Squared PLGA microPlates as injectable depot for the management of Post-Traumatic Osteoarthritis

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INTRODUCTION

Osteoarthritis (OA) is a chronic inflammatory joint disease that affects population across all ages. It is caused by progressive breakdown of joint cartilage and underlying bone, resulting in joint pain, and, eventually, permanent disability. Currently, there is no disease-modifying drug available to reverse the progression of the OA and conventional therapies are palliative, providing only a temporary relief from the symptoms[1].

SYNTHESIS AND PHYSICAL-CHEMICAL CHARACTERIZATION OF µPLs

Within this context, a top-down strategy was used for synthetizing squared-poly (D,L-lactide-co-glycolide) (PLGA) microPlates (µPLs) [2,3] for prolonged release of Dexamethasone (DEX)[2] and matrix metalloproteinase 13 (MMP-13) RNA interference nanoparticles (siMMP13-NPs)[3]. A full characterization of the physical-chemical and mechanical properties of both formulations was performed. µPLs exhibited an edge length of 20 µm and a height of 10 µm and a stiffness value of about 3.1 ± 0.9 MPa (Fig. 1).

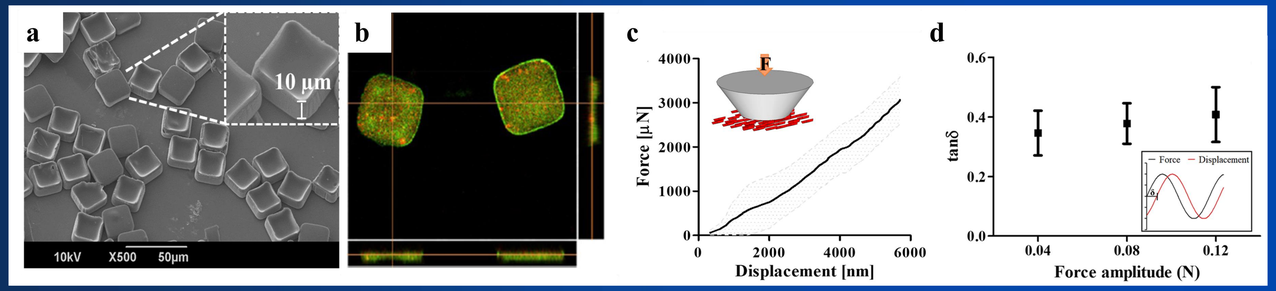

Fig 1. a. SEM images of the empty µPLs released from the PVA template; b. Confocal microscopy image of Cy5-siNP loaded Curcumin-µPLs; c. Force-displacement curve for a flat punch indentation experiment on an ensemble of µPLs (average curve and standard deviation).; d. Energy dissipation ability of µPL upon cyclic mechanical loading (frequency 5 Hz) as a function of the force oscillation amplitude.

BIOPHARMACOLOGICAL CHARACTERIZATION OF µPLS

In addition, the bio-pharmacological properties of µPLs were investigated (Fig. 2). Under the sink conditions, 80% of DEX was released from µPLs within the first 10 days (Fig. 2a). Conversely, under confined conditions, DEX µPLs provided a sustained drug release for several months, with ~20% of DEX released in 1 month (Fig. 2b)[2]. Equally, *in vitro* siMMP13-NP release profile from µPLs in PBS (0.75 mg of µPLs in 1.5 mL) showed prolonged release over the 5-week experiment (Fig 2c and d) [3].

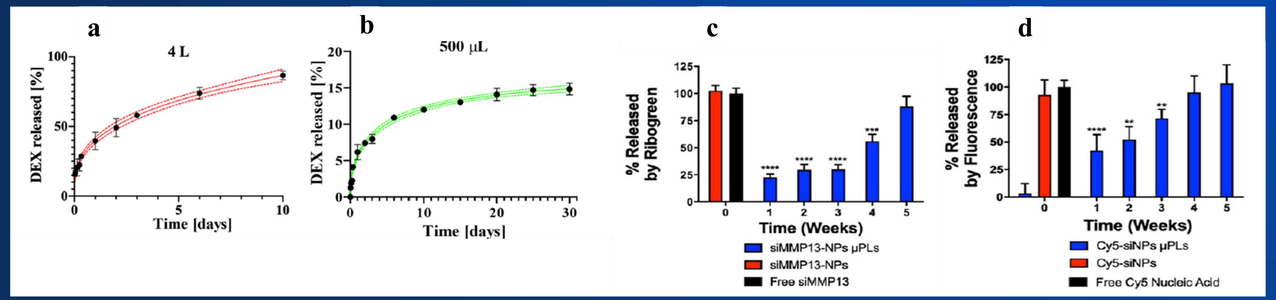

Fig 2. a. DEX release profile from µPLs under sink conditions (4 L, red line) and fit to the Weibull empirical drug release model (red line; 95% confidence margin: red margin); b. DEX release profile from µPLs under confined conditions, mimicking the synovial volume (500 µL, Weibull: green line with 95% confident band); c. siMMP13-NP release profile from µPLs as measured by Quant-iT Ribogreen assay; d. Cy5-siNP release kinetics from µPLs as measured by Cy5 fluorescence.

