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Creep-resistant porous structures based on stereo-complex forming triblock copolymers of 1,3-trimethylene carbonate and lactides

ZHENG ZHANG, DIRK W. GRIJPMA, JAN FEIJEN*
Institute for Biomedical Technology (BMTI), Department of Polymer Chemistry and Biomaterials, Faculty of Science and Technology, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands
E-mail: j.feijen@ct.utwente.nl

Stereo-complexes (poly(ST–TMC–ST)) of enantiomeric triblock copolymers based on 1,3-trimethylene carbonate (TMC) and L- or D-lactide (poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA)) were prepared. Films of poly(ST–TMC–ST) could be prepared by solvent casting mixtures of equal amounts of poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA) solutions and by compression moulding co-precipitates. Although compression moulding was performed at 191 °C, thermal degradation was not apparent and materials with good tensile properties could be obtained. For compression-moulded poly(ST–TMC–ST) specimens containing approximately 16 mol % lactide, the values for *E*-modulus, yield stress and elongation at break were respectively 17, 1.7 MPa and 90%. Also a very low long-term creep rate of $2.2 \times 10^{-7}\,\mathrm{s}^{-1}$ was determined when specimens were loaded to 20% of the yield stress. When compared with compression-moulded poly(TMC), poly(ST–TMC–ST) specimens deform at a rate that is one to two orders of magnitude lower. Furthermore, poly(ST–TMC–ST) specimens showed complete dimensional recovery within 24 h after loading to 20% and 40% of the yield stress for 40 and 5.5 h, respectively. Highly porous poly(TMC) and poly(ST–TMC–ST) structures with interconnected pores were prepared by a method combining co-precipitation, compression moulding and salt leaching. After prolonged compressive deformation, solid and porous poly(ST–TMC–ST) discs showed significantly better recovery behaviour than poly(TMC) discs.

1. Introduction

In cell culturing and tissue engineering research, it has been recognised that mechanical stimulation of cells under conditions that mimic the *in vivo* environment [1] has a pronounced influence on cell phenotype [2], proliferation and apoptosis behaviour [3]. Moreover, in comparison with static culturing of cells in the engineering of muscle and cartilage tissues, structurally and functionally superior tissues were obtained when cyclic tensile conditions were applied to smooth muscle cells seeded in cross-linked collagen sponges [4, 5] and cyclic compressive forces to chondrocytes in agarose gels [6]. To resist the deformations during long-term cell cultures in tissue engineering, the ideal scaffolding structure should be elastic and resistant to creep.

High molecular weight poly 1,3-trimethylene carbonate (poly(TMC)) is an amorphous polymer with a low glass transition temperature (T_g) at approximately $-20\,^{\circ}\mathrm{C}$. It degrades *in vivo* by surface erosion without the release of acidic compounds [7]. Several cell types have been successfully cultured on TMC-based copolymers [8, 9]. These properties make it an interesting material for use in cell culturing and tissue engineering. Unfortunately, the creep-resistance of poly(TMC) is rather low.

Stereo-complexation of poly L-lactide (poly(LLA)) and poly D-lactide (poly(DLA)) occurs upon co-precipitation [10] or solvent casting [11] of mixed solutions containing equal amounts of polymers. Previously, we prepared and characterised biodegradable thermoplastic elastomers based on stereo-complexation of enantiomeric triblock copolymers of TMC and L- or D-lactide (poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA)). Solvent-cast films of the stereo-complex (poly(ST–TMC–ST)) showed good mechanical properties and excellent creep-resistance [12].

We have developed a versatile technique to prepare well-controlled porous structures from low T_g polymers that involves polymer and salt co-precipitation, compression moulding and leaching [8, 13]. The aim of this study was to prepare creep-resistant, porous scaffolds by this

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*Author to whom all correspondence should be addressed.

thermal processing method. It is known that the melting temperature of the stereo-complex of equal amounts of poly(LLA) and poly(DLA) is very high, approximately 230 °C [10], which might lead to thermal degradation of the polymer. Therefore, to investigate the effect of the high processing temperature on the properties of poly(ST–TMC–ST) specimens, the tensile and creep properties of films prepared by compression moulding were compared with those of films prepared by solvent casting.

Porous poly(ST–TMC–ST) structures were prepared, and their recovery behaviour (resistance to creep) after long-term compressive deformation was evaluated and compared with that of high molecular weight porous poly(TMC) specimens.

