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Oligo(trimethylene carbonate)-Based Supramolecular Biomaterials

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ABSTRACT: A supramolecular polymer system is presented in which relatively short trimethylene carbonate (TMC) prepolymers are linked via reversible quadruple hydrogen bonding ureidopyrimidinone (UPy) moieties. Several UPy-modified bifunctional and trifunctional TMC polymers were synthesized. Tunability of the mechanical and thermal properties was achieved by mixing different trifunctional UPy−TMC with bifunctional UPy−TMC polymers. The concentration of UPy groups influenced the crystallization behavior of the materials. The UPy-modified TMC polymers displayed melting endotherms, which are caused by aggregation of the UPy units into small crystallites. It is assumed that the UPy dimers stack in the lateral direction due to urethane hydrogen bonding. Atomic force microscopy confirmed the presence of fiberlike stacks. These crystallites give the material its mechanical strength and prevent flowing of the material after processing into 3D scaffolds. More importantly, these polymers can be processed quite easily at slightly elevated temperatures. Because of the strong temperature dependence of the reversible nature of the hydrogen bonds, their melt viscosities are low, while at temperatures below 50 °C excellent mechanical properties are found as a result of supramolecular, physical cross-linking. The UPy−TMC polymers are shown to be biocompatible and fibroblasts proliferate well on drop cast films of UPy−TMC. Thus, these novel supramolecular UPy−TMC polymers are very promising, biocompatible, tunable, and easily processable biomaterials for applications such as tissue engineering.

Introduction

Polycarbonates are promising polymers to be used in several biomedical applications. Trimethylene carbonate (TMC) can be polymerized chemically[1] or enzymatically.[2] Also star-shaped copolymers containing TMC have been synthesized.[3,4] Biodegradable ionomers have been produced via end-functionalization of PTMC with phosphoryl−choline.[5,6] Different copolymers of TMC and lactides or ϵ-caprolactone have been examined for their use in tissue engineering.[7−9] The material properties of PTMC are strongly dependent on the molecular weight of the polymers.[10] It has been shown that very high molecular weight poly(trimethylene carbonate) (HMW−PTMC), with M_n above 200 kg/mol, has excellent mechanical properties,[10] is biocompatible, and is extensively degraded in vivo after 3 weeks.[11] At lower molecular weights the materials are tacky and have low Young's moduli and low strains at yield.[10]

Processing of this high molecular weight polymer is quite difficult due to the very high viscosities in the melt or in solution. Furthermore, to retain the shape of a device after processing, the PTMC material needs to be cross-linked to minimize creep and flow.[10] Chemical cross-linking has been achieved by γ-irradiation[10] or by photocross-linking of prepolymers containing methacrylates and fumaric acid monoethyl esters.[12] Besides that, physical cross-linking by stereo-complexation of lactide segments in triblock copolymers containing TMC has been shown.[13,14] These methods are accompanied by several disadvantages, such as changes in material properties due to processing at elevated temperatures, the potential decrease in molecular weight, the generation of degradation products, and the need to irradiate and cross-link the materials in their final shape. Therefore, the processing of these materials into tissue engineering scaffolds[15] with rapid prototyping techniques such as fused deposition modeling (FDM)[16] cannot be done readily.

The use of supramolecular units as physical chain-extending and cross-linking moieties is a possible solution to these difficulties. End-functionalization of relatively short prepolymers with quadruple hydrogen bonding 2-ureido-4[1*H*]-pyrimidinone (UPy)[17−19] moieties has been shown to be promising.[20] Dimer formation between the UPy units by strong noncovalent hydrogen bonding (association constant, $K_a = 10^6 - 10^7$ L·mol^{-1}) yields chain extension of relatively short UPy-functionalized prepolymers which results in high virtual molecular weights (Figure 1).[21] The strong temperature dependence of the reversible nature of these hydrogen bond interactions (with lifetimes between 0.1 and 1 s at room temperature) gives rise to low melt viscosities and easy processing from the melt. After processing, the material becomes supramolecularly cross-linked by lateral association of the UPy dimers. The material properties can also be tuned by mixing bifunctional and trifunctional UPy-modified polymers (Figure 1). In this way a modular approach can be applied to make UPy-based materials with different material properties. To investigate whether these supramolecular polymers, based on TMC-oligomers and quadruple hydrogen bonding UPy units, are suitable as materials for tissue engineering, different UPy-PTMC polymers were synthesized, characterized, mixed and processed. Also, their mechanical behavior, thermal properties, biocompatibility and degradability were studied.

Results and Discussion

Synthesis and Characterization. Five different UPy-functionalized TMC-based polymers were synthesized starting from trimethylene carbonate (TMC) with hexanediol as initiator for the two bifunctional polymers $\mathbf{1_{a,b}}$ and glycerol as initiator for

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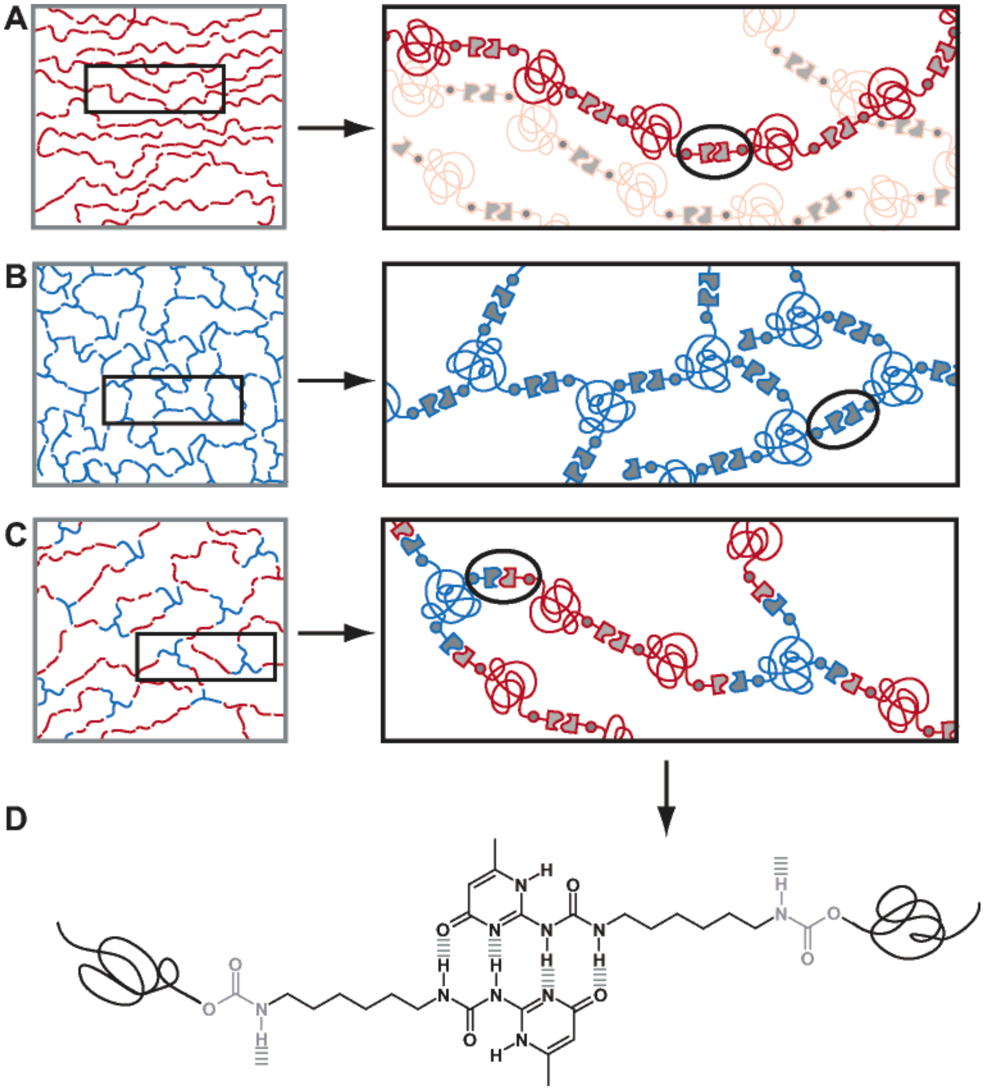
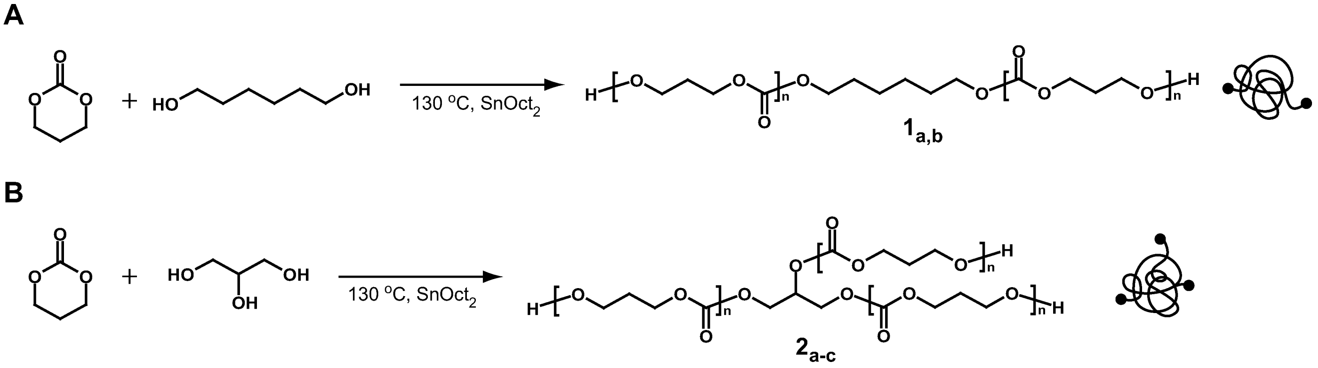


