  $M_n$  of PTMC and the composition of the copolymer, the  $M_n$  of the triblock copolymers can now be calculated as well.



**Fig. 2** - Characteristic  $^1\text{H}$ -NMR spectra of (A) synthesized PTMC oligomers and (B) PCL-b-PTMC-b-PCL triblock copolymers. (C) Characteristic  $^{13}\text{C}$ -NMR spectrum of PCL-b-PTMC-b-PCL triblock copolymers. The spectra shown are of a  $\text{PCL}_{20}\text{-PTMC}_{40}\text{-PCL}_{20}$  triblock copolymer, see Tab. I.

**TABLE I** - Characteristics of the synthesized PTMC oligomers and PCL-*b*-PTMC-*b*-PCL triblock copolymers as determined by <sup>1</sup>H-NMR

Target structure	PTMC oligomer		Triblock copolymer		Triblock copolymer after purification	
	TMC conversion (%)	M <sub>n</sub> (kg/mol)	ε-CL conversion (%)	TMC: ε-CL (weight ratio)	Targeted M <sub>n</sub> (kg/mol)	Obtained M <sub>n</sub> (kg/mol)
PCL <sub>10</sub> -PTMC <sub>20</sub> -PCL <sub>10</sub>	99.8	18.3	98.9	54:46	40	35.4
PCL <sub>15</sub> -PTMC <sub>30</sub> -PCL <sub>15</sub>	99.7	25.8	99.2	55:45	60	47.1
PCL <sub>20</sub> -PTMC <sub>40</sub> -PCL <sub>20</sub>	99.8	35.2	98.9	57:43	80	62.2

**TABLE II** - Thermal properties of the synthesized PCL-*b*-PTMC-*b*-PCL triblock copolymers and PCL as determined by DSC

	TMC: ε-CL (weight ratio)	T <sub>g1</sub> (°C)	T <sub>g2</sub> (°C)	T <sub>m</sub> (°C)	ΔH (J/g)
PCL <sub>10</sub> -PTMC <sub>20</sub> -PCL <sub>10</sub> <sup>a)</sup>	54:46	-57.8	-19.4	51.9	33.4
PCL <sub>15</sub> -PTMC <sub>30</sub> -PCL <sub>15</sub> <sup>a)</sup>	55:45	-60.1	-19.2	52.7	39.7
PCL <sub>20</sub> -PTMC <sub>40</sub> -PCL <sub>20</sub> <sup>a)</sup>	57:43	-58.3	-20.3	58.4	42.7
PCL <sub>80</sub>	0:100	-59.6	-	54.1	52.8

<sup>a)</sup> A description of the nomenclature of the polymers used for the study is given in the Materials and methods section; characteristics of the polymers are given in Tab. I.

Figure 2C shows a <sup>13</sup>C-NMR spectrum of the carbonyl region of the obtained triblock copolymer. Single peaks at 155.2 and 174.0 ppm, respectively corresponding to carbonyl carbons of the PTMC blocks and of the PCL blocks, confirm the block structure of the triblock copolymers. A more random structure, resulting for example from transesterification reactions, would lead to a splitting of these signals (15).

An overview of the characteristics of the synthesized precursors and triblock copolymers is given in Table I. It can be seen that the TMC conversion was essentially complete and that the M<sub>n</sub> values of the PTMC were close to the targeted values. In the second polymerization step, the conversion of ε°-CL was very high as well, although the composition of the resulting triblock copolymer was somewhat richer in TMC than the feed composition. While the synthesis procedure allowed control of the characteristics of the triblock copolymers, their molecular weight was somewhat lower than intended.

The thermal properties of the PCL-*b*-PTMC-*b*-PCL triblock copolymers are determined by those of the PCL-segments and the PTMC segments. Table II gives an overview of the thermal properties of the prepared PCL-*b*-PTMC-*b*-PCL triblock copolymers obtained after precipitation and drying as determined by DSC in the second heating scan after quenching from the melt. The table also shows that PCL<sub>80</sub> is a semi-crystalline polymer with a melting temperature of approximately 54°C and a glass transition temperature of approximately -60°C.

