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In situ forming acyl-capped PCLA–PEG–PCLA triblock copolymer based hydrogels

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ABSTRACT

Sustained intra-articular drug delivery opens up new opportunities for targeted treatment of osteoarthritis. In this study, we investigated the *in vitro* and *in vivo* properties and performance of a newly developed hydrogel based on acyl-capped PCLA–PEG–PCLA specifically designed for intra-articular use. The hydrogel formulation consisted of a blend of polymers either capped with acetyl, or with 2-(2′,3′,5′,-triiodobenzoyl, TIB) moieties. TIB was added to visualize the gel using μCT, enabling longitudinal quantification of its degradation. Blends containing TIB-capped polymer degraded *in vitro* (37 °C; pH 7.4 buffer) through dissolution over a period of ~20 weeks, and degraded slightly faster (~12 weeks) after subcutaneous injection in rats. This *in vivo* acceleration was likely due to active (enzymatic) degradation, shown by changes in polymer composition and molecular weight as well as the presence of macrophages. After intra-articular administration in rats, the visualized gel gradually lost signal intensity over the course of 4 weeks. Good cytocompatibility of acetyl-capped polymer based hydrogel was proven *in vitro* on erythrocytes and chondrocytes. Moreover, intra-articular biocompatibility was demonstrated using μCT-imaging and histology, since both techniques showed no changes in cartilage quality and/or quantity.

1. Introduction

Osteoarthritis (OA) is a common joint disease that affects approximately 30% of the elderly population [1]. Current treatment is mainly based on pain prevention through orally administered drugs, as often non-steroidal anti-inflammatory drugs [2], since disease modifying drugs (DMOADs) are not (yet) available. The development and application of DMOADs are hindered by the fact that it is difficult to obtain sufficient intra-articular (i.a.) concentrations, while high systemic exposure of some putative drugs leads to unwanted side-effects [2,3]. The best local therapies for OA so far are i.a. injections (of hyaluronan or corticoid steroids), thereby circumventing the problem of sub-therapeutic local drug concentrations. However, i.a. injections also provide limited effects, due to the rapid i.a. drug turnover leading to a fast decline of the local drug concentrations to therapeutically inactive levels. For example, i.a. administration of Kenalog® (triamcinolone acetonide suspension) allows for local delivery [4], but only for a limited period of ~1 week [4–6]. Therefore, often multiple i.a. injections are given [7], leading to the risk of cartilage and joint damage and/or infections [2,5,7,8].

Ideally, a single i.a. injection of a local drug delivery system (DDS) for OA would provide sustained drug concentrations in a joint in a controlled way for at least one month. Suitable drug delivery systems are easy to inject, show high encapsulation efficiency with

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low burst release, tunable release kinetics, and full recovery of the loaded drug. Ideal systems release a drug of interest for weeks while maintaining a therapeutically effective concentration at the target site. DDSs developed for i.a. use have, up to now, been mainly based on liposomes or polymeric nano/microparticles [9,10]. Liposomes for local intra-articular treatment (and other non-vascular routes) usually show a short drug release duration which is hardly adjustable [11], although biological stability of liposomes can be improved by surface PEGylation [12,13]. On the other hand, microparticle-based systems show a more controllable and sustained release [11,14]. The main drawback of both liposome- and microparticle-based systems is their costly and non-straightforward manufacturing mainly because up scaling is challenging [11,15,16].

