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Degradation, intra-articular retention and biocompatibility of monospheres composed of [PDLLA-PEG-PDLLA]-b-PLLA multi-block copolymers

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ABSTRACT

In this study, we investigated the use of microspheres with a narrow particle size distribution ('monospheres') composed of biodegradable poly(DL-lactide)-PEG-poly(DL-lactide)-*b*-poly(L-lactide) multiblock copolymers that are potentially suitable for local sustained drug release in articular joints. Monospheres with sizes of 5, 15 and 30 μm and a narrow particle size distribution were prepared by a micro-sieve membrane emulsification process. During *in vitro* degradation, less crystallinity, higher swelling and accelerated mass loss during was observed with increasing the PEG content of the polymer. The monospheres were tested in both a small (mice/rat) and large animal model (horse). *In vivo* imaging after injection with fluorescent dye loaded microspheres in mice knees showed that monospheres of all sizes retained within the joint for at least 90 days, while the same dose of free dye redistributed to the whole body within the first day after intra-articular injection. Administration of monospheres in equine carpal joints caused a mild transient inflammatory response without any clinical signs and without degradation of the cartilage, as evidenced by the absence of degradation products of sulfated glycosaminoglycans or collagen type 2 in the synovial fluid. The excellent intra-articular biocompatibility was confirmed in rat knees, where μCT-imaging and histology showed neither changes in cartilage quality nor quantity. Given the good intra-articular retention and the excellent biocompatibility, these novel poly(DL-lactide)-PEG-poly(DL-lactide)-*b*-poly(L-lactide)-based monospheres can be considered a suitable platform for intra-articular drug delivery.

Statement of Significance

This paper demonstrates the great potential in intra-articular drug delivery of monodisperse biodegradable microspheres which were prepared using a new class of biodegradable multi-block copolymers and a unique membrane emulsification process allowing the preparation of microspheres with a narrow particle size distribution (monospheres) leading to multiple advantages like better injectability, enhanced reproducibility and predictability of the *in vivo* release kinetics. We report not only on the synthesis and preparation, but also *in vitro* characterization, followed by *in vivo* testing of intra-articular biocompatibility of the monospheres in both a small and a large animal model. The favourable intra-articular biocompatibility combined with the prolonged intra-articular retention (>90 days) makes these monospheres an interesting drug delivery platform. What should

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also be highlighted is the use of horses; a very accurate translational model for the human situation, making the results not only relevant for equine healthcare, but also for the development of novel human OA therapies.

1. Introduction

To this date, disease modifying drugs are not available for the treatment of OA (osteoarthritis), the most common joint disease [1] and the current treatment is mainly based on pain prevention/reduction with orally administered drugs, often non-steroidal anti-inflammatory drugs (NSAIDs)[2]. Due to the chronic nature of OA in combination with the short half-life and poor distribution of drugs in general, and in this case more specifically NSAIDs to the joint [3–5], oral medication has to be taken daily for a long period and in high dosages. This high systemic exposure can in its turn lead to unwanted side-effects [2,6]. To circumvent these adverse effects, intra-articular injections (mainly hyaluronan or corticosteroids) have been used quite extensively in clinical practice, but this route of drug delivery has as a major drawback that the injected substance is rapidly released from the joint with daily synovial fluid turnover [7–9], making multiple injections necessary [7]. Ideally, a single intra-articular injection of a local drug delivery system (DDS) for OA would provide sustained drug concentrations in the injected joint in a controlled way for a longer period of action. Drug delivery systems can significantly improve pharmacokinetics of therapeutic compounds, which is especially relevant for the treatment of chronic diseases and for compounds with a narrow therapeutic window, since systemic plasma concentrations can be reduced with concurrent reduction of undesirable side-effects [10,11]. Moreover, the use of registered drugs and drug candidates, which generally are inactivated and eliminated from the body before even entering the joint, would benefit for therapeutic outcome when administered locally to the tissue of interest. Biodegradable DDSs are attractive for clinical applications since their degradation products are eliminated via metabolic routes and/or excreted by the kidneys, obviating the need for surgical removal. To date, several biodegradable DDSs for intraarticular delivery have been developed, including liposomes, hydrogels and polymeric nano/microparticles [12–15]. Poly(DL-lactide) (PDLLA) and poly(DL-lactide-co-glycolide) (PLGA) are the most widely used biodegradable polyesters for use in sustained release microparticles. However, a limitation of PDLLA and PLGA is that acidic degradation products that are formed upon hydrolysis of the ester bonds accumulate in the polymer matrix due to which the in situ pH in the microparticles may drop significantly [16,17]. The acidic micro-environment has been reported to negatively impact the stability of pH sensitive therapeutic agents such as proteins [18] and cause irregular release profiles of encapsulated actives [19] as well as dose-dumping of acidic degradation products which may evoke significant foreign body reactions [20]. Controllable and sustained drug release has been observed with microparticle-based systems prepared by different manufacturing processes [12,21], where emulsification/solvent evaporation is the most commonly used method [22–24]. Nevertheless, the difficulty to control particle size with this technique and the broad particle size distribution of the obtained microspheres leads to difficulties in formulation reproducibility and poor injectability [25,26].

