



Photo-crosslinked networks prepared from fumaric acid monoethyl ester-functionalized poly(D,L-lactic acid) oligomers and N-vinyl-2-pyrrolidone for the controlled and sustained release of proteins

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ABSTRACT

Photo-crosslinked networks were prepared from fumaric acid monoethyl ester-functionalized poly(D,L-lactic acid) oligomers and N-vinyl-2-pyrrolidone. Two model proteins, lysozyme and albumin, were incorporated into the network films as solid particles and their release behavior was studied. By varying the NVP content and macromer molecular weight the degradation behavior and protein release profiles of the prepared networks could be tuned. The more hydrophilic and less densely crosslinked networks released albumin and lysozyme at a faster rate. Although active lysozyme was released from the networks over the complete release period, lysozyme release was often incomplete. This was most likely caused by electrostatic and/or hydrophobic interactions between the protein and the degrading polymer network.

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1. Introduction

In the past decade several researchers have investigated the use of photo-crosslinked biodegradable polymer networks for controlled drug delivery applications [1,2]. A major advantage of photo-crosslinking is that a drug can easily be entrapped in a network by dispersing or dissolving it in a macromer solution prior to crosslinking. In this way large amounts of drug can be loaded at high efficiencies. Furthermore, photo-polymerization is rapid and can be accomplished with minimal heat generation, allowing the incorporation of heat-sensitive compounds such as proteins.

Poly(D,L-lactic acid) (PDLLA) is a well-known polymer that has been studied extensively for application in biodegradable drug delivery systems [3]. Networks based on PDLLA can be prepared by photo-initiated crosslinking of functionalized PDLLA oligomers. Besides the frequently used methacrylate derivatives, fumaric acid derivatives are also attractive compounds for endfunctionalization reactions. It can be expected that residual unreacted fumarate

endgroups will not lead to toxicity upon implantation since fumaric acid is a compound naturally found in the body [4]. The relatively low reactivity of fumarate endgroups can be overcome by choosing an appropriate co-monomer for the photo-initiated crosslinking reaction, such as N-vinyl-2-pyrrolidone (NVP) [5].

Many photo-crosslinked polymer networks have been studied as drug delivery systems. These include photo-crosslinked highly swollen hydrogels [6–8] and more hydrophobic networks based on PDLLA, poly(ε-caprolactone), poly(trimethylene carbonate) (PTMC) or co-polymers of these three [9–12]. Drug release profiles can be tuned by varying the crosslink density or by adjusting the hydrophilicity of crosslinked polymer networks. Several authors have used the incorporation of poly(ethylene glycol) (PEG) to increase network hydrophilicity and adjust drug release profiles [13–15]. Increasing the NVP content of networks prepared from fumaric acid derivatives was shown to also increase network hydrophilicity and drug release rates [16–18].

Controlled and sustained release of protein drugs is challenging due to the large size and the relative instability of these molecules. During preparation, storage and release a range of conditions may affect the stability of a protein [19,20]. Important in this respect is the possible presence of residual organic solvents. When incorporating protein drugs in photo-crosslinked polymer networks care

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should be taken to prevent side reactions of free radicals with the protein [21]. However, when solid protein particles are dispersed in an organic phase containing the macromer and photo-initiator contact with free radicals is limited since the protein remains in the solid form during the photo-polymerization reaction [22].

In previous research we prepared biocompatible tissue engineering scaffolds by stereolithography from fumaric acid monoethyl ester (FAME) functionalized PDLLA oligomers and NVP [18]. Furthermore, we investigated PTMC oligomers functionalized with FAME endgroups for the controlled release of model drugs and proteins [5,23]. Due to slow degradation of the PTMC-based networks only low molecular weight model drugs and proteins could be released over a time period of several months. To allow for the release of high molecular weight proteins in a controlled and sustained way in this paper we describe the preparation of photo-crosslinked polymer networks from PDLLA-FAME macromers and NVP. The crosslink density and hydrophilicity of the networks were varied by using macromers of different molecular weights and different NVP concentrations. The degradation behavior of the networks and the release of two model proteins, lysozyme and bovine serum albumin, from the prepared networks were studied.

2. Materials and methods

2.1. Materials

D,L-Lactide (DLLA) was purchased from Purac Biochem (The Netherlands). Tin 2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$), trimethylol propane (TMP), fumaric acid monoethyl ester (FAME), deuterated chloroform, bovine serum albumin, lysozyme, *Micrococcus lysodeikticus* and acetate buffer solution (pH 4.7) were obtained from Sigma Aldrich (St. Louis, MO). N-Vinyl-2-pyrrolidone (NVP) and 1,3-dicyclohexylcarbodiimide (DCC) were purchased from Fluka (Switzerland). 4-Dimethylaminopyridine (DMAP) was purchased from Merck (Germany). Irgacure 2959 (2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone) was obtained from Ciba Specialty Chemicals (Switzerland). The BCA (bicinchoninic acid) protein assay kit was obtained from Thermo Fisher Scientific (Waltham, MA). Phosphate-buffered saline (PBS), pH 7.4 was obtained from B. Braun (Germany). Analytical grade dichloromethane (DCM) was obtained from Biosolve (The Netherlands). DCM was dried over CaH_2 and distilled. The other solvents were of technical grade and were used as received (Biosolve, The Netherlands).