Alternatively, an *in situ* forming depot containing highly concentrated solutions (400 mg/ml) of the hydrophobic drug celecoxib in poly(ethylene glycol) 400 (PEG_{400}) has recently been developed [17]. Upon i.a. injection PEG_{400} is diluted and celecoxib precipitates/crystallizes, allowing a sustained release by slow dissolution (~10 days). This shows the potential i.a. use of *in situ* forming depots, but modulating release rates and durations using this system are not possible [18]. Temperature-responsive gelling systems (composed of ABA triblock copolymers with a PEG middle block flanked by polyester blocks of divers compositions, dispersed in aqueous medium) do meet desirable DDS properties including high encapsulation efficiency, low burst and good drug recovery. They can be injected as a solution and transform into a gel after injection [19–24]. Moreover, terminal hydroxyl-end group modification of the polyester–PEG–polyester triblock copolymers enables further modulation of rheological and degradation/dissolution properties of aqueous and temperature-responsive gelling systems [25,26]. Indeed, we previously showed that the rheological properties of aqueous systems containing acyl-capped poly(ε-caprolactone-*co*-lactide)-*b*-poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-lactide) (PCLA–PEG–PCLA) are easy to modulate [27].

Getting more insight into the *in vivo* behavior of DDSs is a crucial step towards clinical applications for treatment in a joint. In that respect, it is pivotal to determine whether a DDS forms a depot that is retained at the injection site and to investigate its degradation kinetics. Fluorescent particles [28,29] and dye-loaded microparticles [28] are examples of visible DDSs used previously. For both systems, the initial distribution of the particles after administration can be visualized, however due to diffusion and release of the dyes from the DDSs, biodegradation kinetics cannot be investigated. To circumvent this problem, dyes have been covalently bound to polymeric particles [30]. This method facilitates visualization, but only through *ex vivo* sectioning, hence lacking longitudinal follow-up possibilities of particle quantification and spatiotemporal distribution. Non-invasive *in vivo* imaging of DDSs can be achieved by, for instance, computed tomography (CT) using iodine containing moieties that confer a degree of X-ray opacity [31,32]. Indeed, others have shown that different systems, for instance polymethacrylate-based microparticles [33] and hydrogels [34], containing covalently bound 2-(2′,3′,5′,-triiodobenzoyl) moieties (TIB) are suitable for *in vivo* visualization.

In the current study, we investigated the potential of biodegradable, temperature-responsive gelling systems made of an aqueous acyl-capped PCLA–PEG–PCLA triblock copolymer dispersion for *in vivo* use, and in particular i.a. application. Primarily, we assessed the real time degradation kinetics of the gel both *in vitro* and *in vivo* (subcutaneous and i.a.) in a non-invasive manner. For this, we modified the hydroxyl end groups of PCLA–PEG–PCLA with 2-(2′,3′,5′,-triiodobenzoyl) moieties (TIB) to obtain radiopaque gels suitable for long-term *in vivo* visualization using μCT. Secondly, the cytocompatibility and i.a. biocompatibility of the gels were tested.

2. Methods and materials

2.1. Materials

L-Lactide was obtained from Purac Biochem BV, The Netherlands. Hexabrix 320®, a clinical iodine-based contrast agent, was obtained from Guerget, The Netherlands. All other chemicals were obtained from Aldrich and used as received.

2.2. Synthesis of TIB chloride

2-(2′,3′,5′,-Triiodobenzoyl) (TIB) chloride was synthesized as described previously [35]. Briefly, 2,3,5-triiodobenzoic acid (10.5 g; 21 mmol) was dissolved in dichloromethane (100 ml), followed by the addition of a catalytic amount of dimethylformamide (10 mg, 0.14 mmol). An excess of oxalyl chloride (10 ml; 79 mmol, –COCl/COOH = ~8 mol/mol) was added drop wise and the mixture was stirred for two days at room temperature. Volatiles were evaporated under reduced pressure and the remainder was stripped with toluene three times to yield 9.3 g (85%) of TIB chloride. Characterization of TIB chloride dissolved in $CDCl_3$ was done with ^{1}H NMR using a Varian Oxford, operating at 300 MHz. ^{1}H NMR spectra were referenced to the signal of chloroform at 7.26 ppm.

