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Celecoxib-loaded PEA microspheres as an auto regulatory drug-delivery system after intra-articular injection

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ABSTRACT

In this study, we investigated the potential of celecoxib-loaded polyester amide (PEA) microspheres as an auto-regulating drug delivery system for the treatment of pain associated with knee osteoarthritis (OA). Celecoxib release from PEA microspheres and inflammation responsive release of a small molecule from PEA was investigated *in vitro*. Inflammation responsive release of a small molecule from PEA was observed when PEA was exposed to cell lysates obtained from a neutrophil-like Hl-60 cell line. Following a short initial burst release of ~15% of the total drug load in the first days, celecoxib was slowly released throughout a period of >80 days. To investigate biocompatibility and degradation behavior *in vivo*, celecoxib-loaded PEA microspheres were injected in OA-induced (ACLT + pMMx) or contralateral healthy knee joints of male Lewis rats. Bioactivity of celecoxib from loaded PEA microspheres was confirmed by PGE_2 measurements in total rat knee homogenates. Intra-articular biocompatibility was demonstrated histologically, where no cartilage damage or synovial thickening and necrosis were observed after intra-articular injections with PEA microspheres. Degradation of PEA microspheres was significantly higher in OA induced knees compared to contralateral healthy knee joints, while loading the PEA microspheres with celecoxib significantly inhibited degradation, indicating a drug delivery system with auto regulatory behavior. In conclusion, this study suggests the potential of celecoxib-loaded PEA microspheres to be used as a safe drug delivery system with auto regulatory behavior for treatment of pain associated with OA of the knee.

1. Introduction

Osteoarthritis (OA) is the most common form of arthritis and constitutes a large medical healthcare economic burden worldwide, leading to pain and physical disability [1]. Systemic treatment with non-steroidal anti-inflammatory drugs (NSAIDs) has shown to provide effective pain relief in patients with knee OA, but its systemic use is associated with gastro-intestinal and cardiac adverse effects [2,3]. This limits its potential use in a chronic disease such as OA, where long term treatment is required. Therefore, an intra-articular drug delivery system (DDS) is necessary, which circumvents side effects associated with systemic treatment and allows prolonged local drug residence time [4]. Because OA is a chronic disease characterized by a variation in inflammation intensity [4] an attractive therapeutic approach would be intra-articular injection with a DDS which is inflammation-responsive. Loading an inflammation-responsive DDS with an anti-inflammatory drug may result in an auto regulatory DDS: the level of inflammation will impact degradation of the DDS and drug release, quenching inflammation, decreasing degradation and thus dosing the release.

Importantly, a DDS which can be used as an intra-articular treatment for OA should present a set of properties [5]. Firstly, the DDS should be responsive to the osteoarthritic disease process and able to slowly release a drug throughout time. Secondly, the DDS should be biocompatible and able to safely degrade in a knee joint. Finally, drug release from the DDS would be desired to slow the rate of its own release.

A candidate for such a DDS with auto regulatory behavior is a polyester amide (PEA) based injectable microsphere formulation. PEA polymers are based on α-amino acids, aliphatic dicarboxylic acids, and aliphatic α-ω diols [6]. The presence of amino acids in PEA makes it susceptible to enzymatic degradation by proteolytic enzymes. Several studies have reported this mechanism of degradation of PEA by enzymes such as α-chymotrypsin, elastase, papain and protease K, which are enzymes belonging to the serine protease family [7–11]. Since serine

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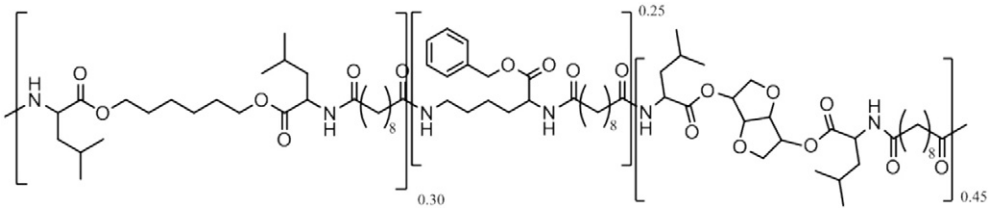

Fig. 1. Structure of PEA III Ac Bz, random copolymer consisting of building blocks A, B and C.

proteases are present in synovial fluid and a key component of the inflammatory response, drug release from a PEA based DDS is potentially reactive to the disease process in inflammation related conditions such as OA [12,13].

A candidate anti-inflammatory drug to incorporate into PEA microspheres is the COX-2 inhibitor celecoxib, which is an anti-inflammatory drug that has been shown to be an effective analgesic for OA related pain [14]. Celecoxib, when administered systemically, has been reported to raise the risk for cardiovascular events, however incorporating the drug in a PEA based DDS for intra-articular administration can circumvent these side effects [15]. PEA has already been demonstrated to have a good biocompatibility, is applied clinically in drug eluting stents and is being investigated for ophthalmologic indications [16,17]. However its use in the treatment of arthritic diseases has not yet been described.