Figure 1. Modular approach to mixed supramolecular polymer systems (**C**) using bifunctional (**A**) and trifunctional (**B**) UPy polymers. Supramolecular polymers are formed via quadruple hydrogen bonding between ureidopyrimidinone (UPy) units (**D**).

Scheme 1. Synthesis of the Hydroxy-Terminated (A) Bifunctional $1_{a,b}$ and (B) Trifunctional 2_{a-c} TMC Prepolymers

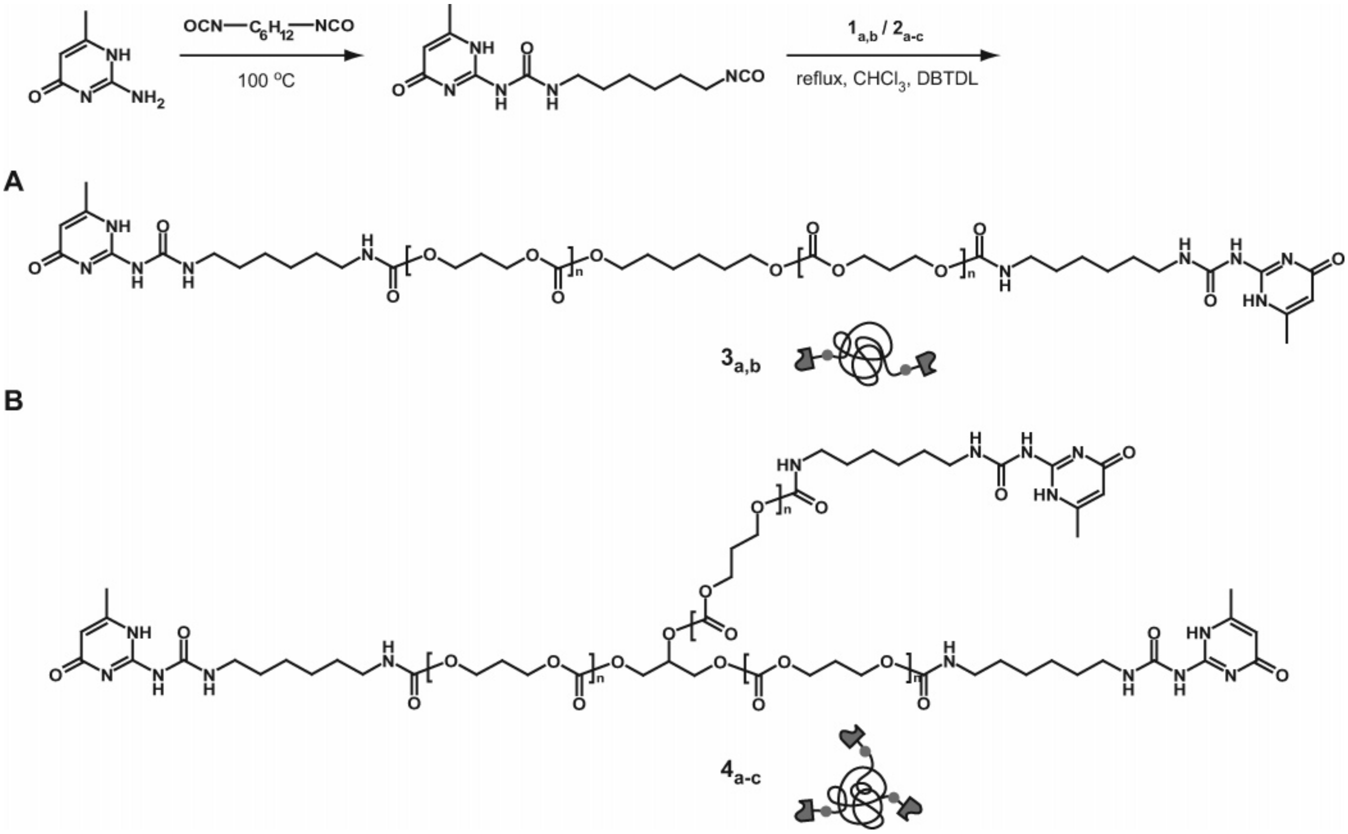


the three trifunctional polymers $\mathbf{2}_{a-c}$ using tin octanoate ($SnOct_2$) as catalyst (Scheme 1).[14] This oligomerization resulted in relatively short hydroxy-terminated TMC prepolymers with different molecular weights varying between 2 and 4 kg/mol for the bifunctional, and between 1 and 12 kg/mol for the trifunctional TMC prepolymers, as determined with GPC and ^1H NMR end group determinations (Table 1). ^1H NMR showed the presence of small amounts of remaining TMC monomer, but this did not interfere with the subsequent UPy-functionalization reaction. As a reference, high molecular weight poly-trimethylene carbonate (**HMW−PTMC**; $M_n = 291$ kg/mol) was synthesized.[10] The hydroxy-terminated TMC prepolymers were functionalized with UPy units using a UPy−hexyl isocyanate molecule as synthon, which was synthesized from methylisocytosine and an excess of hexyldiisocyanate (Scheme 2).[21] Upon UPy-functionalization of the hydroxy-terminated prepolymers using dibutyl tin dilaurate (DBTDL) as catalyst, the reaction mixtures became viscous, indicating the formation of intermolecular quadruple hydrogen-bonded UPy dimers. The UPy-modified materials were obtained in relatively good yields by precipitation into hexane, removing the residual TMC monomer concentration to less than 0.01 wt %. The recovered yields were