2. Materials and methods

2.1. Materials

Polymer grade TMC (Boehringer Ingelheim, Germany), LLA and DLA (Purac Biochem, The Netherlands) were used without further purification. Stannous octoate ($SnOct_2$) (stannous 2-ethylhexanoate) (Sigma, USA) and 1,6-hexanediol (Aldrich, Germany) were used as received. Solvents were of analytical grade (Biosolve, The Netherlands).

Polymers were synthesised by ring-opening polymerisation under inert atmosphere using stannous octoate as a catalyst at 130 °C. High molecular weight poly(TMC) ($\overline{M}_n = 2.3 \times 10^5$, $T_g = -21\,°\mathrm{C}$) was synthesised and purified as previously described [14]. ABA triblock copolymers, where A is a poly(LLA) or poly(DLA) hard block and B is a poly(TMC) rubber block (abbreviated as poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA)) were prepared by initiation of the TMC (0.2 mol) ring-opening polymerisation with 1,6-hexanediol (0.82 mmol), followed by subsequent ring-opening polymerisation of either LLA or DLA (0.067 mol) using the α-ω hydroxy terminated poly(TMC) pre-polymer as initiator [12]. The synthesised triblock copolymers were purified by precipitating chloroform solutions of the polymers into methanol and characterised as previously described [12] by nuclear magnetic resonance (^{1}H- NMR) spectroscopy (300 MHz, Varian Inova, USA), gel permeation chromatography (GPC, using chloroform as eluent) and differential scanning calorimetry (DSC, Perkin Elmer, Pyris 1, at a scan rate of $10\,°\mathrm{C\,min}^{-1}$) after drying *in vacuo*.

A stereo-complex (poly(ST–TMC–ST)) of equal amounts of poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA) triblock copolymers was formed by co-precipitation or solvent casting methods using polymer solutions (10 wt per vol %) in chloroform [12].

2.2. Mechanical properties of poly(TMC) and poly(ST–TMC–ST)

Solvent-cast poly(ST–TMC–ST) films were prepared by casting mixtures of equal amounts of 10 wt/vol % poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA) solutions in chloroform onto glass plates, using a casting knife of 750 μm thickness. After drying, this resulted in films with a thickness of 60–90 μm, from which strips measuring 5 mm × 100 mm (ASTM D882-91) were cut.

Poly(TMC) and poly(ST–TMC–ST) was compression moulded in stainless steel moulds to yield films measuring 500 μm × 5 mm × 100 mm (ASTM D882-91) and discs measuring 13 mm in diameter and 6 mm in height (ASTM D395-89B) using a laboratory press (Fonteijne THB008, The Netherlands). The moulding temperatures were 140 °C for poly(TMC) and 191 °C for poly(ST–TMC–ST). The poly(ST–TMC–ST) specimens were annealed at 50 °C in the press for 30 min before they were cooled down to room temperature.

Tensile tests were carried out in duplicate at room temperature according to ASTM D882-91 specifications using solvent-cast and compression-moulded strips. A Zwick Z020 universal tensile tester equipped with a 10 N load cell was operated at a crosshead speed of 50 mm/min. The specimen deformation was derived from the grip-to-grip separation, which initially was 50 mm. The determined values of the Young's modulus are only an indication of the stiffness of the different materials.

Short-term static tensile creep tests in accordance to ASTM D2990-95 were also carried out using the tensile tester. Specimens were loaded to a stress corresponding to 10% of the yield stress, and the creep rates were determined within 1 h as the minimal slope of the strain–time curves [15]. Long-term static tensile creep tests were performed by attaching a dead weight corresponding to 20% or 40% of the yield stress to vertically mounted 5 mm × 100 mm strips [16]. The elongation in time was measured with a cathetometer (Heerbrugg, Switzerland), by measuring the distance between two marks initially 50 mm apart, at an accuracy of 0.1 mm. The plateau creep rates were determined from the slope of the linear part of the strain–time curves. To measure the elasticity of the specimens, the permanent deformation (after removal of the weights) was measured after a recovery period of 24 or 100 h.

2.3. Preparation and characterisation of porous structures

Poly(TMC) or equal amounts of poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA) were dissolved in chloroform to a final concentration of 5 wt/vol %. NaCl was sieved to a particle size range of 106–250 μm and added to the vigorously stirred polymer solutions. The amount of salt was adjusted to give a salt to polymer ratio of 80 : 20 vol/vol. The salt-containing suspensions were precipitated into a 10-fold volume of isopropanol and the formed fibrous polymer/salt composites were dried and compression moulded into discs according to ASTM D395-89B specifications (see above). Porous poly(TMC) and poly(ST–TMC–ST) structures were obtained after leaching of the compression-moulded, salt-containing polymer precipitates [13] with water at 4 °C. Removal of the water from the scaffold was done by freeze-drying.