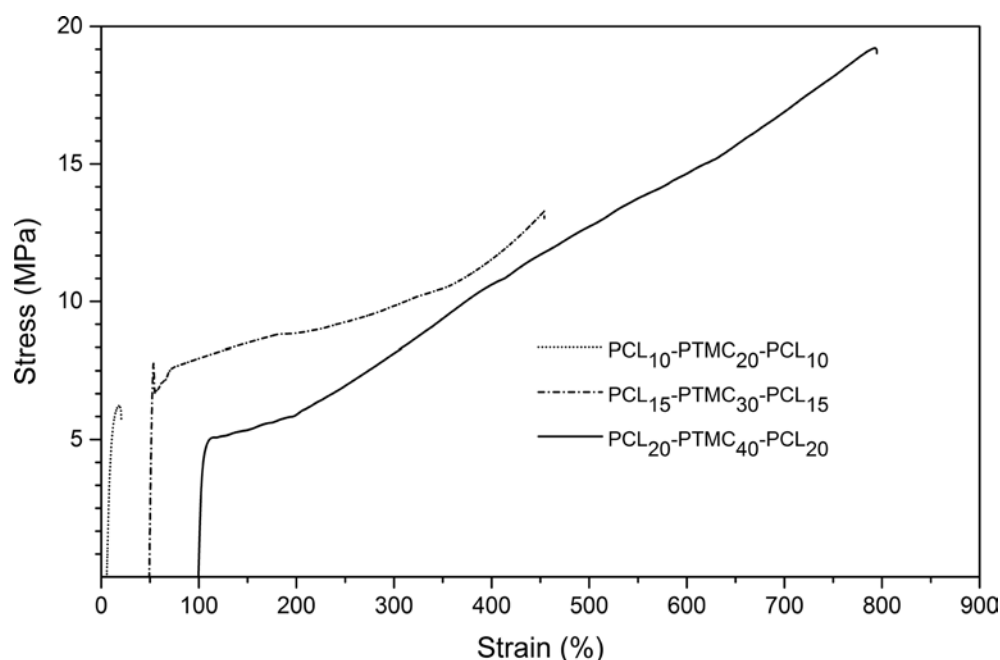
For all compositions, the triblock copolymers showed 2 glass transition temperatures and a single melting temperature, indicating a phase separated system. The higher glass transition temperatures at -19 to -20°C are related to the PTMC segments of the triblock copolymers. As mentioned before, PTMC is an amorphous polymer with a glass transition temperature of approximately -17°C. The lower glass

transition temperatures at -60°C to -58°C and the melting temperature of the crystalline fraction at 52°C to 58°C are due to the PCL segments in the block copolymer. It can be seen in the table that with increasing lengths of the PTMC- and PCL blocks of the copolymer phase, separation becomes more pronounced. For comparable monomer contents, the glass transition temperatures of the amorphous phases decrease, while the melting temperature and heat of fusion of the crystalline phases increase. These values approach those of the PCL<sub>80</sub> homopolymer.

The phase separated morphology of these triblock copolymers is a very important feature, as it renders the polymer matrix a physically cross-linked material. Such thermoplastic elastomers have the characteristics of a rubber-like material and the melt processability (and solvent solubility) of a thermoplast. Indeed, when these materials are cast into films by dissolution in chloroform followed by evaporation of the solvent, transparent supple films are obtained. Characteristic tensile stress-strain diagrams at room temperature of these films are shown in Figure 3.

It can be seen that with increasing molecular weights of the PTMC- and PCL blocks and the overall molecular weight of the PCL-*b*-PTMC-*b*-PCL triblock copolymers, the maximum tensile strength and elongation at break increase. See also Table III. The toughness of the block copolymers, determined from the area under the stress-strain curves, increases with increasing molecular weights as well. Earlier work has described the synthesis of PCL-*b*-PTMC-*b*-PCL triblock copolymers, but polymers of sufficiently high molecular weight that would yield materials with interesting mechanical properties were not obtained (16).

The values of the elasticity modulus of the triblock copolymers are significantly lower than that of PCL. When



**Fig. 3** - Characteristic stress-strain diagrams of PCL-*b*-PTMC-*b*-PCL triblock copolymers determined at room temperature, illustrating the effect of the molecular weight of the weights of the PTMC- and PCL blocks on their tensile properties. A description of the used nomenclature of the polymers is given in the experimental part, characteristics of the polymers are given in Table I.