Table 1. (A) GPC Results (Number-Average M_n in kg/mol, Weight Average M_w in kg/mol and Polydispersity PD) and NMR Determinations (Number-Average M_n in kg/mol) of the Hydroxy-Terminated TMC Polymers and (B) NMR Determinations of the UPy-Functionalized TMC Polymers

	A				B	
	M_n(GPC)	M_w(GPC)	PD	M_n(NMR)		M_n (NMR)
1$_a$	2.9	5.8	2.0	2.0	**3**$_a$	2.6
1$_b$	4.0	8.4	2.1	3.0	**3**$_b$	4.8
2$_a$	1.0	2.6	2.6	1.9	**4**$_a$	2.8
2$_b$	4.1	6.4	1.6	3.7	**4**$_b$	4.5
2$_c$	11.8	17.9	1.5	12.9	**4**$_c$	15.2
HMW−PTMC	291	425	1.5			

Scheme 2. Hydroxy-Terminated Polymers Reacted with the UPy−Isocyanate Synthon[a]



[a] This resulted in (A) bifunctional 3$_{a,b}$ and (B) trifunctional 4$_{a-c}$ UPy-modified TMC polymers.

68% and 54% for the bifunctional polymers **3**$_{a,b}$, respectively, and 76%, 87%, and 23% for the trifunctional polymers **4**$_{a-c}$, respectively. The lower yield found for **4**$_c$ might be caused by the difficulties that were encountered when filtrating the very viscous reaction mixture; gel formation occurred on the filter. The polymers were characterized with NMR and IR spectroscopy. The characteristic peaks of the hydrogen-bonded protons are clearly visible at 13.1, 11.9, and 10.1 ppm for all five UPy-modified materials measured in deuterated chloroform. IR showed that the UPy-moieties are present as keto-tautomer with characteristic bands at 1699, 1669, 1587, and 1526 cm^{-1}.[22]

Mechanical Properties. The material properties of the TMC prepolymers changed dramatically upon functionalization with UPy-moieties. The hydroxy-terminated TMC prepolymers **1**$_{a,b}$ and **2**$_{a,b}$ were viscous liquids and **2**$_c$ was a waxy, slightly white solid. They became strong and flexible upon functionalization (**3**$_{a,b}$ and **4**$_{a-c}$). Tensile testing measurements on bifunctional **3**$_{a,b}$ showed a decrease in Young's modulus (*E*) when the TMC prepolymer chain between the UPy units is longer, indicating that the material becomes more flexible. In this case the relative concentration of UPy groups is lower. The respective *E* values are 77 and 26 MPa for **3**$_a$ and **3**$_b$ (Figure 2; Table 2). At the same time the elongation at break increases, from 37 to 218% for **3**$_a$ and **3**$_b$, respectively. This implies that the material becomes more tough. The films of trifunctional **4**$_{a,b}$ were too brittle to test, probably caused by strong physical network formation. The trifunctional UPy−polymer **4**$_c$ containing a large TMC part could be strained for more than 250%, but *E* turned out to be very low; 1.2 MPa. Polymer **4**$_c$ starts to flow during and after stretching of the material. The UPy-modified TMC polymers do not show the strain-induced crystallization which occurs for the **HMW−PTMC** at higher strains (Figure 2; Table 2).[10] Comparison of linear PTMC with an M_n of 53 kg/mol (data not shown)[10] with supramolecular **3**$_b$ with an M_n of 4.8 kg/mol shows the usefulness of the UPy moiety. Whereas *E* is 3 MPa for the covalent polymer, *E* is much higher for **3**$_b$, 26 MPa. Even the stress at break is higher for **3**$_b$, 1.8 MPa, than for the covalent PTMC, 0.2 MPa. Also the yield stress turned out to be much higher for **3**$_b$. The differences in material properties between the covalent and supramolecular polymer are striking.

The behavior of **3**$_b$ was also tested under continuous cyclic loading using strains up to 10% and 20% (Supporting Information). The deformation was in the same order of magnitude as measured for **HMW−PTMC**;[10] 20 cycles strained up to 10%

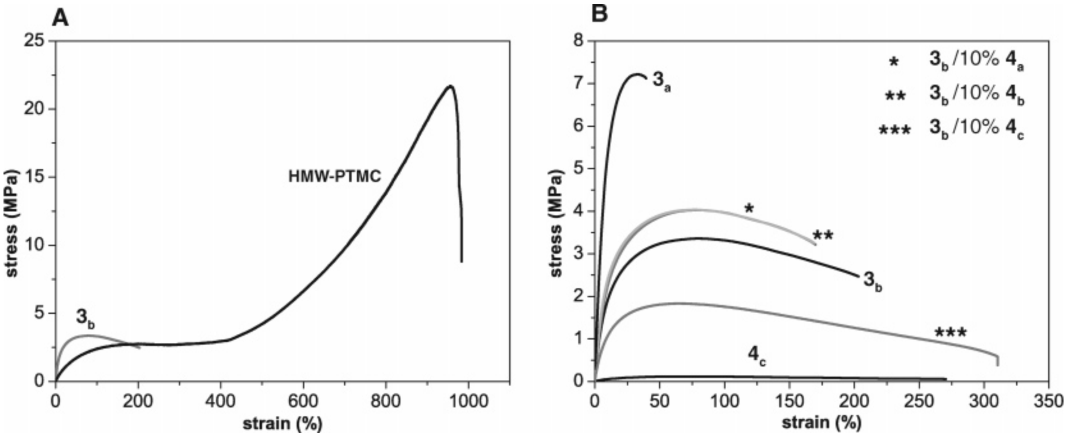


Figure 2. Tensile testing graphs of the UPy-functionalized TMC polymers and of mixtures of these UPy polymers. (A) Bifunctional UPy-modified PTMC $\mathbf{3_b}$ was compared with **HMW−PTMC**. (B) Stress−strain behavior of the bifunctional and trifunctional UPy polymers and of mixtures of these polymers. The curves of $\mathbf{3_b}$/10% $\mathbf{4_a}$ (∗) and $\mathbf{3_b}$/10% $\mathbf{4_b}$ (∗∗) are laying on top of each other.

Table 2. Tensile Test Data of the UPy-Functionalized TMC Polymers and of Mixtures of These Polymers[a]

	E (MPa)	σ_{yield} (MPa)	ϵ_{yield} (%)	σ_{break} (MPa)	ϵ_{break} (%)	$\sigma_{\max}$ (MPa)
$\mathbf{3_a}$	77 ± 3	7.0 ± 0.2	9.7 ± 0.4	7.3 ± 0.2	37.4 ± 2.2	7.4 ± 0.3
$\mathbf{3_b}$	26 ± 4	3.1 ± 0.1	16.2 ± 1.6	1.8 ± 0.7	218 ± 20	2.7 ± 0.6
$\mathbf{4_c}$	1.2 ± 0.7	0.3 ± 0.2	18.9 ± 2.9	0.06 ± 0.02	284 ± 15	0.16 ± 0.08
$\mathbf{3_b}$/10% $\mathbf{4_a}$	36 ± 1	3.8 ± 0.1	16.4 ± 0.8	3.3 ± 0.1	162 ± 12	4.1 ± 0.1
$\mathbf{3_b}$/10% $\mathbf{4_b}$	38 ± 1	3.7 ± 0.2	15.1 ± 1.6	3.1 ± 0.3	172 ± 6	3.9 ± 0.2
$\mathbf{3_b}$/10% $\mathbf{4_c}$	17 ± 1	1.8 ± 0.1	28.7 ± 3.6	0.75 ± 0.21	310 ± 9	1.7 ± 0.1
HMW−PTMC	5.7 ± 0.9	2.7 ± 0.2	56.2 ± 6.0	15 ± 4	882 ± 98	19 ± 4

[a] The Young's modulus (E in MPa), yield-stress (σ_{yield} in MPa), yield-strain (ϵ_{yield} in %), stress at break (σ_{break} in MPa), elongation at break (ϵ_{break} in %), and the maximum stress ($\sigma_{\max}$ in MPa) are shown.