The porosity of the prepared porous structures was determined in triplicate by measuring their dimensions and mass. The density of non-porous compression-moulded poly(TMC) is $1.33 \pm 0.03\,\mathrm{g/cm^3}$ and that of poly(ST–TMC–ST) is $1.21 \pm 0.02\,\mathrm{g/cm^3}$. The average pore size and pore interconnectivity of the structures was evaluated by scanning electron microscopy (SEM) using a Hitachi S800 (Japan) field emission scanning electron microscope operating at 3–5 kV. Cross-sections were coated with a gold layer (Polaron E5600 sputter-coater, UK).

To determine the elasticity of the polymer and the porous polymer scaffolds after prolonged compressive deformation, the compression set of compression-moulded solid and porous discs were determined in duplicate according to ASTM D395-89B. A compressive deformation of 25% was applied to the discs for 24 h at ambient conditions. After removal of the specimen from the compression device, the residual deformation was measured after 30 min, one day and one week. The compression set is defined as the ratio of the residual deformation and the initially applied deformation.

3. Results and discussion

The characteristics of the synthesised and purified TMC and LLA or DLA ABA triblock copolymers (poly(LLA–TMC–LLA) and LLA or DLA ABA triblock copolymers poly(DLA–TMC–DLA)) and of the stereo-complex (poly(ST–TMC–ST)) obtained after co-precipitation of equal amounts of the enantiomeric triblock copolymers are given in Table I.

From Table I, it can be seen that poly(ST–TMC–ST) precipitates show a high melting temperature of 191 °C. To be able to process this material in the melt, high temperatures need to be applied which might result in thermal degradation of the lactide containing polymers and a deterioration in mechanical properties.

Preliminary experiments revealed that it was possible to compression mould these stereo-complexes at 191 °C, that is, at the peak melting temperature of poly(ST–TMC–ST). To assess the effect of compression moulding at this temperature on thermal degradation of poly(ST–TMC–ST), molecular weight determinations by GPC cannot be performed, as the stereo-complex is not well soluble in chloroform. Therefore, the effect of temperature on the tensile properties and creep behaviour of the compression-moulded specimens was investigated. Table II shows a comparison of the mechanical properties in tensile tests and creep rates between compression-moulded and solvent-cast poly(ST–TMC–ST) films. Also the properties of high molecular weight poly(TMC) (as a reference material) are given in Table II.

For all specimens, Table II shows that solvent-cast films have higher values of *E*-modulus, yield stress, elongation at yield and maximal tensile strength than compression-moulded materials. This can probably be related to differences in thickness of the specimens. Although the *E*-modulus values of solvent-cast and compression-moulded poly(ST–TMC–ST) specimens are higher than those of poly(TMC), these materials are still very flexible with a low yield stress and a high elongation at break. Most remarkable is the significantly reduced creep rates in short-term experiments of poly(ST–TMC–ST) compared with those of poly(TMC).

The long-term static creep behaviour of compression-moulded poly(TMC) and poly(ST–TMC–ST) specimens was investigated. In Table III the loading conditions of the specimens and the resulting times to fracture, plateau creep rates ($\dot{\varepsilon}_{\text{plateau}}$) and the permanent deformation at a given loading time are presented.

To both polymers a stress corresponding to 20% and 40% of σ_{yield} was applied resulting in constant creep of the material. From the long-term strain–time curves, constant creep rates could be determined for both materials. These creep rates increase with increasing load.

TABLE I Characteristics of TMC and LLA or DLA ABA triblock copolymers and of their stereo-complex obtained by co-precipitation

Triblock copolymer	TMC pre-polymer	Purified ABA triblock copolymer				
	$\overline{M}_n$ ($\times 10^3$)[a]	LA (mol-%)[b]	$\overline{M}_n$ ($\times 10^3$)[a]	Poly(lactide) block length ($\times 10^3$)[c]	T_g[d] (°C)	T_m[d] (°C)
Poly(LLA–TMC–LLA)	16.4	17.5	23.8	2.7	– 12.4	150.3
Poly(DLA–TMC–DLA)	16.5	14.2	21.8	2.1	– 15.0	139.9
Poly(ST–TMC–ST)[e]	—	—	—	—	– 15.8	190.9

[a] Determined by GPC.

