Acta Biomaterialia 31 (2016) 80–88

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

# Tough biodegradable mixed-macromer networks and hydrogels by photo-crosslinking in solution

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ARTICLE INFO

*Article history:*
Received 24 July 2015
Received in revised form 30 October 2015
Accepted 9 December 2015
Available online 10 December 2015

*Keywords:*
Single-macromer networks
Mixed-macromer networks
Photo-crosslinking
Crosslinking in solution
Hydrogels

ABSTRACT

The preparation of polymeric networks that are both tough and biodegradable remains a challenge. Here we show a very straightforward method to produce tough biodegradable networks from low molecular weight macromers for applications such as tissue engineering. Photo-crosslinking combinatorial mixtures of methacrylate-functionalized poly(1,3-trimethylene carbonate) (PTMC), poly(D,L-lactide) (PDLLA), poly(ε-caprolactone) (PCL) and poly(ethylene glycol) (PEG) oligomers in propylene carbonate (PC) allowed the preparation of network films with excellent tensile characteristics and resistance to tearing. This method enabled the production of both very tough mixed-macromer elastomers as well as mixed-macromer hydrogels. A mixed-macromer hydrogel prepared from 33 wt.% PTMC, 33 wt.% PCL and 33 wt.% PEG had a very high tearing energy of 0.81 kJ/m$^2$, which is comparable to tearing energies determined for articular cartilage.



## 1. Introduction

Tough biodegradable polymer networks have great potential to be used as load bearing implants, for example in tissue engineering applications. Specifically elastomeric polymer networks are of interest. They can be designed to have mechanical properties that match those of load bearing tissues like cartilage and withstand dynamic loading [1]. Much attention has been paid to improve the toughness of biodegradable elastomers, but simultaneously achieving biodegradability and toughness remains a challenge.

To obtain high toughness and tear strength in polymer networks, the growth of micro-cracks should be hindered [2]. This has been studied extensively for natural and synthetic elastomers. Micro-crack growth has been slowed down by the addition of filler particles, the introduction of crystallizable domains in the polymer network, crosslinking chains with bimodal chain length distributions, and crosslinking in a diluted state [3–8]. Much of the fundamental research on toughening has been conducted using non-biodegradable elastomers like polyisoprene and poly (dimethyl siloxane) (PDMS). However, the development of tough and tear-resistant biodegradable elastomers is very limited.

We have prepared flexible, elastic and tough networks that are biocompatible and biodegradable by γ-irradiation of high molecular weight poly(1,3-trimethylene carbonate) (PTMC) [9–11] and by photo-crosslinking methacrylate-functionalized PTMC oligomers (PTMC macromers) [12,13]. In the latter case, toughness was found to improve with increasing molecular weight of macromers. As such, PTMC networks were found to show even higher toughness than high molecular weight linear PTMC. Furthermore, the tear propagation strength was found to increase from 1.9 N/mm to 9.3 N/mm when high molecular weight PTMC compounded with pentaerythritol triacrylate (PETA) was γ-irradiated [14]. Amsden and co-workers photo-crosslinked an acrylated poly(ε-caprolactone-*co*-D,L-lactide) P(CL-*co*-DLLA) oligomer dissolved at different concentrations in dichloromethane. The obtained networks became less rigid and strong, and more extendable with decreasing macromer concentrations during the crosslinking [15]. Photo-crosslinking fumarate-functionalized copolymers with different contents of TMC, DLLA and CL yielded elastomers with a wide range of mechanical properties [16].

Hydrogels (hydrophilic polymer networks) are another class of networks that are of great interest for use in tissue engineering. In aqueous environments these networks have high water contents and low rigidity, and as a rule are fragile and very weak [17]. Toughening of polyvinylalcohol (PVA), polyacrylamide (PAA), poly(hydroxyethylmethacrylate) (PHEMA) or polyethylene glycol (PEG) hydrogels has been done by preparing interpenetrating

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http://dx.doi.org/10.1016/j.actbio.2015.12.014
1742-7061/

networks, ionic networks or composite networks [18]. Although the tensile strengths and tearing energies of the hydrogels are much improved compared to the unmodified hydrogels, these hydrogels are still less tough than load bearing tissues. They are also non-biodegradable. Extremely high toughness in PAA hydrogels was achieved by Gong and co-workers. Interpenetrating double networks comprising a loosely crosslinked PAA network and a densely crosslinked poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) network were prepared, yielding hydrogels with exceptionally high strengths and toughnesses [19]. PAA is, however, non-biodegradable and residues of the acrylamide monomer are potentially toxic. Recently, we prepared tough biodegradable hydrogels based on PEG by crosslinking methacrylate-functionalized PTMC-PEG-PTMC [20] and PDLLA-PEG-PDLLA [21] triblock copolymers. Although the hydrogels were shown to be quite resilient, their mechanical properties were not extensively studied.

It is clear that considerable work has been done to prepare (co) polymer networks with optimized mechanical properties for specific applications. However, the design and the syntheses of the networks is time-consuming and the number of network compositions that can be prepared is limited. The discovery of new materials may be more efficient if a combinatorial chemistry approach is taken. Combinatorial chemistry has been successfully used to discover new drugs and polymeric biomaterials [22,23]. However, the preparation of tough combinatorial networks based on combinations of macromers (mixed-macromer networks) has not yet been reported.