2.3. Synthesis of acetyl-capped ($PCLA_{2\times1700}CL_{2.5}Acet$) and TIB-capped PCLA–PEG–PCLA ($PCLA_{2\times1700}CL_{2.5}TIB$ and $PCLA_{2\times750}CL_{5.7}TIB$) polymers

The acetyl-capped and TIB-capped PCLA–PEG–PCLA triblock copolymers ($PCLA_{2\times1700}CL_{2.5}Acet$ and $PCLA_{2\times1700}CL_{2.5}TIB$, respectively) used in this study were essentially synthesized and characterized as described previously [27]. In short, L-lactide and ε-caprolactone dissolved in toluene were polymerized with PEG_{1500}-diol as a macroinitiator in the presence of tin(II) 2-ethylhexanoate as a catalyst. The exact amounts used in the synthesis are summarized in Table 1. For $PCLA_{2\times1700}CL_{2.5}$ and $PCLA_{2\times750}CL_{5.7}$, a caproyl/lactoyl (CL/LA) molar ratio of 2.5/1 and 5.7/1 mol/mol, respectively were used. Subsequently, acylation of the hydroxyl end-groups using an excess of acetyl chloride or TIB chloride (ratio chloride/OH groups = 4 mol/mol) resulted in the formation of acetyl-capped and TIB-capped PCLA–PEG–PCLA respectively, with a yield of ~85%.

TIB-end group: 8.40–7.60 ppm ($I_{8.0}$, m, 2H, Ar H) [36–38].

Acetyl-end group: 2.14–2.12 ppm ($I_{2.13}$, C**H$_3$**–CO–O–CH(CH_3)–); 2.03–2.05 ppm ($I_{2.04}$, C**H$_3$**–CO–O–$(CH_2)_5$–) [26,39–42] and 2.10–2.08 ppm ($I_{2.09}$, C**H$_3$**–CO–O–$C(H_2)_2$–O–) as shown in Fig. S1.

The composition of acyl-capped PCLA–PEG–PCLA was established from integral of signals belonging to methine protons of LA subdivided in four quadruplets ($I_{5.1}$), methylene protons of PEG ($I_{3.6}$ at 3.72–3.55 ppm), methylene protons of CL subdivided in two triplets ($I_{2.4} + I_{2.3}$). The degree of modification was calculated from the ratio between protons of the end groups and methylene protons of PEG ($I_{3.6}$ at 3.72–3.55 ppm). TIB-end group ($I_{8.0}$) at 8.40–7.60 ppm ($I_{8.0}$, m, 2H, Ar H) [36–38] as previously described [25].

Table 1
Characteristics of acetyl-capped and TIB-capped PCLA–PEG–PCLA triblock copolymers.

Polymer	Acetyl-capped $PCLA_{1700}$–PEG_{1500}–$PCLA_{1700}$	TIB-capped $PCLA_{1700}$–PEG_{1500}–$PCLA_{1700}$	TIB-capped $PCLA_{750}$–PEG_{1500}–$PCLA_{750}$
Abbreviation	$PCLA_{2\times1700}CL_{2.5}Acet$	$PCLA_{2\times1700}CL_{2.5}TIB$	$PCLA_{2\times750}CL_{5.7}TIB$
PEG feed [g]	50	50	50
ε-Caprolactone feed [g]	88	88	45
Lactide feed [g]	10	10	5
Acetyl chloride feed [g]	10	0	0
TIB chloride feed [g]	0	9.3	9.3
Aimed $M_{n,PCLA}$ [g mol^{-1}]	4900	4900	3200
PCLA/PEG[a]	2.1/1	2.1/1	1.1/1
CL/LA [mol/mol][b]	4.9/1	5.7/1	4.4/1
DM [%][c]	93	90	90
M_n[d] [g mol^{-1}]	4700	3100	3400
PDI[e]	1.4	1.2	1.3

[a] Weight ratio of PCLA to PEG determined by ^{1}H NMR.
[b] Weight ratio of ε-caprolactone to L-lactide determined by ^{1}H NMR.
[c] Degree of modification represents the number of end groups per triblock copolymer determined by ^{1}H NMR.
[d] M_n determined by GPC.
[e] Polydispersity index determined by GPC.