[b] Lactide content in the purified triblock copolymer as determined by ^{1}H-NMR.

[c] Poly(lactide) block length, calculated from the lactide content and $\overline{M}_n$ of the purified triblock copolymer.

[d] Determined by DSC in the 1st heating scan at a rate of 10 °C/min.

[e] Stereo-complex formed by co-precipitation of equal amounts of poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA).

TABLE II Tensile properties of solvent-cast and compression-moulded poly(TMC) and poly(ST–TMC–ST) specimens

	E-modulus (MPa)	σ_{yield} (MPa)	$\varepsilon_{\text{yield}}$ (%)	$\sigma_{\max}$ (MPa)	$\varepsilon_{\text{break}}$ (%)	$\dot{\varepsilon}^{d}$ ($\times 10^{-5}\,\text{s}^{-1}$)
Poly(TMC)[a, b]	6.8	2.6	139	24	820	n.d.[e]
Poly(TMC)[c]	5.9 ± 0.1	1.8 ± 0.1	132 ± 3	3.1 ± 0.3	995 ± 50	2.9
Poly(ST–TMC–ST)[a]	28.3 ± 0.5	3.3 ± 0.1	85 ± 4	3.3 ± 0.1	170 ± 10	0.17
Poly(ST–TMC–ST)[c]	17.3 ± 2.6	1.7 ± 0.1	63 ± 12	1.7 ± 0.1	90 ± 20	0.13

[a] Solvent-cast films.

[b] Data from Pêgo *et al.* [14].

[c] Compression-moulded films.

[d] Short-term creep rate, $\dot{\varepsilon}$, measured after 1 h upon loading the specimen to 10% of σ_{yield}.

[e] Not determined.

TABLE III Long-term creep behaviour of compression-moulded poly(TMC) and poly(ST–TMC–ST) specimens

	Polymer	Applied stress (% of σ_{yield})	Time (min)	$\dot{\varepsilon}_{plateau}$ ($10^{-5}\,s^{-1}$)	Strain[c] (%)	Permanent deformation (%)
1	Poly(TMC)	20%	2360[a]	1.6	1824	fracture
2	Poly(TMC)	20%	480[b]	1.9	78	12[d]
3	Poly(ST–TMC–ST)	20%	2400[b]	0.022	8	0[e]
4	Poly(TMC)	40%	294[a]	10.0	1022	fracture
5	Poly(TMC)	40%	120[b]	9.0	87	3[d]
6	Poly(ST–TMC–ST)	40%	330[b]	0.47	21	0[e]

[a] Specimens fractured at this time.

[b] Creep experiment was stopped to determine permanent deformation.

[c] Strain at which the creep experiment was stopped (or fracture occurred).

[d] Permanent deformation measured after 100 h recovery.

[e] Permanent deformation measured after 24 h recovery.

When loaded to 20% of σ_{yield}, poly(TMC) specimens creep at a rate of 1.6 to $1.9 \times 10^{-5}\,s^{-1}$, fracturing after 2360 min (39 h). At loading to 40% of σ_{yield}, the creep rate increases to 9.0 to $10.0 \times 10^{-5}\,s^{-1}$ and the specimens fracture after 294 min (5 h).

In comparison, although the polymers have comparable values of yield stress (see Table II), poly(ST–TMC–ST) specimens show much improved creep-resistance under the same loading conditions. The plateau creep rate of compression-moulded poly(ST–TMC–ST) is one to two orders of magnitude lower than that of poly(TMC). Furthermore, poly(ST–TMC–ST) specimens did not break in the time required for poly(TMC) specimens to fracture. At this time point the specimens were still deforming at a very low constant creep rate.

The data in Table III also show that the ability of poly(ST–TMC–ST) to recover its original dimensions after removal of the load is much better than that of poly(TMC) specimens. After a recovery period of 24 h, the permanent deformation of poly(ST–TMC–ST) was negligible under the applied loading conditions. After the experiment was stopped, the specimens were allowed to recover for a period of 100 h, the permanent deformation of poly(TMC) specimens was 12% upon loading to 20% of σ_{yield} for 480 min and 3% upon loading to 40% of σ_{yield} for 120 min.

With the intention of using these polymers in tissue engineering scaffolds, highly porous structures were prepared by leaching of compression-moulded, salt-containing polymer precipitates as described in the experimental part. Using this method porous structures could readily be prepared from poly(TMC) and poly(ST–TMC–ST).

