# Optimization of Punicalagin-Releasing In Situ forming Microparticles (PRISM) for Treatment of Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA), characterized by inflammation and degradation of articular cartilage, is a prevalent joint disease (7.6% of the global population in 2020[1]). Many OA patients receive intra-articular injections of corticosteroids or hyaluronic acid, but these treatments are merely palliative. Disease-modifying OA drugs (DMOADs) and systems for sustained drug release are urgently needed. Punicalagin (PUN), the major polyphenol present in pomegranate, is a good DMOAD candidate because it inhibits production of pro-inflammatory cytokines through attenuation of the NF-κB/iNOS/COX-2/TNF-α and MAPK signaling pathways. It also inhibits secreted collagenases such as MMP-13. We hypothesize that punicalagin-releasing in situ forming microparticles (PRISM) are a suitable means of sustained intra-articular drug delivery. The purpose of this study is to optimize the formulation of PRISM based on the in vitro PUN release kinetics, injectability, and microparticle size. The objectives are a) minimal burst release b) slow long-term release rate c) 5-10 µm particle size[2] d) injection force of < 38 N.[3]

METHODS: To make PRISM, a polymer/drug solution is emulsified into sesame oil using a handheld homogenizer. When the emulsion is injected into an aqueous environment, solvent diffuses out of the droplets, and the polymer and drug precipitate and form microparticles. Table 1 shows the factors and levels of the independent variables in the PRISM formulation. Optimization will be performed based on 4 responses that will fit to a quadratic response surface model: 24-hr burst release, long-term release rate, particle size, and injection force. Design of Experiment (DoE) software (Design Expert, Statease 22.0.5) was used to determine the number of batches (runs) required for optimization, and it yielded 25 different PRISM formulations. Each run consisted of approximately 1 mL of polymer solution, with oil volumes of 1, 2, or 4 mL. To determine PUN release kinetics, the emulsions were pipetted into PBS-containing dialysis bags (MW cutoff = 14,000) from which PUN escaped by diffusion into surrounding PBS. The system was maintained in an orbital shaker (100 rpm) at 37 °C, and the PUN release was determined by monitoring the optical absorption at 378 nm. Injectability was determined using a stepper motor driven micromechanical testing machine to expel the emulsion solution. Each emulsified run was pipetted into a 5cc syringe fitted with a 21G needle. Using the micromechanical testing machine, the force required to expel the solution at a constant 0.25 mL/s was recorded. To determine the microparticle size using Dynamic Light Scattering (DLS), the emulsified solution was pipetted into distilled water, which was gently stirred on a stir plate for 24 hours. Some particles were isolated on a 0.2 µm filter for viewing on a Scanning Electron Microscope (SEM).

RESULTS: PUN release kinetics for all 25 runs have been completed (examples in Fig. 1A), while determination of particle size and injectability is ongoing. A one-way ANOVA revealed that polymer concentration ($p$ = 0.0074) and PCL ($p$ = 0.0264) contents had significant effects on burst release of PUN, whereas oil and benzyl benzoate concentrations did not. Burst is negatively correlated with polymer concentration and positively correlated with PCL content. There were no significant model factors regarding long-term release rate; however, there was a trend of faster long-term release with higher PCL concentration ($p$ = 0.08). Injectability tests conducted to date indicate that some formulations fall in an acceptable range whereas some may require an excessive initial force > 64 N (Fig. 1B). Preliminary data for particle size indicates that the majority fall between 100-1000 nm and occasionally exceed 1000 nm (Fig. 1C). SEM shows some spherical and some irregular particles (Fig. 1D).

DISCUSSION: The system is tunable with respect to burst release, which averaged about 15% of the total PUN loaded. The statistical model demonstrates that polymer concentration has the greatest effect on burst release, with higher concentration leading to less burst. On the other hand, burst release increases with increasing PCL content. Tuning long-term release rate has proven to be more challenging; however, results suggest that higher PCL content promotes faster long-term release. Neither water miscibility of the solvent, determined by dioxane: benzyl benzoate ratio, nor polymer concentration significantly affected PUN release. These results contrast with PLGA-based in situ forming implants, PUN release from which was strongly influenced by solvent water miscibility and polymer concentration[4]. A clear picture of injectability has yet to emerge. Preliminary results for 10-15% polymer concentration suggest that a lower concentration or larger needle may be required. Data gathered so far indicate that particle sizes are typically smaller than the desired 5-10 µm, and we will explore less vigorous emulsification procedures.

SIGNIFICANCE/CLINICAL RELEVANCE: This study is significant to the treatment of osteoarthritis with an injectable, slow releasing DMOAD.

REFERENCE: 1. *The Lancet Rheumatology* 2023; 5 (9): e508-e522 2. *Int J Pharm* 2016; 498 (1-2): 119-129 3. *Adv. Healthcare Mater.* 2020 (9): 1901521 4. *Int J Pharm* 2024; 652: 123842

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*Table 1. Factors and levels of independent variables used by DoE for PRISM formulations.*

| | FACTORS | | | |
|---|---|---|---|---|
| | **Polymer Concentration (% w/w)** | **PLGA*: PCL** Ratio** | **Dioxane: Benzyl Benzoate Ratio** | **Oil: Polymer Ratio** |
| **LEVELS** | 5 | 100:0 | 100:0 | 50:50 |
| | 10 | 75:25 | 85:15 | 67:33 |
| | 15 | 50:50 | 70:30 | 80:20 |
| | | 25:75 | | |
| | | 0:100 | | |

*PLGA = poly(lactic-co-glycolic acid), 75:25 lactide:glycolide, ester end-capped, 7,000-20,000 MW

**PCL = polycaprolactone, 14,000 average MW

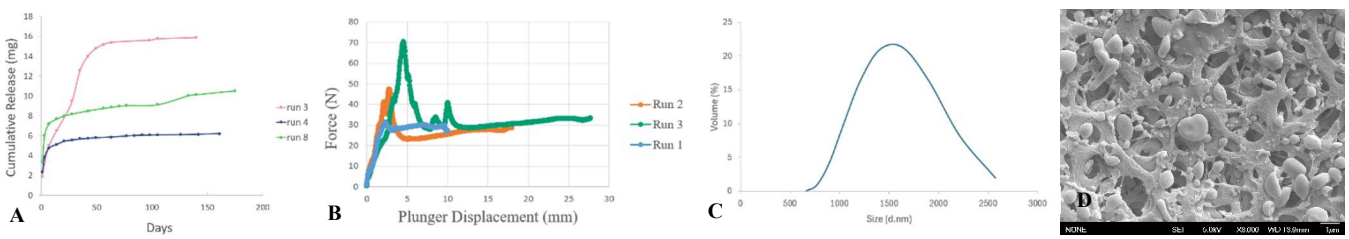


*Figure 1. A) PUN release kinetics of selected Runs; B) Preliminary injection force data; C) Representative DLS data (PRISM Run 3); D) SEM image of PRISM Run 3 (scale bar = 1* µm*). Run 1:15% PCL, dioxane; Run 2:10% PLGA, dioxane/BB 85/15; Run 3 10% PCL/PLGA 75/25, dioxane/BB 85/15*