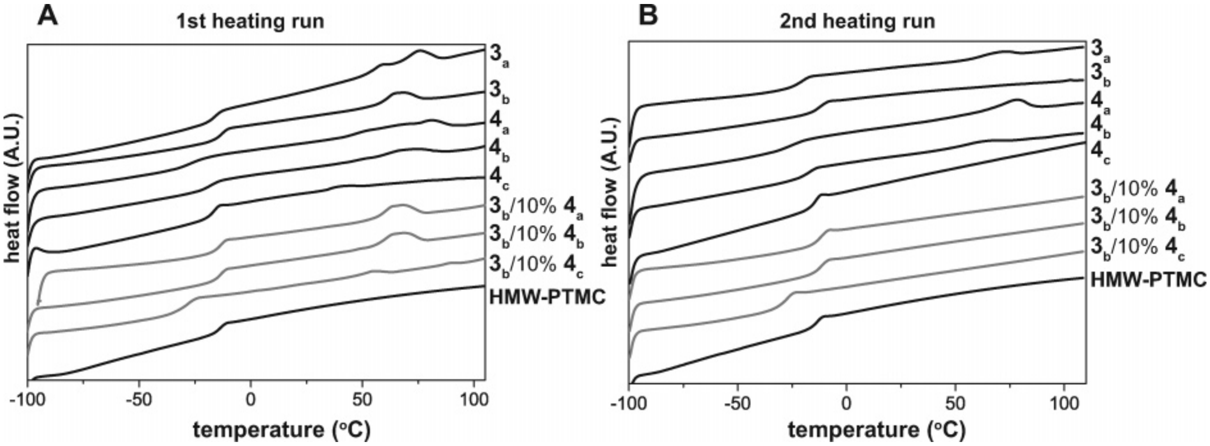


Figure 3. Thermal properties of the UPy-functionalized TMC polymers and mixtures of these polymers: (A) first heating run (20 °C/min); (B**)** second heating run (20 °C/min).

showed approximately 3% strain deformation and 20 cycles strained up to 20% showed approximately 6% strain deformation.

The material properties of $\mathbf{3_b}$ could be tuned by mixing in 10 mol % of the different trifunctional polymers, $\mathbf{4_{a-c}}$. The differences in mechanical properties between $\mathbf{3_b}$ and the mixtures of $\mathbf{3_b}$ with $\mathbf{4_{a,b}}$ are small; the Young's moduli increase slightly from 26 to approximately 37 MPa. However, when $\mathbf{3_b}$ is mixed with $\mathbf{4_c}$, the Young's modulus decreases to 17 MPa, and the elongation at break increases to 310%.

Thermal Properties. Differential scanning calorimetry (DSC) measurements were performed to study the thermal behavior of the polymers and the mixtures. The effect of storage of the polymers was investigated by studying the first heating runs (Figure 3A; Table 3). It is shown that the glass transitions (T_g) shift to higher temperatures when the length of the PTMC chain increases. The heat capacity (ΔC_p) increases with increasing M_n. A similar shift in T_g is seen for the hydroxy-terminated TMC prepolymers, while ΔC_p stays more or less the same with increasing M_n (Supporting Information). The mixtures of $\mathbf{3_b}$ with 10 mol % of $\mathbf{4_{a,b}}$, behave similarly as $\mathbf{3_b}$, except for the mixture of $\mathbf{3_b}$ with 10 mol % $\mathbf{4_c}$. In the latter case the T_g becomes lower. Melting endotherms in the first heating runs are found for all UPy-modified polymers and mixtures. The heat of fusion (ΔH_m) becomes lower with increasing M_n and lower concentration of UPy moieties. The melting temperatures become lower as a consequence of the presence of longer chains. The longer amorphous TMC-parts possibly induce formation of less thermodynamically stable crystalline aggregates. It is assumed that the endotherms are caused by small UPy crystallites that are responsible for the material properties. The **HMW−PTMC** and hydroxy-terminated TMC prepolymers $\mathbf{1_{a,b}}$ and $\mathbf{2_{a,b}}$ do not readily crystallize (Supporting Information).

Table 3. Thermal Properties of the UPy-Modified TMC Polymers and Mixtures of These Polymers[a]

A					B				
1st heating run	T_g (°C)	ΔC_p (J/g·°C)	T_m (°C)	ΔH_m (J/g)	2nd heating run	T_g (°C)	ΔC_p (J/g·°C)	T_m (°C)	ΔH_m (J/g)
$\mathbf{3_a}$	−16	0.37	60, 75	9.9	$\mathbf{3_a}$	−17	0.41	72	2.3
$\mathbf{3_b}$	−13	0.41	65	5.3	$\mathbf{3_b}$	−12	0.43		
$\mathbf{4_a}$	−25	0.30	80	9.5	$\mathbf{4_a}$	−24	0.25	78	6.0
$\mathbf{4_b}$	−18	0.37	69	5.7	$\mathbf{4_b}$	−18	0.35	64	2.1
$\mathbf{4_c}$	−17	0.43	43	1.3	$\mathbf{4_c}$	−17	0.46		
$\mathbf{3_b}$/10% $\mathbf{4_a}$	−14	0.42	67	6.0	$\mathbf{3_b}$/10% $\mathbf{4_a}$	−13	0.43		
$\mathbf{3_b}$/10% $\mathbf{4_b}$	−14	0.39	66	5.9	$\mathbf{3_b}$/10% $\mathbf{4_b}$	−12	0.38		
$\mathbf{3_b}$/10% $\mathbf{4_c}$	−29	0.42	55	1.0	$\mathbf{3_b}$/10% $\mathbf{4_c}$	−28	0.43		
HMW−PTMC	−14	0.40			**HMW−PTMC**	−14	0.51		

[a] The first (A) and second (B) heating runs (20 °C/min) are shown. The glass transition temperature (T_g), the heat capacity (ΔC_p), the melting temperature (T_m) and the heat of fusion (ΔH_m) are depicted.

For the second heating runs, which reflect the intrinsic thermal properties of the polymers, comparable results were found concerning the T_g and ΔC_p (Figure 3B; Table 3). In contrast to the first heating runs, endotherms in the second heating run are only detected for $\mathbf{3_a}$ and $\mathbf{4_{a,b}}$ (Figure 3B; Table 3). The bifunctional $\mathbf{3_a}$ polymer, with the shorter TMC part, shows an endotherm with a ΔH_m of 2.3 J/g. Also for the trifunctional polymers where the TMC part is relatively short, endotherms are found; for $\mathbf{4_a}$ and $\mathbf{4_b}$, ΔH_m are 6.0 and 2.1 J/g, respectively. The longer bifunctional $\mathbf{3_b}$ and trifunctional $\mathbf{4_c}$ polymers do not show a melting peak. Also, the mixtures do not show melting peaks, indicating that 10 mol % of $\mathbf{4_{a,b}}$ is not enough to induce crystallization during the DSC measurements. Also, for the **HMW−PTMC** and the low molecular weight hydroxy-terminated TMC prepolymers no endotherms are visible, indicating that these materials are completely amorphous (Supporting Information). It is clear from the second heating runs that the formation of UPy crystallites does not occur for all UPy polymers during the DSC measurements, indicating that this crystallization process takes longer than a couple of minutes. The DSC results demonstrate that the thermal behavior of the UPy-modified polymers depends on the length of the TMC chains and that introduction of the UPy units to some extent induces crystallinity in the materials. This lateral aggregation of UPy dimer units gives these modified PTMCs their excellent mechanical properties.[23]

