Journal of Controlled Release 253 (2017) 64–72

Contents lists available at ScienceDirect

Journal of Controlled Release

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

# Prolonged inhibition of inflammation in osteoarthritis by triamcinolone acetonide released from a polyester amide microsphere platform

Imke Rudnik-Jansen [a,1], Sascha Colen [a,1,2], Julien Berard [b], Saskia Plomp [c], Ivo Que [d], Mattie van Rijen [a], Nina Woike [b], Annelies Egas [e], Gerjo van Osch [f], Erik van Maarseveen [e], Ken Messier [b], Alan Chan [d], Jens Thies [b], Laura Creemers [a,*]

[a] Department of Orthopaedics, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands
[b] DSM Biomedical, Koestraat 1, 6167 RA Geleen, The Netherlands
[c] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80163, 3508 TD Utrecht, The Netherlands
[d] Department of Radiology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands
[e] Division Laboratory and Pharmacy, Clinical Pharmacy, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands
[f] Department of Orthopaedics & Otorhinolaryngology, Erasmus Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

ARTICLE INFO

*Article history:*
Received 6 January 2017
Received in revised form 6 March 2017
Accepted 7 March 2017
Available online 9 March 2017

*Keywords:*
Osteoarthritis
Microspheres
Corticosteroids
Intra-articular
Inflammation

ABSTRACT

Controlled biomaterial-based corticosteroid release might circumvent multiple injections and the accompanying risks, such as hormone imbalance and muscle weakness, in osteoarthritic (OA) patients. For this purpose, microspheres were prepared from an amino acid-based polyester amide (PEA) platform and loaded with triamcinolone acetonide (TAA). TAA loaded microspheres were shown to release TAA for over 60 days in PBS. Furthermore, the bioactivity lasted at least 28 days, demonstrated by a 80–95% inhibition of $PGE_2$ production using TNFα-stimulated chondrocyte culture, indicating inhibition of inflammation. Microspheres loaded with the near infrared marker NIR780-iodide injected in healthy rat joints or joints with mild collagenase-induced OA showed retention of the microspheres up till 70 days after injection. After intra-articular injection of TAA-loaded microspheres, TAA was detectable in the serum until day seven. Synovial inflammation was significantly lower in OA joints injected with TAA-loaded microspheres based on histological Krenn scores. Injection of TAA-loaded nor empty microspheres had no effect on cartilage integrity as determined by Mankin scoring. In conclusion, the PEA platform shows safety and efficacy upon intra-articular injection, and its extended degradation and release profiles compared to the currently used PLGA platforms may render it a good alternative. Even though further *in vivo* studies may need to address dosing and readout parameters such as pain, no effect on cartilage pathology was found and inflammation was effectively lowered in OA joints.



## 1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease, burdening patients worldwide, with the knee being one of the commonly affected joints [1,2]. The burden for OA is expected to increase, given the rapid increase in prevalence of its risk factors, such as age and obesity [2,3]. The aetiology of OA is still not fully understood, but both cartilage and synovial tissue pathogenesis contribute to this joint disease [4]. Synovial inflammation and the inflammation mediators produced have found to be elevated in OA patients and influences OA processes and symptoms [4–6]. Corticosteroids administered *via* intra-articular (IA) injections are being used to relieve pain in OA patients [7]. Their effects are of limited duration since they are cleared out of the synovial space and human body relatively fast [8–10]. Repeated applications are therefore needed to sustain the pain relief over a longer period of time, although these are associated with adverse side effects, such as infection, muscle weakness and hormone imbalance [7,11–13].

