A novel single precursor-based biodegradable hydrogel with enhanced mechanical properties†

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A mechanically tough biodegradable hydrogel is developed from a single precursor comprising poly(ethylene glycol) and oligo(trimethylene carbonate), where both the crosslink density and swelling properties of the polymer network are independently controlled through $\bar{M}_c$ and hydrophilic-hydrophobic balance. These highly cost effective hydrogels are also biocompatible and can be degraded both hydrolytically and enzymatically.

Synthetic polymer hydrogels have attracted great interest in the biomedical field as their structural and functional properties are comparable to many of the soft tissues found in the human body.[1–3] However, poor mechanical properties of hydrogels, mainly attributed to their chemically crosslinked network structure, limit their widespread applications. The crosslink density and the polymer chain length between the crosslinking points both play important roles in determining the mechanical properties of hydrogels. Commonly used approaches to increase the mechanical strength of hydrogel include increasing either the crosslink density or the concentration of the precursors. However, these manipulations lead to brittle hydrogels with low swelling ratios, thus impeding their biomedical applications such as scaffolds for tissue engineering, temporary soft tissue substituents, and wound dressing materials. Nevertheless, recent advances in gel science have resulted in hydrogels with improved mechanical properties. These involve double network hydrogels,[4–6] nanocomposite hydrogels,[7–10] polyrotaxane-based hydrogels,[11,12] and hydrogels with giant crystalline domains.[13] The mechanical properties of these hydrogels vary depending on their structural characteristics. Some of these hydrogels exhibit high resistance to extension while others show a high degree of resistance to compression. For example, typical double network hydrogels, which are made from 2-acrylamido-2-methylpropanesulfonic acid and acrylamide monomers *via* a two-step polymerization process, can have a compressive fracture stress as high as 17.2 MPa at a fracture strain of 92%.[4] Nanocomposite hydrogels comprising of poly(N-isopropylacrylamide) and laponite nano-particles can resist an elongation as high as 1000%.[9]

In contrast to synthetic hydrogels, biological tissues with structural properties similar to hydrogels ("biological hydrogels") have excellent mechanical properties such as elongation, compression, and toughness. For example, human articular cartilage has a compressive modulus ranging from 0.4 to 0.8 MPa and an ultimate tensile strain of 10–40%.[14] The higher mechanical performance of these "biological hydrogels" has been attributed to various molecular and structural interactions.[15–17] There has been a surge of interest in the development of mechanically robust, easily processed hydrogel systems similar to biological hydrogels from a single precursor for their potential biomedical applications. Compared with systems developed from two or more monomers/precursors, single precursor-based hydrogel systems can be handled relatively easily and do not require elaborate purification and processing in hydrogel synthesis, thus offering a simple one-step procedure for their applications. In this study, we report the synthesis and characterization of a new class of biodegradable hydrogels made from a single precursor with enhanced mechanical properties and can be used for both invasive and non-invasive applications. These hydrogels are formulated based on principles from gel network theory which is summarized below.

As seen from Fig. 1a, when the average molecular weight of polymer chains between crosslinks ($\bar{M}_c$) is low, the highly hydrophilic polymer chains between the crosslinks are highly stretched and highly susceptible to fracture. In a stretched state, the hydrogel mimics the stress-strain behavior of a weak linear elastic material up to the point of fracture that gives rise to their fragile/brittle nature, especially in highly crosslinked networks (Fig. 1a). In contrast, Fig. 1b represents a network wherein the polymer chains between the crosslinks adopt a random conformation, thus resisting the fracture of the chains under stress. Such a random conformation of the chains can be achieved by manipulating the length of the polymer chain between crosslinks and/or controlling the interaction between the polymer chains and the water molecules (*i.e.*, swelling behavior). With this in

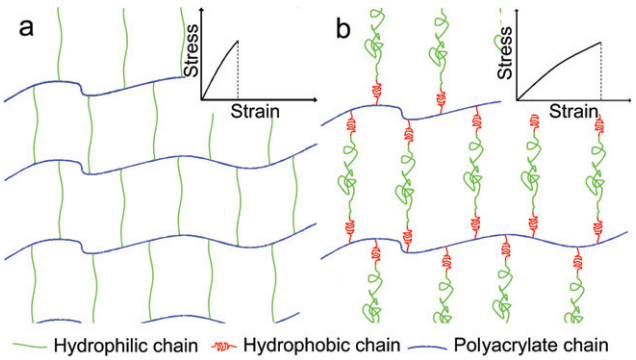

Fig. 1 A schematic illustrating the effect of network structure on mechanical properties of hydrogels. (a) Hydrogels having network structure comprising of lower $\bar{M}_c$ and higher crosslink density demonstrate brittle nature, the inset shows a representative stress-strain curve. (b) Networks having higher $\bar{M}_c$ and small hydrophobic moieties assume a more random conformation, and the inset shows a representative stress-strain curve of such hydrogels.