Material Morphology. The morphology of bifunctional $\mathbf{3_b}$ and trifunctional $\mathbf{4_c}$ was investigated with atomic force microscopy (AFM). The phase image of $\mathbf{3_b}$ clearly shows fibers which appear bright in the phase image, and therefore are attributed to hard segment stacks in the polymer (Figure 4). The fiber dimensions of $\mathbf{3_b}$ are estimated to be approximately 5 nm in diameter and a length up to 100 nm. It is likely that these fibers consist of UPy dimer stacks, stabilized in the lateral direction via hydrogen bonding between the urethane groups (Figure 5).[23] At present, we can only speculate about the three-dimensional structure of the stacks. The stacks can be twisted or tilted and even several stacks can be aggregated together. Further research is needed to clarify the molecular structure of the stacks observed. However, the stacks give the material its strength similar as for a thermoplastic elastomer. There might be stack formation in the trifunctional $\mathbf{4_c}$ polymer but this is not observed in the samples investigated. The longer TMC chains possibly cause tackiness and softness. This is also reflected in the DSC results showing that $\mathbf{4_c}$ clearly displays a significantly lower ΔH_m than $\mathbf{3_b}$. Besides that, its starlike morphology might create a more random structure. As a control **HMW−PTMC** was measured, which shows a complete lack of structure. These AFM measurements show the importance of the UPy units in cooperation with the urethane groups. Hence, in these supramolecular polymers, a double supramolecular interaction is taking place; first the quadruple hydrogen bonding takes care of chain extension and second crystallization of these dimerized UPy units furnish lateral, reversible cross-links. Since both processes are strongly temperature dependent, an ideal processing-material properties relationship is present.

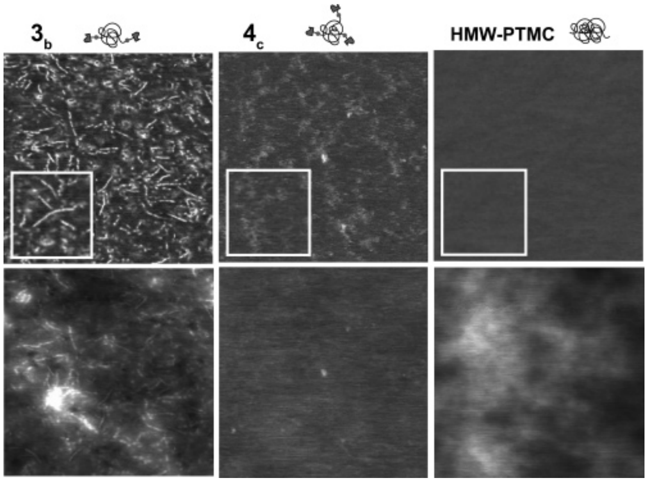


Figure 4. AFM topography (bottom) and phase (top) images at 500 nm scan size (zoom is 150 nm) of the bifunctional $\mathbf{3_b}$ and trifunctional $\mathbf{4_c}$ polymers and of **HMW−PTMC**. Data were obtained in tapping mode; the z range is 5 nm and $\Delta\varphi$ is 10° for all images.

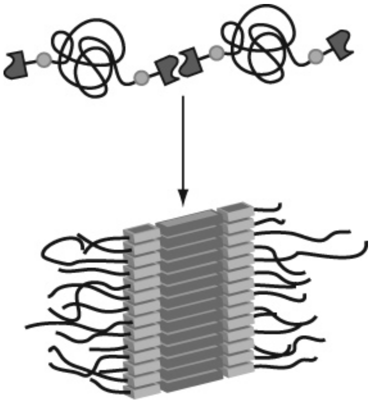


Figure 5. Schematic representation of the lateral UPy−UPy dimer stacks due to additional hydrogen bonding between the urethane groups.

Processing of Materials. Tissue engineering requires the use of scaffolds[15] that can be easily produced. Fused deposition modeling (FDM)[16] is a rapid prototyping technique that is eminently suitable for building up 3D structures. Using this technique, polymers are extruded in the melt through a small diameter nozzle. Bifunctional $\mathbf{3_b}$ and trifunctional $\mathbf{4_c}$ could be easily processed into FDM scaffolds at temperatures of 110 °C and 120 °C, respectively. **HMW−PTMC** was more difficult to process due to its higher melt viscosity. Temperatures around

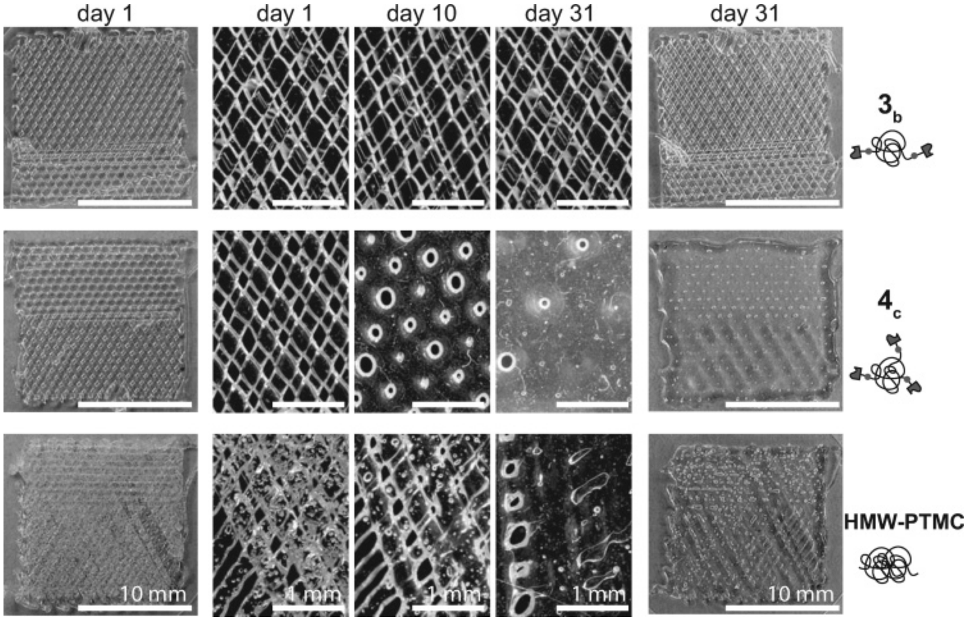


Figure 6. Fused deposition modeling (FDM) scaffolds of bifunctional $\mathbf{3_b}$ and trifunctional $\mathbf{4_c}$ followed in time. The scale bars on the photographs of the entire scaffolds (left and right) indicate 10 mm. The optical micrographs (in the middle) show scale bars of 1 mm.

220 °C were required to process **HMW−PTMC**. The lower processing temperatures of the UPy polymers are due to the lower viscosity of the melts caused by the reversible nature of the hydrogen bonds. The formed FDM scaffolds were followed in time at 21 °C by optical microscopy (Figure 6). After 1 day the scaffolds were still intact. However, after 10 days $\mathbf{4_c}$ and **HMW−PTMC** started to lose their 3D structures and began to flow. The scaffolds of $\mathbf{4_c}$ and **HMW−PTMC** had totally lost their structures after 31 days. They were clearly not dimensionally stable. **HMW−PTMC** was degraded during processing at such high temperature (of 220 °C), as shown by ^{1}H NMR; the M_n was reduced from 291 to approximately 30 kg/mol. Another explanation of this flow behavior is that **HMW−PTMC** gets its material properties from strain-induced crystallization. It is assumed for $\mathbf{4_c}$ that the flow behavior is caused by the length of the TMC-part. Nothing changed in the 3D structure of the scaffold made from $\mathbf{3_b}$. This indicates that the TMC-part is short enough and that enough UPy groups are present to form the crystalline fiber domains, which keep the material intact. So instead of covalently cross-linking the polymer chains, as must be performed for **HMW−PTMC**,[10] these supramolecular interactions are strong enough to form a good material that does not show flowing. Additionally, the possibility to process these supramolecular UPy materials at relatively lower temperatures is advantageous.