2.2. Synthesis and characterization of three-armed FAME-functionalized PDLLA oligomers

Three-armed poly(D,L-lactide) oligomers were synthesized by ring opening polymerization of D,L-lactide in the presence of trimethylol propane (TMP) as a trifunctional initiator (Fig. 1). The oligomer syntheses were carried out on the 40 g scale. DLLA, initiator and $\text{Sn}(\text{Oct})_2$ (approximately $0.2 \text{ mmol mol}^{-1}$ monomer) as a catalyst were reacted in the melt at 130°C for 48 h under argon. The targeted molecular weights were 6.1, 9.1 and 12.1 kg mol^{-1} , corresponding to approximately 14, 21 and 28 D,L-lactide units per arm, respectively. To achieve this 42, 63 and 84 mol of monomer were used per mol of initiator.

The oligomers were functionalized by coupling fumaric acid monoethyl ester to the hydroxyl termini of the oligomers (Fig. 1) [24,25]. An amount of oligomer was charged into a three-necked flask and dried for 2 h at 110°C in vacuo and cooled to room temperature under argon. The oligomers were dissolved in dried DCM, and after addition and dissolution of FAME the system was further cooled to 0°C . Then a DCM solution of DCC and DMAP was added

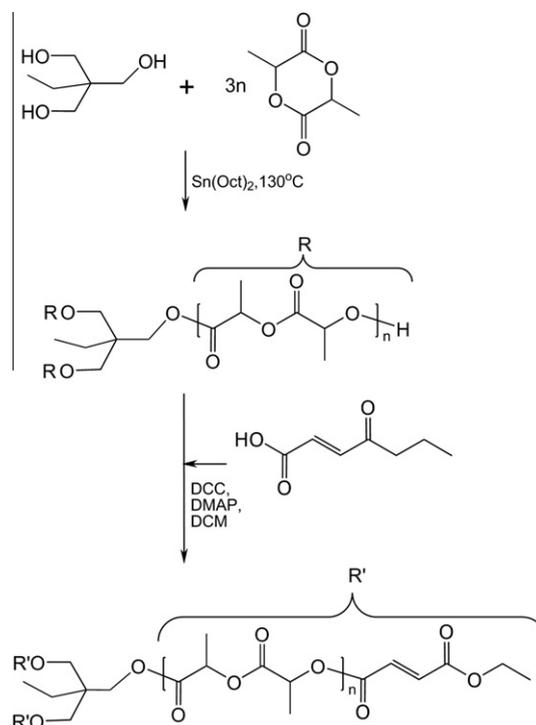


Fig. 1. Synthesis of FAME-functionalized PDLLA oligomers.

drop-wise to the vigorously stirred oligomer solution. In the coupling reaction 1.2 mol of FAME and DCC and 0.03 mol of DMAP were used per mole of hydroxyl endgroups. The coupling reaction was continued overnight, letting the contents slowly warm up to room temperature. After completion of the reaction the formed dicyclohexylurea was removed by filtration. The macromers were purified by precipitation in cold isopropanol, washing with water and freeze-drying.

The macromers are labeled PDLLA 3XMW-FAME, in which 3 is the number of arms (the same for all macromers) and MW is the molecular weight per arm. For example, a PDLLA 3X3K-FAME macromer has a molecular weight of 3000 g mol^{-1} per arm and an overall molecular weight of approximately 9000 g mol^{-1} .

Proton nuclear magnetic resonance ($^1\text{H NMR}$) spectra were recorded in a Varian Inova 300 MHz NMR spectrometer. Deuterated chloroform was used as a solvent. The oligomer and macromer number average molecular weights (M_n) and the degrees of functionalization of the macromers were determined from the spectra.

2.3. Preparation and characterization of PDLLA-FAME/NVP network films

Disk-shaped network specimens were prepared from FAME-functionalized PDLLA oligomers. Macromers were dissolved in NVP at different concentrations (30, 40 and 50 wt.% NVP of the total mass). To each formulation 1 wt.% (with respect to the macromer) of Irgacure 2959 photo-initiator was added. The solutions were poured into a Teflon mold, covered with a thin fluorinated ethylene propylene (FEP) foil, and photo-crosslinked in an Ultralum crosslinking cabinet (365 nm , $3\text{--}5 \text{ mW cm}^{-2}$, 15 min). Disk-shaped samples with a diameter of approximately 10 mm and thickness of approximately 0.5 mm were obtained.

To determine the gel content after crosslinking a network specimen ($n = 3$) was weighed (m_0), extracted with acetone overnight and dried at 90°C until a constant weight was achieved. The mass

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