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Table 1 Properties of TMC20, PEG20, TMC3.4, and PEG3.4 hydrogels

Sample	Toughness (kJ/m^3)	Fracture stress (MPa)	Fracture strain (%)	Compressive modulus (kPa)	Equilibrium swelling ratio	$\bar{M}_c$ (g/mol)	Cross-link density (mol/m^3)
TMC20	215.3 ± 46.4	5.2 ± 1.3	98.2 ± 1.3	14.9 ± 0.2	42.6 ± 0.8	7228	6.01 ± 0.07
PEG20	130.2 ± 45.4	3.3 ± 1.0	98.7 ± 3.5	7.4 ± 0.8	43.2 ± 2.1	7345	3.00 ± 0.33
TMC3.4	10.0 ± 0.9	0.05 ± 0.002	55.4 ± 2.1	36.6 ± 0.3	13.7 ± 0.1	1067	14.24 ± 0.91
PEG3.4	50.6 ± 7.1	0.39 ± 0.08	62.6 ± 1.4	85.0 ± 2.6	12.7 ± 0.5	900	34.30 ± 1.03

mind, we have designed a hydrogel system with enhanced mechanical properties based on two assumptions: (i) the mechanical strength of a hydrogel system can be controlled by manipulating $\bar{M}_c$, wherein the flexible long polymer chains containing hydrophobic moieties form random coil conformation can dissipate energy when force/stress is applied to the network,[18] and (ii) the swelling behavior of the network can be controlled by changing the ratio of hydrophilic to hydrophobic moieties within individual polymer chains.

The hydrogels described in this study were synthesized from a macromolecular precursor with defined molecular weight and hydrophilic-hydrophobic moieties, where both crosslink density and swelling properties of the network were independently controlled through $\bar{M}_c$ and hydrophilic-hydrophobic moieties. Specifically, the precursor is a triblock copolymer, oligo(trimethylene carbonate)-*block*-poly(ethylene glycol)-*block*-oligo(trimethylene carbonate) (OTMC-PEG-OTMC) diacrylate, containing hydrophilic poly(-ethylene glycol) (PEG) moiety and hydrophobic oligo(trimethylene carbonate) (OTMC) with acryloyl groups at both ends. The high molecular weight of the PEG precursor (20 000 g/mol) having small hydrophobic blocks of OTMC groups (with total molecular weight of both TMC end groups of 650 g/mol) allows the confined polymer chains to take a more random-coiled conformation between the crosslinks compared to precursors with low molecular weights (3 400 g/mol). The incorporation of OTMC segments introduces hydrophobic domains into the network; a critical balance of hydrophilic-hydrophobic moieties present within this triblock copolymer results in hydrogels with enhanced toughness, elasticity, and modulus without compromising their swelling ratio. It should also be noted that all the components of this triblock copolymer are biocompatible and have been extensively used in biomedical applications.[19–21] Hence, this single precursor-based hydrogel exhibits highly tunable and improved mechanical properties without compromising the swelling ratio, biocompatibility, and biodegradability.

The triblock copolymer precursors were synthesized through a PEG initiated ring-opening polymerization of trimethylene carbonate (TMC) and subsequent acrylation of the OTMC-PEG-OTMC triblock copolymers (for details refer to ESI, Scheme 1†).[21–23] Hydrogels were synthesized *via* photopolymerization of 10% (wt/v) solution of these precursors (ESI†).[24,25] A nuclear magnetic resonance (NMR) spectrum confirms the chemical structure of the triblock copolymer (ESI†, Fig. S1). In addition to confirming the incorporation of OTMC, NMR data was also used to determine the molecular weight of OTMC units and extent of acrylation of the triblock copolymer (ESI†).