In this paper we describe the preparation of mixed-macromer networks and hydrogels by photo-crosslinking combinatorial mixtures of methacrylate-functionalized PTMC-, PDLLA-, PCL- and PEG-macromers in solution using an inert solvent with low vapor pressure and a high boiling. We anticipated that the crosslinking of mixed-macromer networks would yield networks with high toughness since multiphase networks can possibly contribute to an increase of the tearing strength. Furthermore, crosslinking in solution was also expected to improve the toughness of the networks significantly.

## 2. Experimental

### 2.1. Materials

Polyethylene glycol (PEG) ($M_n$ = 10 kg/mol), stannous octoate ($Sn(Oct)_2$), ε-caprolactone (CL), 1,6-hexanediol, methacrylic anhydride, triethylamine (TEA), deuterated chloroform, trifluoroacetic anhydride (TFAA) were purchased from Sigma–Aldrich (The Netherlands). Trimethylene carbonate (1,3-dioxan-2-one, TMC) was obtained from Huizhou Foryou Medical Devices Co (China). D,L-Lactide (DLLA) was obtained from Purac Biochem (The Netherlands). Dichloromethane (DCM) was obtained from Biosolve (The Netherlands). Calcium hydride ($CaH_2$) and propylene carbonate (PC, boiling point 242 °C) were purchased from Merck (Germany). Ethanol was obtained from Assink Chemie (The Netherlands). Diethyl ether was obtained from Fisher Scientific. Irgacure 2959 (2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone) was obtained from Ciba (Switzerland).

### 2.2. Synthesis of dimethacrylate-functionalized PTMC, PDLLA, PCL and PEG oligomers

Linear oligomers were prepared by ring-opening polymerization of TMC-, DLLA- or CL monomers. Polymerization was performed in an inert argon atmosphere in the presence of 1,6-hexanediol as initiator (0.25 mmol/g monomer to obtain an oligomer with $M_n$ = 4 kg/mol, and 0.1 mmol/g monomer to obtain an oligomer with $M_n$ = 10 kg/mol) at 130 °C for two days. Sn ($Oct_2$) was used as catalyst (0.02 mmol/g monomer). The oligomers were dried at 120 °C under vacuum for 2 h, and cooled to room temperature under argon. Dry DCM (dried over $CaH_2$ and distilled, 3 mL/g oligomer) was added, then TEA (4 mol/mol oligomer) and methacrylic anhydride (4 mol/mol oligomer) were added. The functionalization reaction proceeded for five days at room temperature. The dimethacrylate (dMA)-functionalized macromers were purified by precipitation. PTMC-dMA, PDLLA-dMA, and PCL-dMA were precipitated in cold ethanol (−25 °C) and PEG-dMA was precipitated in cold diethylether (−25 °C). The macromers were subsequently dried under vacuum at 40–50 °C for 3 days.

### 2.3. Preparation of photo-crosslinked networks

PTMC-dMA, PDLLA-dMA, PCL-dMA and PEG-dMA single-macromer networks were prepared by crosslinking the individual macromers in propylene carbonate solutions containing 1 wt.% Irgacure 2959 photo initiator. To assess the effect of the macromer concentration during crosslinking, PTMC-dMA was photo-crosslinked in solutions containing 20, 33 and 50 wt.% macromer first. The solutions were cast on a glass plate at a thickness of 0.5 mm and crosslinked by irradiation at 365 nm for 20 min at 10 mW/cm$^2$ in a nitrogen atmosphere at 50 °C. The crosslinked films were extracted with a mixture of acetone and ethanol (50/50 vol./vol.), the solvent was refreshed every 24 h during three days. The extracted films were washed with ethanol and dried under reduced pressure at 50 °C until a constant weight was reached. To crosslink in bulk, a PTMC-dMA solution in DCM that contained 33 wt.% macromer was prepared and cast on glass, then the DCM was allowed to evaporate. Crosslinking, extraction, washing, and drying was performed as described above.

Eleven different mixed-macromer networks were then prepared by crosslinking mixtures of PTMC-dMA, PDLLA-dMA, PCL-dMA and PEG-dMA macromers dissolved in PC. In these mixtures the macromers were present in equal weight percentages and the concentration of macromers was 33 wt.%. The mixed-macromer solutions were cast, crosslinked, dried and extracted as described above. See Table 4 for an overview. Although PCL-dMA 10k is soluble in PC at 50 °C, the solubility of this macromer was much lower in the presence of other macromers. As it was found that in solution mixtures with PCL-dMA 4k were clear, only this macromer was used to prepare mixed-macromer networks.

### 2.4. Characterization of macromers and photo-crosslinked networks

The macromer number average molecular weights ($M_n$) and the degrees of functionalization ($f$) were determined using a Varian Inova 400 MHz $^1$H NMR spectrometer in deuterated chloroform.

Differential Scanning Calorimetry (DSC) was used to determine the glass transition temperature ($T_g$), the melting temperature ($T_m$) and melting enthalpy ($\Delta H_m$) of the macromers and networks. DSC measurements were performed using a liquid nitrogen-cooled Pyris 1 DSC (Perkin Elmer, USA). Samples weighing 5–10 mg were heated to 100 °C at a rate of 10 °C/min, kept for one minute at 100 °C and then cooled to −100 °C at a rate of 200 °C/min. After five minutes at −100 °C, a second scan was recorded from −100 °C to 100 °C at 10 °C/min. The thermal transitions of the specimens, $T_g$ (midpoint), $T_m$ (peak) and $\Delta H_m$ were determined in the second heating scan.

The mechanical properties of the dry and the hydrated networks were assessed using a Zwick Z020 tensile tester (Germany), equipped with a 500 N load cell at room temperature (20 °C). The elongation of the samples was derived from the grip-to-grip separation, which was initially 35 mm. Experiments were conducted in