**TABLE III** - Mechanical properties of synthesized PCL-*b*-PTMC-*b*-PCL triblock copolymers and PCL as determined by tensile testing at room temperature

	E (MPa)	$\sigma_{\text{yield}}$ (MPa)	$\epsilon_{\text{yield}}$ (%)	$\sigma_{\text{max}}$ (MPa)	$\epsilon_{\text{break}}$ (%)	Toughness (N/mm <sup>2</sup> )
PCL <sub>10</sub> -PTMC <sub>20</sub> -PCL <sub>10</sub> <sup>a)</sup>	125 ± 6	6 ± 0.8	13 ± 2	8 ± 3	15 ± 2	41.7 ± 12
PCL <sub>15</sub> -PTMC <sub>30</sub> -PCL <sub>15</sub> <sup>a)</sup>	275 ± 5	8 ± 1.7	6 ± 1	14 ± 5	420 ± 135	611 ± 190
PCL <sub>20</sub> -PTMC <sub>40</sub> -PCL <sub>20</sub> <sup>a)</sup>	120 ± 9	5 ± 0.4	10 ± 2	16 ± 3	620 ± 90	989 ± 251
PCL <sub>80</sub>	313 ± 25	14 ± 1	6 ± 1	30 ± 3	634 ± 102	1568 ± 275

<sup>a)</sup> A description of the used nomenclature of the polymers is given in the Materials and methods section; characteristics of the polymers are given in Tab. I.

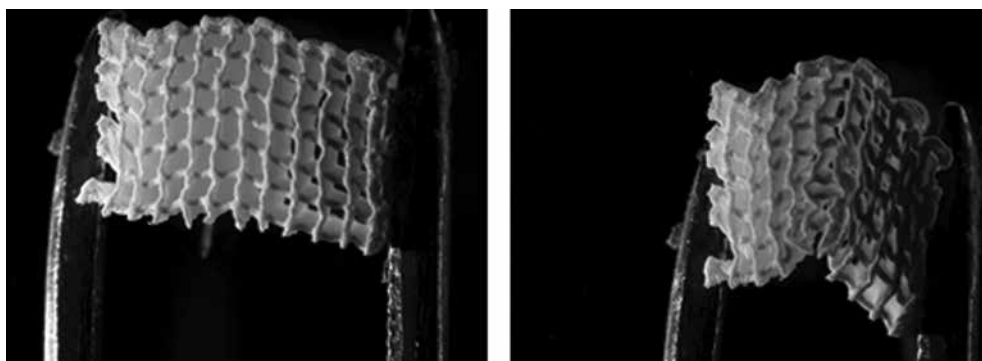
comparing the PCL<sub>20</sub>-PTMC<sub>40</sub>-PCL<sub>20</sub> triblock polymer with PCL, it can be seen that the triblock copolymer has a modulus of 120 MPa while the modulus of PCL is 313 MPa. While the elongations at break are comparable, the yield strength and tensile strength of the triblock copolymer are lower too. Due to the reduced CL content and crystallinity, it can be expected that the degradation characteristics of these triblock copolymers will be more favorable than those of PCL. More experimental work is needed to fully assess the effect of composition and molecular weight of PCL-*b*-PTMC-*b*-PCL triblock copolymers on their thermal-, mechanical- and degradation characteristics.

It should be noted, however, that the mechanical properties of the relatively high-molecular-weight PCL-*b*-PTMC-*b*-PCL triblock copolymers we prepared are a great deal better than those of those of PTMC-*b*-PCL-*b*-PTMC triblock copolymers with similar compositions and molecular weights described in earlier work (17). Therefore, to obtain an effective physically cross-linked network, it seems essential that the low  $T_g$  amorphous PTMC block in the triblock copolymer is flanked by crystallizable PCL ones.