IN VIVO PHARMACOKINETIC STUDY OF µPLS

To evaluate *in vivo* µPLs intra-articular retention time, Cy5 was conjugated on particles surface. After a single intra-articular injection of Cy5-µPLs into a cohort of mice with mechanically induced osteoarthritis (PTOA), a time course of intravital imaging, *ex vivo* imaging, and confocal microscopy analyses was performed. Fluorescent µPLs were detected in the joint, for up to 30 days (Fig. 3a) and a surface erosion evident by loss of fluorescence (heterogenous) was observed in a significant number of individual Cy5-µPLs at days 25 and 30 as compared to days 1 and 5, as showed in Fig. 3b [2,3].

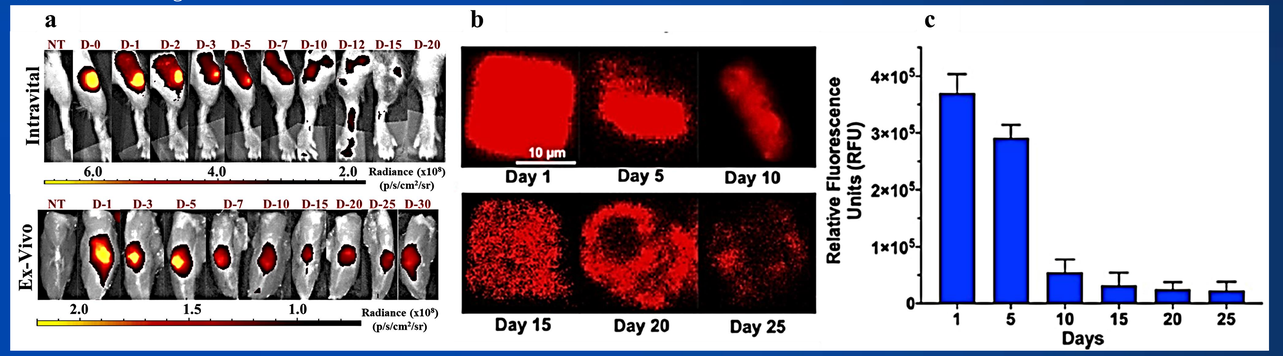

Fig 3 a. Representative pharmacokinetic time course intravital images (skin on) and *ex vivo* knee images (skin off) of Cy5-µPLs injected intra-articularly into PTOA mouse knee joints (D-#, where # represents days after intra-articular injection); **b.** Magnified images of Cy5-µPLs within the joint at multiple time points; c. Quantification of relative fluorescence unites (RFU) of individual Cy5-siNP-µPLs in histological sections over time plotted as mean + SEM.

IN VIVO THERAPEUTIC EFFICACY OF µPLS

The therapeutic efficacy of an intra-articular injection of DEX-µPLs in the PTOA mouse model. In particular, a single injection of DEX-µPLs decreased the expression of IL-1β, TNF-α, IL-6 and MMP-13 by approximately half compared to free DEX at 4 weeks post-treatment (Fig 4a)[2]. In the same animal model, gene silencing efficiency was obtained by siMMP13-NPs released from µPLs for the whole duration of the study (5 weeks), preserving ~50% silencing after 4 weeks (Fig 4b and c).[3].

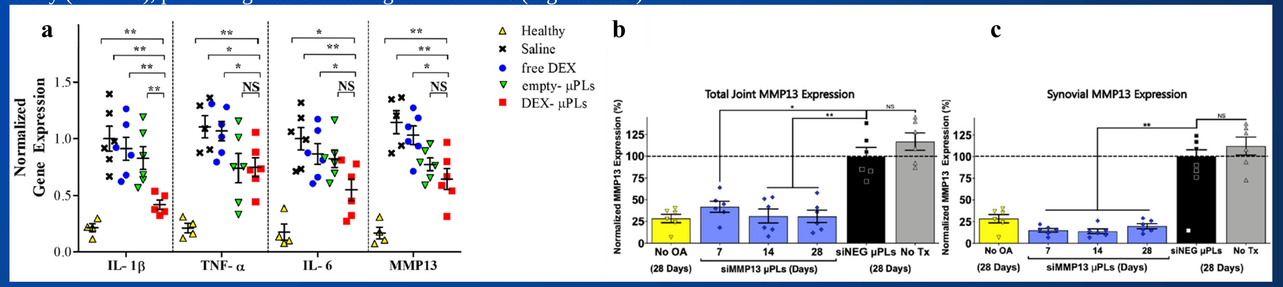

Fig 4. a. ***In vivo expression*** of IL-1β, TNF-α, IL-6, and MMP-13 measured by TaqMan qPCR (for each treatment groups n = 6, while for the healthy group n = 4); b. in vivo longitudinal inhibition of MMP13 and reduction of inflammatory gene expression in the total joint and c. synovium.

CONCLUSIONS

In conclusion, top-down fabrication approach allowed to synthetize shaped-defined µPLs that can act an effective depot system for the local treatment of OA. They provided a sustained drug release for several weeks, reducing inflammation and pain and improving mechanical properties of OA-affected joint.

REFERENCES
1. Martel-Pelletier et al., *Nat Rev Dis Primers*. 2016, 2, 16072.
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3. Bedingfield et al., *ACS nano*, 2021, 15, 9, 14475–14491.