Microsieve membrane emulsification allows the preparation of uniformly sized particles with average size ranging from tens of nanometers to several hundreds of micrometers [27,28]. The advantages of membrane emulsification include 1) excellent control over the particle size and narrow particle size distribution and 2) mild process conditions as no shear forces are needed to form the droplets. Due to the absence of coarse particles, which could potentially block the injection needle, monospheres can be administered less painfully compared to polydisperse microspheres since smaller injection needles can be used [25,29]. Monospheres also lack the presence of a fraction of very small microspheres which can induce particle-induced immunoactivation [25]. Furthermore, size uniformity enables the microspheres to deliver a more precise amount of drug per microsphere, optimization of the drug release kinetics and hence more reproducible and predictable *in vivo* pharmacokinetics [30,31]. The Shirasu Porous Glass (SPG) membranes have been widely used to prepare uniformly sized microparticles [32]. Microsieve™ emulsification is an alternative membrane emulsification technique preparation of monodisperse microspheres (monospheres™) [33]. Contrary to other membrane-based droplet and particle production methods, the droplet size (and thus the particle size) is solely determined by the membrane design and independent of other process parameters. As a consequence, scalability of the process is straightforward and can be achieved by simply increasing the number of pores of the microsieve membrane or by adding more microsieves to the process.

In the present study we used a series of novel phase separated poly(DL-lactide)-PEG-poly(DL-lactide)-*b*-poly(L-lactide) multi-block copolymers ([PDLLA-PEG-PDLLA]-*b*-PLLA) obtained by polymer chain extension of telechelic poly(L-lactide) diol (PLLA) and poly(DL-lactide)-PEG-poly(DL-lactide) diol (PDLLA-b-PEG-b-PDLLA) with 1,4 butanediisocyanate [34]. To prepare microspheres with a narrow size distribution ('monospheres') by means of microsieve membrane emulsification. By varying the ratio of the rigid, semi-crystalline poly(L-lactide) blocks (PLLA) and the soft amorphous poly(DL-lactide)-PEG-poly(DL-lactide) blocks, the hydrophilicity and swelling degree of these multi-block copolymers can be tailored, which allows control over drug release kinetics. Drug release from [PDLLA-PEG-PDLLA]-*b*-PLLA multi-block copolymers is generally controlled by diffusion through the swollen polymer network, which is in contrast to PDLLA and PLGA polymers, where release is in general controlled by degradation of the polymer matrix, Another advantage of the [PDLLA-PEG-PDLLA]-*b*-PLLA multi-block copolymers is that, due to swelling of the polymer matrix, acidic degradation products do not accumulate in the polymer matrix, but are released instead, leading to the preservation of a less acidic micro-environment as compared to PLGA or PDLLA polyesters.

Besides, due to the hydrophilic and swellable nature of the [PDLLA-PEG-PDLLA]-*b*-PLLA multi-block copolymers used in the present study, it is expected that acidic degradation products will not accumulate in the polymer matrix [35], which is anticipated to positively contribute to the preservation of the integrity and bioactivity of the encapsulated therapeutic agents [16,36,37].

In the current study, we investigated the suitability of monospheres composed of biodegradable [PDLLA-PEG-PDLLA]-*b*-PLLA multi-block copolymers with different block ratios as a platform for local intra-articular drug delivery. Intra-articular retention and biocompatibility of these monospheres were assessed in rodents (mice and rats) as well as horses. Like humans, horses suffer from OA, with up to 60% of equine lameness being OA-related