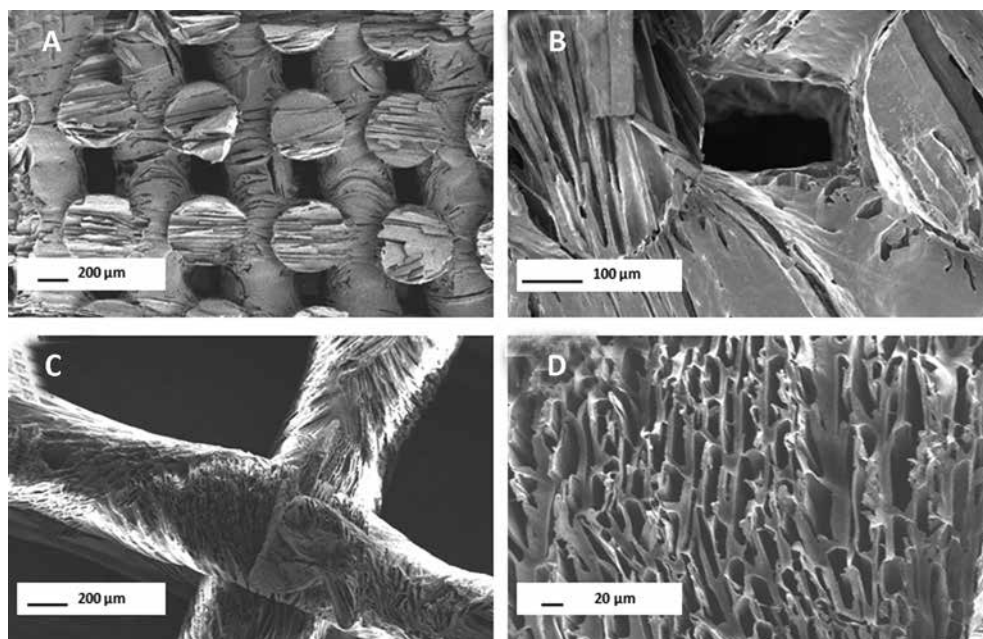
### 3D printing of PCL-*b*-PTMC-*b*-PCL triblock copolymers

3D scaffolding structures were prepared from the PCL-*b*-PTMC-*b*-PCL triblock copolymer with the highest molecular weight. Although the PCL<sub>20</sub>-PTMC<sub>40</sub>-PCL<sub>20</sub> polymer has a low melting temperature of approximately 58°C and can readily be extruded from the melt, we prepared the structures by 3D printing solutions of the triblock copolymer in ethylene carbonate. Ethylene carbonate is a benign water-soluble compound with a melting point of 37°C. By dissolving polymers into this compound at elevated temperatures and subsequently cooling to below its melting temperature, porous polymeric structures can be obtained after extraction with water (18, 19). Therefore, in our case, we will obtain porous structures with microporous features in the fibers upon extraction of the crystallized solvent. The crystallization behavior of ethylene carbonate and thus the characteristics of the resulting microporosity will be largely determined by the concentration of the solvent and the rate of cooling applied.

The PCL<sub>20</sub>-PTMC<sub>40</sub>-PCL<sub>20</sub> triblock copolymer was dissolved in different amounts of ethylene carbonate at 50°C;



**Fig. 4** - Photographic images of a scaffold prepared by extrusion 3D printing of a PCL<sub>20</sub>-PTMC<sub>40</sub>-PCL<sub>20</sub> triblock copolymer after extraction of the crystallized ethylene carbonate solvent and drying.



**Fig. 5** - Scanning electron microscopy images of 3D porous structures prepared by 3D printing of PCL<sub>20</sub>-PTMC<sub>40</sub>-PCL<sub>20</sub> triblock polymer from 25% solutions in ethylene carbonate at 50°C. In (A) and (B) the extruded strands were cooled at room temperature. In (C) and (D) the extruded strands were cooled with dry ice. The images were obtained after extraction of the solvent and drying. See Tab.I for characteristics of the polymer.

clear solutions were obtained. As PCL is insoluble in ethylene carbonate (19), this indicates that no PCL was present in the solution. The solutions were then extruded at 50°C or 60°C through a 100 or 234 μm nozzle at a pressure of 145 kPa, depositing the fibers onto a glass plate in a 0°/90° lay-down pattern. Figure 4 is an image of such a prepared structure. The scaffold is held between tweezers, and can easily be deformed. This illustrates the very high flexibility of the prepared structures.

The SEM images in Figures 5 and 6 show that scaffolds with microporous strands were obtained after extraction. Figures 5A and B shows SEM images of a scaffold prepared from 25% solutions of the polymer in ethylene carbonate upon deposition of the strands at room temperature. The distance between the strands was set to 0.8 mm. Due to die swell, their diameter was approximately 500 μm and the distance between the strands in the built structure ranged between 150 and 250 μm. Because the degree of undercooling for crystallization of the solvent is low at this temperature, relatively large micropores with a width of  $18 \pm 6 \mu\text{m}$  and a length of  $221 \pm 77 \mu\text{m}$  were obtained.