Interestingly, amongst a series of hydrogels with varying molecular weight of OTMC block and PEG block, only those having OTMC units with molecular weight of 650 g/mol and PEG with molecular weight of 20 000 g/mol (hereafter, referred to as TMC20) exhibited good mechanical properties. The corresponding PEG homopolymer hydrogel synthesized from PEG-diacrylate having molecular weight of 20 000 g/mol will be termed as PEG20, PEG homopolymer hydrogels synthesized from PEG with molecular weight of 3 400 g/mol will be termed as PEG3.4, and the PEG3.4 with TMC moieties (570 g/mol) will be referred to as TMC3.4. The equilibrium swelling of these hydrogels and their $\bar{M}_c$ values, relative molecular mass between crosslinks, calculated using Peppas-Merrill model are given in Table 1.[26] The crosslink density (Table 1), active network chains per unit volume, of the hydrogels were calculated using the following equation:[10,27]

$$\sigma = \nu_e kT\left[\alpha - \frac{1}{\alpha^2}\right]$$

where σ is the stress, ν_e is the number of elastically active chains, k is the Boltzmann constant, T is the absolute temperature in Kelvin, and α is the strain.

As seen from Fig. 2, the equilibrium swollen TMC20 hydrogel exhibited large deformations under stress and recovered from deformation upon removal of the stress similar to an elastomer (Fig. 2b). Being a soft gel, PEG20 also deformed under stress and recovered back slowly but broke at higher stress (Fig. 2a). In contrast, pressing between the fingers easily broke PEG3.4 hydrogels (data not shown). The same holds true for TMC3.4 hydrogels. Both PEG20 and TMC20 hydrogels can be made into knots without introducing any macroscopic cracks or damage to the hydrogels at equilibrium swollen conditions (Fig. 2c–d). Note that both PEG20 and TMC20 exhibited similar equilibrium swelling ratios of 43.2 ± 2.1 and 42.6 ± 0.8, respectively (Table 1). The PEG20 knots were bigger in size and the hydrogel were broken into pieces upon slight stretching (Fig 2c). In contrast, the TMC20 knots were smaller in size and could withstand stretching and bending deformations (Fig 2d). PEG3.4 and TMC3.4 hydrogels exhibited brittle fracture and could not be made into a knot (data not shown).

As determined by the compression tests (Table 1), the equilibrium swollen PEG20 hydrogel showed a compressive modulus of 7.4 ± 0.8 kPa; while the compressive modulus of TMC20 hydrogel was 14.9 ± 0.2 kPa. The higher modulus of the TMC20 hydrogel could be attributed to an overall increase in crosslink density which accounts for both chemical crosslinking and secondary interactions from the interpolymer hydrophobic interactions. The fracture stress of TMC20 hydrogels was found to be 5.2 ± 1.3 MPa, which is higher than that of PEG20 hydrogels (3.3 ± 1.0 MPa), suggesting that the incorporation of OTMC blocks enables the hydrogels to sustain higher stresses. The fracture strain of the TMC20 was 98.2 ± 1.3%, which is quite similar to that of PEG20 hydrogels. Moreover, the toughness of the TMC20 and PEG20 hydrogels were measured to be 215.3 ± 46.4 kJ/m^3 and 130.2 ± 45.4 kJ/m^3, respectively. The toughness of TMC20 hydrogels is substantially higher than that of PEG20 hydrogels, and is also much higher than most of the

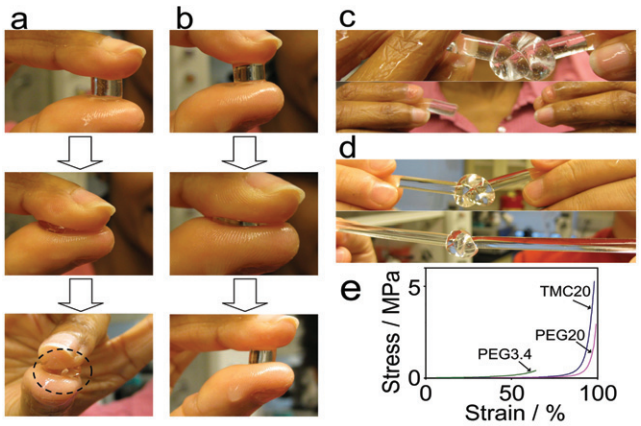

Fig. 2 Photographs demonstrating how the TMC20 hydrogels sustain compression, knot formation and stretching: (a) PEG20 hydrogels deformed under compression but broken into pieces at higher stress. The dotted circle shows the broken hydroegl pieces; (b) deformation and recovery of TMC20 hydrogel under stress; (c) knots formed from PEG20 hydrogels (top) were broken into pieces upon stretching (bottom); (d) TMC20 hydroegls knots (top) were able to withstand stretching (bottom); (e) stress-strain profiles of the hydrogels under uniaxial compression.

hydrogels synthesized from a single precursor. The photo-gelation (for details, see ESI†) of TMC20 hydrogels required less than 5 minutes, making them amenable to various biomedical applications such as cell encapsulation, cell delivery, tissue filler, and minimally invasive applications.