Applying CS in controlled release platforms can prevent fast clearance and overcome the disadvantages of repeated injections. Ideally, biomaterials used for these purposes show little or no local inflammatory responses and have a slow degradation profile. Biodegradable polymers have already been widely used in orthopaedic applications, but foreign body reaction to the biomaterial leading to further tissue degeneration are a safety concern [14–16]. Moreover, most of the platforms described give sustained release over a relative short period of days, compared to the clinical need of months to years. One of the FDA-

* Corresponding author.
*E-mail address:* l.b.creemers@umcutrecht.nl (L. Creemers).
[1] Shared first author.
[2] Present address: Orthopädische Gemeinschaftspraxis Bad Bentheim, Paulinenweg 1, 48455 Bad Bentheim, Germany.

http://dx.doi.org/10.1016/j.jconrel.2017.03.014
0168-3659/

approved biomaterial platforms commonly used for controlled release is poly(lactic-co glycolic acid) (PLGA). PGLA is a commonly used biomaterial to achieve controlled release of drugs in OA [17–20]. Typically *in vivo*, release was demonstrated for 4–32 days [17,20–22]. Recently, in a clinical trial with osteoarthritis patients, a PLGA-based controlled release formulation of TAA resulted in a more profound pain reduction than a single intra-articular bolus injection [23]. However, the analgesic effect from the sustained TAA release was only superior to bolus injections between five and ten weeks after injection. A further disadvantage of PGLA is its degradation into lactic and glycolic acid, with a risk of inducing toxic local pH levels [18,24].

Recently, a polyester amide (PEA) biomaterial platform was described that induced a minor foreign body reaction in the eye, knee joint and the intervertebral disc [25–27]. Furthermore, it has been shown to be biocompatible upon injection in those tissues with slow clearance rates due to the avascular environment, with the eye and intervertebral disc known to be immunoprivileged [28]. This platform is built of amino acid moieties and hence degradation will not result in toxic conditions [29–31]. PEA particle erosion is dependent on serine protease activity and hence no erosion occurs *in vitro* in PBS [32]. More importantly, PEA microspheres are a suitable platform for prolonged release of small molecule drugs such as corticosteroids, as shown by their *in vitro* release of celecoxib of 80 days [27]. In addition, PEA microspheres were injected into a rat degenerative articular joint, a location that is readily accessible to cells from the acquired and the innate immune system and hence more likely to show inflammatory responses to biomaterials. Whether the clinically used corticoid TAA, applied in sustained release, is safe and effective in reducing inflammatory processes still has to be determined. Hence, in the current study, PEA microspheres loaded with TAA were characterized in terms of loading and release *in vitro*, using a cell-based model of induced inflammation [19]. Furthermore, the release of TAA into the systemic circulation and its effects on articular cartilage and synovial lining inflammation in a rat model of osteoarthritis was compared to a bolus of TAA.

## 2. Materials and methods

### 2.1. Preparation and loading of polyester amide microspheres

PEA was synthesized in accordance to procedures reported previously [25,26,29]. For the preparation of microspheres, PEA was dissolved in dichloromethane (Merck Millipore). 20 wt% triamcinolone acetonide was added to the solution and homogenized by ultrasound. The suspension was added to 20 ml of an aqueous solution containing surfactants for stabilization (1 wt% of poly(vinyl alcohol and 2.5 wt% NaCl, Sigma Aldrich) under high shear, using an ultra-Turrax. After a stable suspension was obtained the particles were allowed to harden in 100 ml of water containing 1 wt% of poly(vinyl alcohol) and 2.5 wt% NaCl for 12 h. Excess of water and surfactant was removed by rinsing and centrifugation. Finally, particles were frozen and dried. Preparation procedures of microspheres loaded with IR-780 iodide are exactly the same, but are loaded with 0.2% w/w of IR 780 iodide instead of TAA and dissolved in DCM. Size distribution of PEA particles was measured by static light scattering and ranged from 8 to 50 μm.

### 2.2. TAA release by PEA microspheres in vitro

#### 2.2.1. In vitro release of TAA in PBS buffer

Release kinetics of TAA from PEA microspheres in PBS buffer were determined by incubation in a volume of 50 ml at 37 °C, of which 45 ml buffer was renewed. Buffer exchange was performed twice the first day, every day up to day 3 and from there every 3–4 days up to day 24. After that, the buffer was renewed on a weekly basis. Size distribution of TAA-loaded particles was determined with Static Light scattering, using a Malvern Mastersizer 2000. Approximately 10 mg of microparticles were accurately weighted and dissolved in appropriate amount of methanol and shaken until a clear solution was obtained. The samples were analysed for TAA concentration by High performance Liquid chromatography (HPLC), using a Waters e2695 Alliance HPLC with UV detector. The method was obtained from the pharmacopoeia collection.