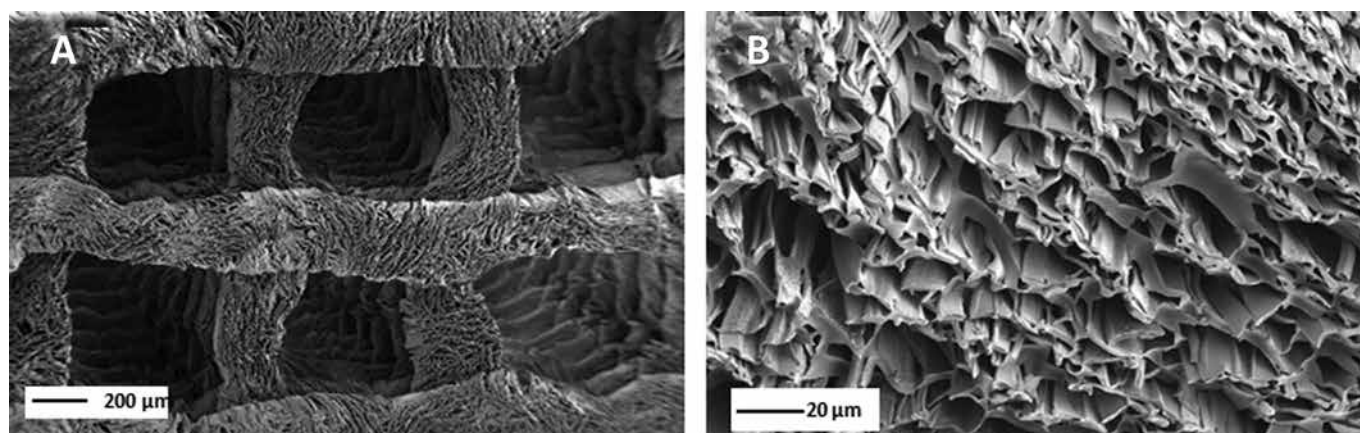
In Figures 5C and D, images of scaffolds prepared from the same solutions are presented. The same lay-down pattern

was used, except that the distance between the strands was 2 mm. It can be seen that here, too, significant die-swell had occurred. In this case, however, the strands were cooled with dry ice, and it is clear that the morphology of the microporosity is much finer. As the undercooling is much higher, smaller ethylene carbonate crystals are formed, resulting in smaller micropores after extraction. These micropores had a width of  $13 \pm 3 \mu\text{m}$  and a length of  $58 \pm 12 \mu\text{m}$ .

Figure 6 shows SEM images of prepared scaffolds using 20% solutions heated to 60°C and cooled using dry ice. Here the nozzle diameter was 234 μm. In this case microporous structures were obtained as well, but the morphology of the microporosity differed and pores with 2 size distributions could be observed. The larger pores have widths of  $4 \pm 2 \mu\text{m}$  and lengths of  $20 \pm 1 \mu\text{m}$ , the smaller pores with widths of  $2 \pm 1 \mu\text{m}$  and lengths of  $6 \pm 3 \mu\text{m}$ .

## Conclusions

We have shown that PCL-*b*-PTMC-*b*-PCL triblock copolymers can successfully be prepared by sequential polymerization of trimethylene carbonate and ε-caprolactone with



**Fig. 6** - Scanning electron microscopy images of 3D porous structures prepared by 3D printing of PCL<sub>20</sub>-PTMC<sub>40</sub>-PCL<sub>20</sub> triblock polymer from 20% solutions in ethylene carbonate at 60°C and cooling with dry ice. The images were obtained after extraction of the solvent and drying. See Tab. I for characteristics of the polymer.

2,2-dimethyl 1,3-propanediol as initiator and diphenyl phosphate as catalyst. For polymers containing approximately equal amounts of the monomers, excellent mechanical properties are obtained when the molecular weight is sufficiently high (approximately 60 kg/mol). The tensile modulus, tensile strength and elongation at break of these tough materials are respectively 120 MPa, 16 MPa and 620%.

These thermoplastic materials with melting points close to 58°C can readily be extruded. Using low melting ethylene carbonate as a crystallizable solvent, tissue engineering scaffolds with different microporosities can easily be prepared by extrusion-based 3D printing. Although the composition and molecular weight of these polymers remains to be optimized, in relation to their degradation characteristics as well, the results demonstrate that PCL-*b*-PTMC-*b*-PCL triblock copolymers are promising biomaterials for use in extrusion-based 3D printing of biomedical implants and tissue engineering scaffolds. Variation of the characteristics of the morphology of the micropores could be a useful way to tailor the behavior of cells cultured in or on such 3D printed tissue engineering scaffolds (20). However, the compatibility of these 3D printed scaffolds with cells should be evaluated in *in vitro* and *in vivo* experiments first.

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