The equilibrium swollen PEG3.4 hydrogels showed the usual brittle behavior of cross-linked networks with a high compressive modulus. The PEG3.4 exhibited a compressive modulus of 85.0 ± 2.6 kPa, which is much higher than those of both TMC20 and PEG20 hydrogels. This higher compressive modulus of PEG3.4 is a manifestation of lower $\bar{M}_c$ and higher crosslink density (Fig. 1a). Other than the compressive modulus, PEG3.4 showed poor toughness, lower fracture stress, and strain. The toughness of PEG3.4 hydrogels was calculated to be 50.6 ± 7.1 kJ/m^3 and the fracture stress and strain were 0.39 ± 0.08 MPa and 62.6 ± 1.4%, respectively. These values are much lower than those seen in PEG20 and TMC20. These results indicate the effect of $\bar{M}_c$ on the overall mechanical properties of the hydrogels; larger $\bar{M}_c$ values enhance the toughness and the fracture stress and strain, and decrease the compressive modulus. These findings additionally suggest the importance of structure-property relationships and the need of utilizing precursors with varying molecular weights depending upon the targeted applications.

The ability of PEG20 hydrogels to withstand stress and undergo deformation upon stress indicates that the "long" flexible chains between the crosslinking points promote their resistance to compressive stress (*i.e.* hydrogels are less brittle) without compromising the structural integrity of the network. The incorporation of a short OTMC blocks was found to enhance the overall mechanical properties (compressive modulus, fracture stress, toughness) of these hydrogels (TMC20) over their PEG20 counterparts without compromising the swelling ratio and ability to deform under stress before breakage with near-complete recovery. However, precursors containing PEG chains with the same molecular weight (20 000 g/mol) but OTMC blocks having molecular weight higher than 650 g/mol failed to create robust hydrogels. These hydrogels were very soft and could not maintain their shape under their own weight. Precursors with OTMC units having molecular weights of 3680 g/mol and above could not be dissolved in water at ambient temperature, and therefore were not investigated in this study.

The incorporation of OTMC units into PEG3.4 (TMC3.4 hydrogels) did not improve their mechanical properties. In fact they showed inferior mechanical properties compared to their PEG3.4 counterparts (Table 1). Increasing further the molecular weight of incorporated OTMC units to 1200 g/mol (*i.e.* double that of TMC3.4) did not have any significant effect on hydrogel mechanical properties as they exhibited almost similar performance as that of TMC3.4 (data not shown). These findings indicate that the incorporation of OTMC units alone cannot result in hydrogels with enhanced mechanical properties. These findings additionally suggest that the enhanced mechanical properties observed in the case of TMC20 are a result of synergistic contributions from both $\bar{M}_c$ and the hydrophobic-hydrophilic moieties within the polymer network.

The presence of OTMC segments not only imparts hydrophobic domains into the network but also makes the hydrogels biodegradable both *in vitro* and *in vivo*. Homopolymers of TMCs are known to undergo both hydrolytic and enzymatic degradation;[21,28] the degradation products, CO_2 and 1,3-propane diol, do not provoke any inflammatory reaction. The degradation of TMC20 hydrogels was evaluated by incubating the hydrogels for 3 weeks in phosphate buffered saline (PBS) (hydrolytic degradation) and lipase solution (enzymatic degradation; 5000 IU/mL in deionized water; lipase from *thermomyces lanuginosus*, E. C. 3.1.1.3.) at 37 °C,[28] and gravimetrically measuring the weight changes as a function of time (see ESI, Fig. S2†). No significant degradation of TMC20 hydrogels was observed initially (week 1) in the absence of enzyme. However by week 2, loss of approximately 10% of weight was observed in PBS. In contrast, three times more weight loss was observed in the presence of enzymes during the same incubation time. Incubation of TMC20 for 4 weeks caused the TMC20 hydrogels to degrade by 24.9 ± 0.7% and 40.1 ± 3.0%, hydrolytically and enzymatically, respectively; however, the hydrogels still maintained their shape. After 8 weeks of incubation, near-complete degradation of the hydrogels was observed. The enzymatic degradation of TMC20 hydrogels left a very soft mass, which did not disappear even after 12 weeks, whereas the TMC20 in PBS disappeared completely with no residual mass after 8 weeks. The preservation of structural integrity during the initial degradation is an important requirement for various applications such as tissue engineering, material guided healing, drug and cell delivery devices, and temporary tissue fillers and *in vivo* sensors.