Biocompatibility and Degradability. The biocompatibility of UPy-functionalized TMC $\mathbf{3_b}$ was tested in vitro with an indirect LDH (lactate dehydrogenase) viability assay[24] with 3T3 mouse fibroblasts. This test is based on the leakage of LDH enzymes through the cell membrane which might become permeable due to toxic compounds in the culture medium. The activity of these enzymes is measured. Medium was incubated with compression molded films of the selected materials $\mathbf{3_b}$ and **HMW−PTMC** (Figure 7A). Latex (C1) and UHMWPE (C2) were used as controls. The viability of the cells in culture medium (C3) was set at 100% viability. The LDH test showed that the viability of the cells stayed above 90% for $\mathbf{3_b}$. As a comparison **HMW−PTMC** was also tested, showing that the viability of the cells was around 95%. This test shows that the synthesized UPy-modified TMC polymers are not toxic.

The proliferation of fibroblasts was investigated on films drop cast from either tetrahydrofuran (THF) or chloroform (Figure 7B). Two cell densities (5.7×10^3 cells/cm^2 and 2.3×10^4 cells/cm^2) were used, which gave similar results. The cells were able to proliferate in time to ultimately form a confluent layer. No differences could be seen in proliferation rate of the cells on $\mathbf{3_b}$ and **HMW−PTMC**. In conclusion, fibroblast cells proliferate well on the UPy-functionalized polymers, indicating the biocompatibility of the used materials.

The degradation behavior of the UPy-modified TMC polymers was investigated in vitro. The mass loss of films of $\mathbf{3_b}$ was determined after four months of incubation at 37 °C in PBS buffer. The weight of the samples did hardly change: from less than 1% mass loss during the first three months to 1.8 ± 0.1% after four months. Chain scission could not be detected with GPC. Also, IR and ^{1}H NMR measurements showed that the polymeric structures stayed unchanged upon incubation. Since **HMW−PTMC** is also stable in vitro and easily degraded in vivo, it is expected that the selected supramolecular TMC polymers behave similarly.

Conclusions

Short hydroxy-terminated bifunctional and trifunctional TMC prepolymers were synthesized and functionalized with UPy moieties. High virtual molecular weight polymers were produced by chain extension as a result of the UPy modification. This is reflected in the change in material properties; the viscous liquids became strong, flexible materials. The material properties can be tuned by varying the length of the TMC part and as a result of that the amount of UPy units present in the material. The tunability of this supramolecular system concerning the material properties was demonstrated by a modular approach via mixing bifunctional and trifunctional UPy-modified TMC polymers. It was shown that depending on the length of the TMC part between the UPy units, the UPy groups can dimerize and form lateral stacks of UPy−UPy dimers due to additional urethane hydrogen bonding. It is proposed that these stacks give the material thermoplastic elastomeric properties.

Owing to the reversible nature of these UPy moieties the polymers can be easily processed with FDM at much lower

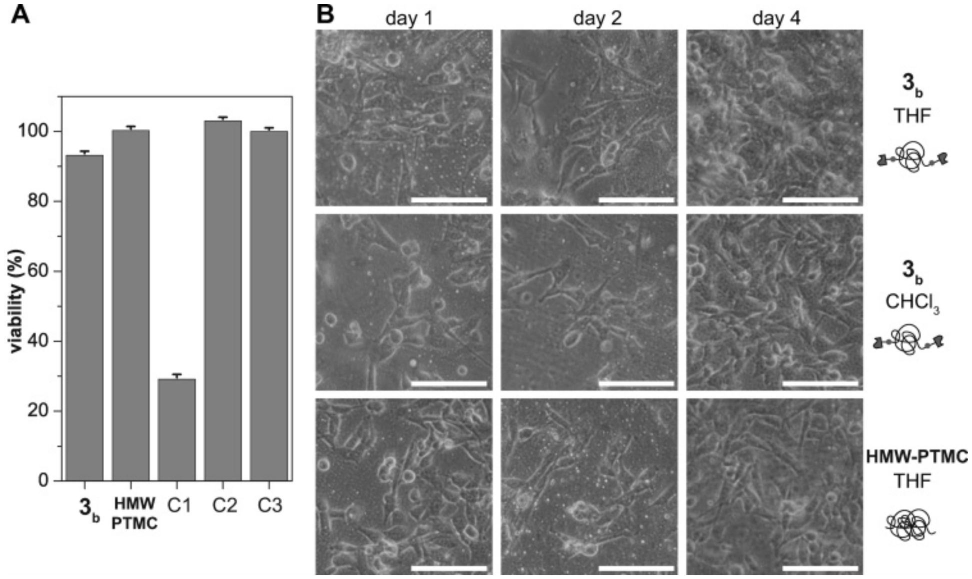


Figure 7. Biocompatibility of bifunctional UPy-modified TMC. (A) Indirect LDH viability test on $\mathbf{3_b}$, on **HMW−PTMC**, on UHMWPE (C2) as a positive control and on latex (C1) as negative control. The viability of the cells in culture medium (C3) was set at 100%. (B) Cell proliferation tests on drop cast films (from chloroform or THF) of $\mathbf{3_b}$ and of **HMW−PTMC** (from THF) as a control. The scale bars indicate 100 μm.

temperatures than their covalent counterparts. Because of the dynamic nature of hydrogen bonding, their melt viscosities are much lower. Besides easy processing, the developed FDM scaffolds can keep their dimensional shape if required due to the supramolecular cross-linking via the UPy groups at room temperature. No additional cross-linking is necessary. It has also been shown that the UPy-modified TMC materials are stable in an aqueous environment and that they are not toxic to fibroblasts.

This supramolecular TMC-system serves multiple purposes, because next to the possibility of easy processing, the system displays the ability to flow or to retain its shape dependent on the length of the TMC chains. This system might find its way in tissue engineering applications, varying from implants that must stay intact to prostheses that can adjust to the implantable location by showing flowing behavior. Next to that the hydrogen-bonding units leave ample room for a modular approach to biofunctionalization[20] of the materials, combining the advantages of tuning the mechanical properties of the material and the nature of the bioactive compound used.

Experimental Section

General Materials. Polymer grade 1,3-trimethylene carbonate (TMC) was purchased from Boehringer Ingelheim (Germany). The catalysts, tin octanoate and dibutyl tin dilaurate (DBTDL), and the initiators, 1,6-hexanediol and glycerol, were purchased from Aldrich. 1,6-diisocyanatohexane was obtained from Fluka. Commercial products were used without further purification. All solvents purchased from Acros Chimica or Sigma-Aldrich were of p.a. quality or HPLC grade. Deuterated chloroform was obtained from Cambridge Isotope Laboratories. Phosphate buffered saline (PBS) tablets were purchased from Sigma (dissolution of the tablets in water resulted in a 0.01 M phosphate buffer with 0.0027 M potassium chloride and 0.137 M sodium chloride, with pH = 7.4). Trypsin-EDTA (200 mg/L EDTA, 500 mg/L trypsin) solution was purchased from BioWhittaker Cambrex Bio Science. Trypan blue was obtained from Biochrom AG. The used cell culture medium consisted of a 1:1 mixture of Ham's F-12 with l-glutamine (BioWhittaker), supplemented with 1% penicillin, 1% streptomycin (Biochrom AG; 1000 u, 10000 μg/mL) and 10% fetal bovine serum (FBS) (Biochrom AG) and of Dulbecco's modified Eagle's medium (DMEM) with 1 g/L glucose without L-glutamine (BioWhittaker), supplemented with 1% penicillin, 1% streptomycin (Biochrom AG; 1000 u/10000 μg/mL), 1% glutamine (BioWhittaker, 200 mM in 0.85% sodium chloride solution), and 10% fetal bovine serum (FBS) (Biochrom AG).