#### 2.2.2. Chondrocyte isolation and culture

Articular cartilage was harvested from knee joints derived from patients undergoing arthroplasty. Anonymous use of redundant tissue from joint surgery used for scientific purposes is part of the standard treatment agreement with patients from the University Medical Center Utrecht [33]. Chondrocytes were isolated by a 3-hour enzymatic digestion in 0.1% pronase (Roche, Mannheim, Germany), followed by an overnight enzymatic digestion in 0.04% collagenase type 2 (Worthington Biochemical, Lakewood, NJ, USA) at 37 °C. Undigested debris was removed using a 70 μm-cell strainer (Becton Dickson, Franklin Lakes, USA). The resulting suspension of cells was washed in PBS and centrifuged. Afterwards, the cells were re-suspended in expansion medium (3000 cells/cm$^2$) consisting of DMEM (Gibco® Life Technologies, Carlsbad, CA, USA) containing 4.5 mg/ml glucose, 0.8 mg/ml glycyl-L-glutamine, 10% foetal bovine serum (FBS) (HyClone® Thermo Fisher Scientific, Waltham, MA, USA), 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco® Life Technologies) and 10 ng/ml basic fibroblast growth factor (bFGF) (R&D Systems, Minneapolis, MN, USA). The cells were cultured at 37 °C and 5% $CO_2$. The culture medium was renewed every 3–4 days. At passage two, cells were frozen in aliquots of 1 million per vial in freezing medium, containing 10% DMSO (Merck) and 20% FBS in DMEM. For each release experiment, cells of the same donor were used at each of the successive time intervals.

#### 2.2.3. Release and bioactivity of TAA from microspheres

One day before the experiment, cells were thawed and seeded onto a 24-well culture plate, at a density of 40,000 cells per well. Cells were cultured in medium containing DMEM (including glucose and glycyl-L-glutamine), 10% FBS and antibiotics. The following day, the medium was renewed before starting the experiment. PEA microspheres, loaded with TAA at 20%, were dispersed in a total of 1 ml culture medium and placed in Transwell® baskets at 8.7 or 0.87 μg per well (0.4 μm pore size, polycarbonate membrane) (Corning, Amsterdam, The Netherlands) over the plated cells. Unloaded microspheres were taken as controls. Cells and microspheres were pre-incubated for 4 h at 37 °C, at 5% $CO_2$ and 95% humidity. Subsequently, tumour necrosis factor alpha (TNFα) (eBioscience, San Diego, CA, USA) was added at a final concentration of 10 ng/ml to the culture medium in all conditions. Cells and microspheres were co-incubated further for another 72 h before the microspheres were transferred to a new 24-well culture plate containing cells seeded according to the procedure described above. This procedure was repeated 9 times accounting to a total release period of 27 days. Cells treated with $10^{-7}$ M or $10^{-8}$ M TAA directly added to the culture medium and continuously renewed, were included as positive control in each time interval. Each condition was analysed in 4 replicates ($n = 4$) and experiments were performed for three different donors. At every 72-hour time point medium was collected and cells were lysed in KDalert™ Lysis Buffer (Ambion®, Life Technologies). Samples were stored at −80 °C until further analysis if not used immediately.

#### 2.2.4. $PGE_2$ release

Cell culture medium was collected at day 3, 6, 9, 12, 15, 18, 21, 24 and 27 and stored at −80 °C; media were brought to room temperature immediately prior to $PGE_2$ measurement. $PGE_2$ was measured using the enzyme immunoassay Prostaglandin $E_2$ Parameter Assay Kit (R&D Systems) following the manufacturer's instructions. Colorimetric intensity was determined using the Benchmark Microplate Reader (Bio-Rad) at 450 nm. The readings were subtracted by those at 540 nm. The concentration of $PGE_2$ in the samples was determined by using a calibration