Since both PEG and PTMC are biocompatible polymers and have been widely used for biomedical applications,[19–21] TMC20 hydrogels that combine both these moieties are also expected to show biocompatibility. The ability of the TMC20 hydrogels to support cell culture both two dimensional (2D) and three dimensional (3D) was evaluated using human bone marrow derived mesenchymal stem cells (hMSCs). For 2D culture, hMSCs were plated homogenously onto discs of TMC20 and PEG20 hydrogels and cultured. After three hours of incubation, a majority of the cells had adhered and several of these cells (>20%) were spread out on TMC20 hydrogels, while only a few hMSCs were found adhered to PEG20 hydrogels (Fig. 3a–b). After 24 hours, almost all the hMSCs were spread out on the TMC20 hydrogels compared to PEG20 hydrogels, on which only a small

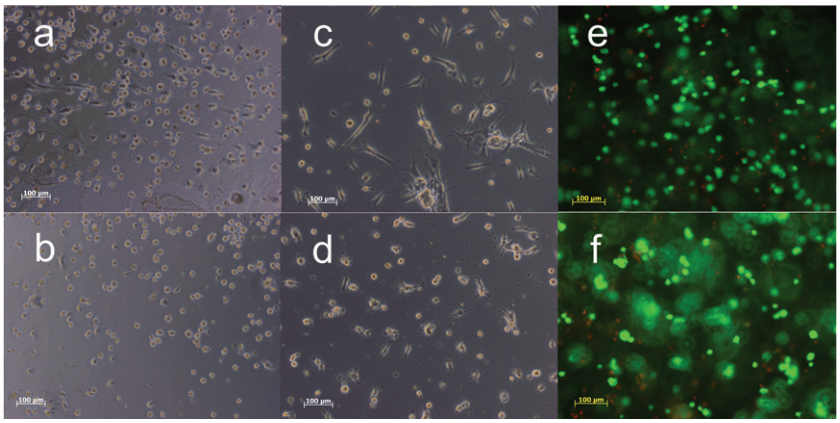

Fig. 3 Bright field images of hMSCs grown on PEG20 and TMC20 hydrogel discs (a–d). After 3 hours of plating, the hMSCs on TMC20 hydrogels start spreading out (a) compared to PEG20 (b). After 24 hours, almost all the hMSCs were spread out on TMC20 hydrogels (c), while only a few cells were found to be spreading on PEG20 hydrogels (d). Fluorescent micrographs of hMSCs encapsulated within TMC20 hydrogels (e) and PEG20 hydrogels (f) after 24 hours of photoencapuslation. Comparable cell viability was observed between the two hydrogels as evidenced by the number of viable cells.

fraction of hMSCs were found to be spreading onto the surface (Fig. 3c–d). The enhanced adhesion and spreading of hMSCs on TMC20 over PEG20 could be attributed to the hydrophobic OTMC domains.[29,30] In fact, various studies have utilized hybrid hydrogels containing hydrophobic components to improve the cell-hydrogel interactions.[30]A report by Wang *et al.* recently showed that the presence of hydrophobic silica particles promotes protein adsorption onto the surface of these hydrogels compared to their non-silica containing counterparts.[29] This enhanced cell adhesion observed in the case of these hybrid hydrogels could be attributed to the enhanced protein adsorption. To further evaluate the applicability of TMC20 for tissue engineering applications, we examined the biocompatibility of TMC20 as a scaffold for 3D cell encapsulation. As seen from Fig. 3f, the TMC20 hydrogels, like PEG20 hydrogels (Fig. 3e), support photo-encapsulation of hMSCs cells and their subsequent *in vitro* culture.

In summary, the TMC20 hydrogels are tougher and more elastic as compared to other existing single precursor-based hydrogels while still retaining a high water content. The synthesis of TMC20 hydrogels is highly cost effective and these materials are easily processed. Additionally, its constituents, PEG and TMC, and their degradation products, are biocompatible and do not exert any adverse effect on cells in both 2D and 3D cultures. The non-toxic degradation product of these hydrogels could be easily removed from the local environment. The above discussed distinct properties of this novel hydrogel system, in conjunction with the long history of the biomedical application of PTMC and PEG, makes it a very promising material for various biomedical applications.

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