General Methods. All syntheses were performed under an atmosphere of argon. Chloroform was dried over 4 Å molecular sieves. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 300 or Mercury 400 MHz spectrometer at 298 K. Chemical shifts are given in ppm (δ) values relative to tetramethylsilane (TMS). Infrared (IR) spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer with a Universal ATR sampling accessory for solids. Gel permeation chromatography was performed in chloroform using a Waters model 510 pump (with Waters Styragel column) with a HP Ti−Series 1050 autosampler, a Waters model 410 Differential refractometer and a Viscotek H502 viscometer detector, using a universal calibration method. Optical microscopy pictures of the FDM scaffolds and of the fibroblast cells were taken on a Zeiss Axiovert 25 microscope with a Sony (Cybershot, 3.3 Megapixels) digital still camera DSC−S75 (Carl Zeiss ACC Terminal). Normal photographs of these scaffolds were also taken with the Sony digital still camera DSC−S75.

Synthesis. TMC prepolymers ($\mathbf{1_{a,b}}$ and $\mathbf{2_{a-c}}$) were synthesized by ring-opening polymerization of TMC using tin octanoate (0.2 mmol per mol TMC) as catalyst and a specific amount of 1,6-hexane diol or glycerol as initiator. The polymerizations were conducted in a vacuum-sealed ampule at 130 °C for 48 h.[14] Subsequently, these TMC prepolymers ($\mathbf{1_{a,b}}$ and $\mathbf{2_{a-c}}$) were functionalized with UPy units ($\mathbf{3_{a,b}}$ and $\mathbf{4_{a-c}}$) using the UPy−isocyanate synthon.[21]

UPy—Isocyanate Synthon. The synthesis was performed as described before.[21] 2-amino-4-hydroxy-6-methylpyrimidine (0.23 mol; 29.1 g) was dissolved in 1,6-diisocyanatohexane (1.6 mol; 272.3 g) and heated at 100 °C for 16 h. The reaction mixture was cooled and pentane was added. The resulting precipitate was filtered and thoroughly washed with pentane. The product was dried at 50 °C in vacuo, yielding a white powder. Yield: 98%, 66.8 g, 0.23 mol. M.p.: 185 °C. ^{1}H NMR (CDCl$_3$): $\delta = 13.14$ (s, 1H), 11.87 (s, 1H), 10.19 (t, 1H), 5.82 (s, 1H), 3.27 (m, 4H), 2.23 (s, 3H), 1.61 (m, 4H), 1.41 (m, 4H) ppm. ^{13}C NMR (CDCl$_3$): $\delta = 173.1$, 156.7, 154.8, 148.4, 106.8, 43.0, 39.9, 31.3, 29.4, 26.3, 26.2, 19.0 ppm. IR (ATR): $\nu = 2931$, 2262 (NCO stretch), 1698 (UPy), 1667 (UPy), 1577 (UPy), 1519 (UPy), 1461, 1356, 1310, 1255 cm^{-1}. Anal. Calcd. (%) for $C_{13}H_{19}N_5O_3$: C, 53.2; H, 6.5; N, 23.9. Found (%): C, 53.2; H, 6.2; N, 24.0.

TMC Prepolymers ($\mathbf{1_{a,b}}$ and $\mathbf{2_{a-c}}$). As an example, the synthesis of PTMCdiol ($\mathbf{1_b}$) is described. TMC was polymerized (0.46 mol;

47.3 g) using 1,6-hexane diol (16 mmol; 1.9 g) as initiator, which resulted in a clear, viscous solution as product. GPC (chloroform): $M_n = 4.0$ kg/mol; $M_w = 8.4$; PD = 2.1. ^{1}H NMR ($CDCl_3$): δ = 4.28 (t, 4H), 4.22 (t, 4nH), 4.15 (t, 4H), 3.73 (t, 4H), 2.05 (m, 2nH), 1.90 (m, 4H), 1.69 (m, 4H), 1.41 (m, 4H) ppm. $M_n = 3.0$ kg/mol (calculated from ^{1}H NMR). 0.1 wt % (3 mol %) TMC monomer present (calculated from ^{1}H NMR: 4.47 (t, 4H), 2.17 (m, 2H) ppm).^{13}C NMR ($CDCl_3$): δ = 155.0, 154.9, 154.7, 67.7, 64.9, 64.1, 63.9, 58.6, 31.4, 28.3, 27.8, 25.1 ppm. IR (ATR): ν = 2971, 2910, 1736 (C═O stretch), 1587, 1461, 1404, 1362, 1331, 1219 (C−O stretch), 1141, 1092, 1028 cm^{-1}.

UPy-Functionalized TMC Polymers ($3_{a,b}$ and 4_{a-c}). As an example, the synthesis of PTMCdiUPy (**3_b**) is described. PTMCdiol (**1_b**) was reacted with the UPy−isocyanate synthon making use of a similar procedure as described before.[21] Polymer **1_b** (15.3 mmol; 46.0 g) was dissolved in dry chloroform, after which the UPy-isocyanate synthon (38.5 mmol; 11.3 g) was added. Dibutyl tin dilaurate was added (0.3 mL) and the solution was stirred at 80 °C for 16 h. The completeness of the reaction was checked with ^{1}H NMR and ^{13}C NMR for the presence of hydroxy-end groups. The solution was filtrated and 20 g silica kieselgel 60 and two drops of dibutyl tin dilaurate were added. The mixture was heated at 80 °C for 2 h after which additionally 5 g silica and two drops of dibutyl tin dilaurate were added. This mixture was stirred again for 2 h at 80 °C. With IR the absence of the UPy−isocyanate synthon was checked and it was found to be absent. The silica was removed by filtration over Hyflo after dilution of the mixture with chloroform. The chloroform was removed under reduced pressure. The polymer was precipitated from chloroform into hexane and filtrated. The resulting flexible, fibrous material was dried for 48 h at 30 °C in vacuo. Yield: 54%, 39.2 g, 8.2 mmol. ^{1}H NMR ($CDCl_3$): δ = 13.12 (s, 2H), 11.85 (s, 2H), 10.13 (s, 2H), 5.84 (s, 2H), 4.95 (s, 2H), 4.24 (t, 8H, 4nH), 4.16 (t, 4H), 3.24 (m, 4H), 3.16 (m, 4H), 2.25 (s, 6H), 2.04 (m, 2nH), 1.98 (m, 4H), 1.62−1.22 (m, 24H) ppm. $M_n = 4.8$ kg/mol (calculated from ^{1}H NMR). 0.01 wt % (0.8 mol %) TMC monomer present (calculated from ^{1}H NMR: 4.47 (t, 4H), 2.17 (m, 2H) ppm). ^{13}C NMR ($CDCl_3$): δ = 173.1, 156.6, 156.3, 155.1, 155.0, 154.7, 148.3, 106.6, 68.0, 64.8, 64.3, 64.1, 60.9, 58.4, 40.7, 39.6, 29.7, 29.3, 28.5, 28.0, 26.3, 26.1, 25.3, 18.9 ppm. IR (ATR): ν = 2968, 2936, 2860, 1737 (C═O stretch), 1699 (UPy), 1669 (UPy), 1587 (UPy), 1526 (UPy), 1460, 1405, 1362, 1331, 1220 (C−O stretch), 1140, 1096, 1029 cm^{-1}.