Fig. 1 shows the cross-section of a porous structure prepared from poly(ST–TMC–ST). The porosities of the obtained structures were 84.8 ± 1.5% for poly(TMC) and 86.6 ± 0.8% for poly(ST–TMC–ST). Both scaffolds show interconnected pores with an average pore size (determined from SEM) of respectively 120 ± 42 and 123 ± 37 μm. These values are in good agreement with the volume fraction (80%) and the size range (106–250 μm) of salt particles used. Therefore, the porosity and the average pore size can be well controlled when employing this method to prepare porous structures from these flexible polymers.

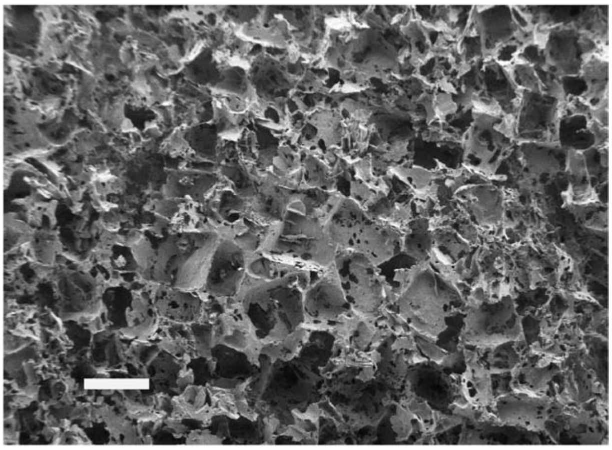

Figure 1 SEM micrograph of a cross-section of a porous poly(ST–TMC–ST) structure (bar = 200 μm).

To determine the elastic properties of the porous structures and their ability to recover to their original dimensions after prolonged deformation, as during the application of mechanical forces to cell-scaffold constructs in tissue engineering, long-term compressive deformation tests were performed.

Fig. 2 shows the compression set of compression-moulded solid and porous poly(TMC) and poly(ST–TMC–ST) discs. Solid and porous poly(TMC) discs show a large permanent deformation after compression of the specimens to 75% of their initial thickness. Even after a recovery period of one week, the compression set is higher than 70%. It is clear from the figure that solid and porous discs of poly(ST–TMC–ST) show much better recovery behaviour, the compression sets are

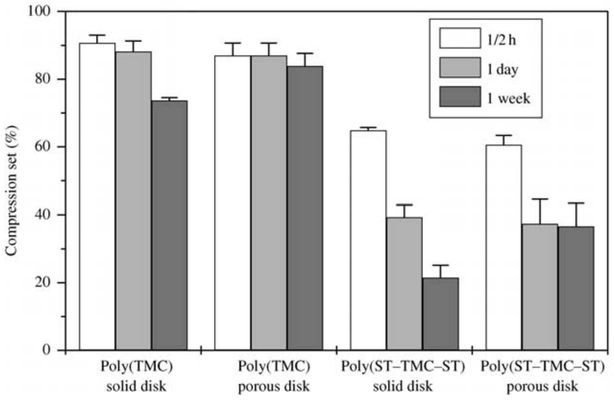

Figure 2 Compression set of solid and porous poly(TMC) and poly(ST–TMC–ST) disks at different time periods of recovery after removal of the applied compressive deformation. (The compression set is a relative measure of the permanent deformation.)

approximately 60% after 30 min and lower than 40% after one week. This shows that it is possible to prepare highly porous structures from elastomeric poly(ST–TMC–ST) materials that possess excellent resistance to creep under long-term loading conditions.

4. Conclusions

It was found possible to prepare poly(ST–TMC–ST) specimens by compression moulding co-precipitated mixtures of poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA). These specimens show good tensile properties, excellent creep-resistance and complete recovery behaviour in long-term static tensile creep tests. Well-defined, highly porous poly(ST–TMC–ST) structures with interconnected pores can be prepared by a method in which poly(LLA–TMC–LLA) and poly(DLA–TMC–DLA) triblock copolymers together with salt particles are co-precipitated, compression moulded and leached with water. These highly porous poly(ST–TMC–ST) disks (porosity 87%, pore size 123 μm) have very good recovery behaviour after prolonged compressive deformation. These results show that poly(ST–TMC–ST) materials and scaffolds are attractive for use in cell culturing and tissue engineering when long-term mechanical deformation of the structures is desired.

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