In this study, we investigated the use of PEA microspheres as an auto-regulatory intra-articular DDS in OA treatment. First, we examined inflammation-responsive release of a small molecule from PEA *in vitro* in the presence or absence of a serine protease inhibitor. Next, biocompatibility, degradation and effects on OA progression of celecoxib-loaded PEA microspheres were investigated in experimental OA *in vivo*.

2. Material & methods

2.1. Synthesis of polymer and preparation of PEA microspheres

PEA was synthesized in accordance to procedures reported previously [16,18,19]. The selected PEA is depicted on Fig. 1 and it comprises three types of building blocks randomly distributed along the polymer chain. Polymer characterization can be found in Table 1. For the preparation of microspheres, PEA was dissolved in dichloromethane (Merck Millipore). 5 wt% celecoxib was added to the solution and homogenized by sonication. The suspension was added to 20 mL of cold water containing 1 wt% of poly(vinyl alcohol) (Sigma Aldrich) under high shear, using an ultra-Turrax. After a stable suspension was obtained the particles were let to harden in 100 mL of water containing 1 wt% of poly(vinyl alcohol) for 12 h. Excess of water and surfactant was removed by rinsing and centrifugation. Finally, particles were frozen, dried under vacuum and stored at −15 °C until being used.

2.2. Determining particle size, particle morphology and loading efficiency of celecoxib in PEA microspheres

Size distribution of particles was measured using a Malvern Mastersizer 2000. Morphological examination of PEA microspheres was performed using SEM (Versa 3D FEG-ESEM). For determination of celecoxib loading efficiency, 10 mg of freeze dried microparticles were weighed and dissolved in methanol and shaken until a clear solution was obtained. Next, samples were subjected to analysis with High Performance Liquid Chromatography (HPLC) using a Waters e2695 Alliance HPLC with a UV detector. The method was obtained from the pharmacopeia collection.

2.3. Release kinetics of PEA based celecoxib-loaded microspheres in vitro

For the evaluation of the *in vitro* release kinetics of celecoxib from PEA microspheres, at least 10 mg of microspheres were placed in centrifuge tubes and immersed in phosphate buffered saline (PBS) at 37 °C under gentle shaking. After centrifugation, part of the buffer was removed and replaced with fresh buffer at defined time points such as 1 h, 4 h, 1 day, 2 days, 4 days, and 7 days until completion of the release study. The PBS solution with released API was transferred to HPLC vials and stored in the freezer until HPLC analysis.

2.4. Hl-60 cell culture and cell lysate

The promyelocytic cell-line Hl-60 (98070106) was purchased at the European Collection of Cell Cultures (ECACC). Hl-60 cells were cultured in RPMI 1640 medium containing 2 mM L-glutamine (Sigma Aldrich R8758). The culture medium was supplemented with 10% sterile filtered fetal bovine serum (Sigma Aldrich F2442) and 100 U/mL streptomycin and 100 U/mL penicillin. Cells were cultured at 37 °C and 5% CO_2 without any shaking and were passaged every 2–3 days to keep cell concentration between $0.1 * 10^6$ and $1 * 10^6$ cells/mL. During passaging, cells were centrifuged a $300 \times g$ and seeded in fresh full medium at a concentration of $0.1–0.2 * 10^6$ cells/mL.

2.4.1. Viability and cell counting

Cells were counted using the Guava Viacount reagent (Merck Millipore # 4000-0040) on a Guava EasyCyte plus flow cytometer according to the manufacturer's instructions. Viability was also determined within the same method.

2.4.2. Differentiation

Hl-60 cells were differentiated toward neutrophil like cells using the DMSO method. For this method cells were incubated in full medium supplemented with 1.25% sterile filtered DMSO for 5 days. Cells were seeded at a concentration of $0.2 * 10^6$ cells/mL at the start of the differentiation process and reached $\pm 1 * 10^6$ cells/mL after 5 days.

2.4.3. Lysates

Cells were lysed by 3–5 freeze-thaw cycles from liquid nitrogen to a 37 °C water bath. Cells were generally kept for 10 min in liquid nitrogen and 10 min in the water bath. Cell lysis was confirmed by microscopic analysis. The process was repeated till ± 100% lysed cells were obtained.

Table 1
Polymer characterization. The relative ratio between the polymer building blocks was determined by ^{1}H NMR. Tg of the polymer was determined under dry conditions.

	Mn (kDa)	Polydispersity index (PDI)	Glass transition temperature (Tg)	Relative ratio A:B:C
PEA III Ac Bz	55	1.6	57 °C	0.31:0.26:0.43