High Molecular Weight PTMC (HMW—PTMC). Very high molecular weight PTMC was synthesized by ring-opening polymerization of TMC in a vacuum-sealed glass ampule at 130 °C for 62 h using tin octanoate (0.2 mmol per mol TMC) as catalyst.[10] The polymer was precipitated from chloroform into hexane, resulting in a white material. GPC (chloroform): $M_n = 291$ kg/mol; $M_w = 425$; PD = 1.46. ^{1}H NMR ($CDCl_3$): δ = 4.24 (4nH), 2.05 (2nH) ppm. ^{13}C NMR ($CDCl_3$): δ = 155.0, 64.0, 28.0 ppm. IR (ATR): ν = 2970, 2922, 1736 (C═O stretch), 1587, 1460, 1404, 1362, 1331, 1219 (C−O stretch), 1141, 1092, 1028 cm^{-1}.

Preparation of Films. Films (**$3_{a,b}$**, **4_{a-c}**, and **HMW−PTMC**) were prepared by casting chloroform solutions in Teflon molds, and slow evaporation of the solvent at room temperature. The samples were dried in vacuo at 37 °C for 1−2 days. Mixtures of **3_b** with 10 mol % **4_a**, 10 mol % **4_b**, or 10 mol % **4_c** were obtained by dissolving both polymers in chloroform and stirring them for 16 h at room temperature. The same procedure was followed as described above.

Mechanical Properties. Tensile testing experiments were performed according to ASTM D1708−96 specifications in air at room temperature on a Zwick Z010 Universal Tensile Tester at an elongation rate of 20 mm/min with a preload of 0.02 N and a load cell of 20 N (or 2.5 kN in the case of **HMW−PTMC**). Tensile bars with a cross section of approximately 1.5 mm^2 were obtained by punching them out of sheets of materials that were prepared by drop casting out of chloroform. The grip-to-grip separation was approximately 20 mm. The measurements were performed at least in quadruplicate. Yield stresses and yield strains were determined by taking the intersection point of the two tangents to the initial and final parts of the load elongation curves around the yield point,[23] due to the shape of the stress−strain curves. An indicative Young's modulus (E) was determined by calculating the slope at 0% strain.

Cyclic tests were carried out using the same equipment as described above. Films of **3_b** were deformed cyclically (1000 cycles) up to 10% and up to 20% strain (see Supporting Information).

Differential Scanning Calorimetry. Thermal properties were investigated with differental scanning calorimetry (DSC) on a Perkin-Elmer differential scanning calorimeter Pyris 1 with Pyris 1 DSC Autosampler and Perkin-Elmer CCA7 cooling element under a nitrogen atmosphere with heating and cooling rates of 20 °C/min. The time elapsed between the runs was 5 min. The temperature range was −100 °C until 120 °C. Samples of 10−12 mg were measured.

Atomic Force Microscopy. Atomic force microscopy was performed in soft tapping mode on thin layer samples (drop cast from chloroform 1 mg/mL) on glass substrates at room temperature with a Digital Instrument Multimode Nanoscope IV using silicon cantilever tips (PPP−NCH-50, 204−497 kHz, 10−130 N/m). Scanner 6007JVH was used with scan rates between 0.5 and 1 Hz. All images were subjected to a first-order plane-fitting procedure to compensate for sample tilt.

Fused Deposition Modeling. The polymers (**3_b**, **4_c** and **HMW−PTMC**) were processed into FDM honeycomb grids with angles of 60° according to the following method: polymer billets were made in an aluminum mold during 1 h at 90 °C for **3_b** and **4_c** and at 150 °C for **HMW−PTMC**. Extrusion of these pellets was performed with the FDM machine; a *xyz*-manipulator, a direct extrusion head (for pellets) with plunger that forces the material through a capillary into the nozzle (width = 0.3 mm) and a heater, were used. Unidex 500 software was used to operate the system. The flow speed was 0.1 mm/s. The obtained scaffolds had a fiber width down to 250 μm. The extrusion took place at approximately 110 °C for **3_b**, at 120 °C for **4_c** and at 220 °C for **HMW−PTMC**.

After processing of **HMW−PTMC**, the presence of hydroxy end groups was detected with ^{1}H NMR in $CDCl_3$; the protons next to the hydroxy group were clearly visible at 3.76 ppm. The M_n of **HMW−PTMC** was calculated from ^{1}H NMR to be 30 kg/mol after processing.

Cell Culture Conditions. 3T3 mouse fibroblasts were cultured on a 1:1 mixture (see above) of Ham's F-12 and Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS). They were cultured in a humidified incubator at 37 °C and 5% CO_2. Before seeding, the cells were washed twice with PBS solution. Then they were trypsinized with a trypsin−EDTA solution, washed with PBS and counted after trypan blue staining in a Neubauer counting chamber. The cells were seeded in the culture medium with FBS. The passage of the cells was always between 4 and 60 and the viability of the cells was always above 97%.

Viability Assay. The cell viability of 3T3 mouse fibroblasts seeded in medium that was incubated with different materials was investigated using the LDH (lactate dehydrogenase) leakage kit (Sigma-Aldrich). Compression molded films of all materials were sterilized with UV (unless otherwise stated) for 2 h on each site, prior to use (^{1}H NMR revealed no sign of degradation of the materials). The polymers **3_b** and **HMW−PTMC** were incubated (0.2 g/mL medium) with medium containing FBS for 24 h at 37 °C. After that the medium was refreshed and the polymers (0.2 g/mL medium) were incubated again in medium with FBS for another 24 h at 37 °C. Latex gloves, which are known to be toxic (sterilized using the autoclave), and ultrahigh molecular weight polyethylene (UHMWPE), which is known to be biocompatible, were used as controls. These materials were treated in the same way. The incubated medium (150 μL/well of a 96-wells plate) was added to the cells (cultured at a starting density of 2.5×10^3 cells/well in a 96-wells plate for 48 h) and the cells were cultured for 48 h. The viability of the cells was tested in triplicate (per material) according to the protocol enclosed by the manufacturer.

Proliferation Assay. Films of **3_b** and of **HMW−PTMC** were made by drop casting the polymers from either THF or chloroform on glass coverslips (diameter = 1.5 mm). The films were dried in vacuo at 37 °C for at least 2 days and sterilized with UV for at least 2 h (^{1}H NMR revealed no sign of degradation of the materials). 3T3 mouse fibroblasts were seeded in two densities (5.7×10^3 cells/cm^2 and 2.3×10^4 cells/cm^2) on these films in duplicate. The cells were followed in time with optical microscopy.

Degradability Study. Drop cast films of $\mathbf{3_b}$ from chloroform (50−65 mg) were put in 10 mL PBS-buffer containing 0.05 w/v% sodium azide. The samples were shaken constantly at 37 °C in quadruplicate. After predetermined periods mass loss was determined after rinsing the samples three times with distilled water and drying them at 40 °C for 1.5 h. The samples were weighed (Sartorius microbalance) before and after degradation. GPC, IR, and ^{1}H NMR were performed.

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Supporting Information Available: Figure showing results of cyclic tensile testing of $\mathbf{3_b}$ strained up to 10% and up to 20% strain and a table giving DSC data for the first and second heating run of the hydroxy-terminated TMC prepolymers $\mathbf{1_{a,b}}$ and $\mathbf{2_{a-c}}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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