

# Fluoride-Doped Mineral Coated Microparticle Platform to Prolong Transfection of mRNA in Fracture Repair

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**Disclosures:** Anna Laura Nelson (N), Gianluca Fontana (N), Josh Choe (N), Johnny Huard (N), William Murphy, Nicole Ehrhart (consultant positions for Onkos Surgical Inc. and Ripple Neuromed Inc.), Chelsea S Bahney (ORS, TERMIS, OTA; Iota BioSciences); \*co-submitting

**INTRODUCTION:** The long term goal of this project is to develop an injectable mRNA delivery platform specifically designed to localize therapeutic expression within a fracture site. Reports of non-union have been found to occur up to 14% within tibial shaft fractures in a large study over the course of two years.<sup>1,2</sup> There are currently no pharmaceutical approaches approved to accelerate fracture healing or to treat non-unions. Current standard of care for non-unions involves surgical intervention to enhance stability and/or to promote regeneration through bone grafting. Delivery of mRNA is an attractive strategy recently popularized by the novel coronavirus vaccine that delivers genetic material without genomic integration. Therapeutic platforms incorporating mRNA have undergone translational limitations due to challenges associated with mRNA stability, toxicity of delivery vesicles, and immunogenicity.<sup>3</sup> Mineral coated microparticles (MCM) comprise a core made of  $\beta$ -Tricalcium Phosphate immersed in modified simulated body fluid (mSBF) to create mineral coatings with tunable porosity and degradation rate. MCMs have been shown to reduce cytotoxicity of non-viral vectors, improve transfection efficiency and kinetics.<sup>4</sup> In this study we aimed to test various mineral compositions to optimize mRNA delivery to the fracture. We hypothesize that MCM can serve as the delivery vehicle for mRNA complexes and fluoride doping will enhance transfection.

**METHODS:** Microparticles were biomimetic using mSBF to create the mineral coating as described previously.<sup>4</sup> Fluoride-doped MCM (FMCM) were formulated using fluoride supplementation in the mSBF. All of the procedures listed in this study were approved by our Institutional Animal Care and Use Committee (IACUC) and ARRIVE guidelines will be followed in reporting *in vivo* results. Delivery platforms were tested *in vivo* using a murine tibial fracture model stabilized with an intermedullary pin and all mRNA platforms were injected into the fracture callus 6 days following fracture. Firefly luciferase mRNA was used as a reporter gene at a concentration of 10 ug/mouse for all studies. IVIS imaging was used to locate and quantify firefly luciferase protein. Osteogenic characterization and inflammatory responses were determined by using qRT-PCR for various osteogenic (*collagen X*, *axin2*, *runx2*, *osterix*, *osteocalcin*) and inflammatory markers (*il-1 $\beta$* , *il-4*). Systemic inflammation was measured by analyzing C-Reactive protein (R&D Systems) within the serum. Transfection kinetics and efficiency were studied using Firefly mRNA (TriLink Biotechnology) as a reporter gene and mRNA was isolated at various timepoints following delivery. Firefly luciferase was used to measure the level of transfection and Hall Brundt's Quadruple (HBQ) Stain was used to assess platform interference with fracture healing.

**RESULTS:** To determine MCM's capabilities for enhancing transfection into a fracture callus, mRNA encoding for firefly luciferase was delivered in Lipofectamine alone or complexed to MCM/FMCM. We first tested whether MCM and FMCM formulations could alter inflammatory or osteogenic markers locally within the fracture callus. Despite no significant differences, FMCM was found to increase pro-inflammatory markers, anti-inflammatory markers and all osteogenic markers tested (**FIG 1**). Despite the increase seen in local inflammatory markers, no significant differences were found between any of treatment groups when testing for systemic inflammation through C-Reactive Protein (CRP) (**FIG 1C**). As there was a slight increase in osteogenic genes within the FMCM group, histomorphometric principles were used to quantify bone and cartilage composition within the fracture callus both 2 and 8 days after treatments (**FIG 2**). No significant differences between any of the treatment groups after 8 days (**FIG 2B-C**). An IVIS imaging time course shows FMCM prolonged signal *in vivo* as compared to Lipofectamine (**FIG 3**).

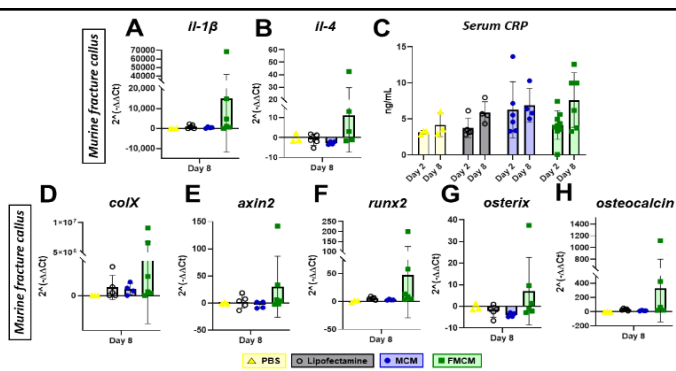
**DISCUSSION:** In this study, we aimed to develop MCMs as a delivery platform to improve therapeutic mRNA delivery for fracture repair. Here we show, that fluoride doped MCM prolongs expression of mRNA within a fracture callus, does not alter or modulate systemic inflammation following treatment and may promote osteogenesis when delivered locally. Despite the slight trend seen with FMCM enhancing osteogenic genes, it did not appear to hinder or accelerate fracture repair. Future directions of this study should perform an FMCM dose response to determine if increased doses accelerate fracture repair while maintaining minimal cytotoxic effects.

**SIGNIFICANCE/CLINICAL RELEVANCE:** There exists an unmet clinical need to stimulate bone regeneration through a non-surgical delivery platform. In order to combat the precedence of non-union and delayed healing in fractures, we are developing FMCMs as an injectable delivery platform to deliver candidate mRNA locally to the fracture callus to prolong mRNA expression, minimize cytotoxicity and to promote osteogenesis locally.

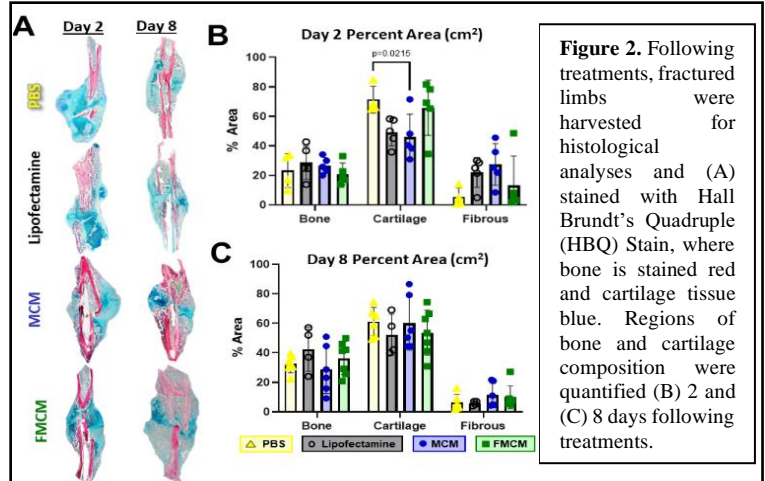
**REFERENCES:** 1). Collaborators\*, G.F., The Lancet Healthy Longevity, 2021. 2). Cauley, J.A., The global burden of fractures. Lancet Healthy Longev, 2021. 3). Sultana, N. et al. Optimizing Cardiac Delivery of Modified mRNA, 2017. 4). Fontana, G. et al. Mineral-Coated Microparticles Enhance mRNA-Based Transfection.

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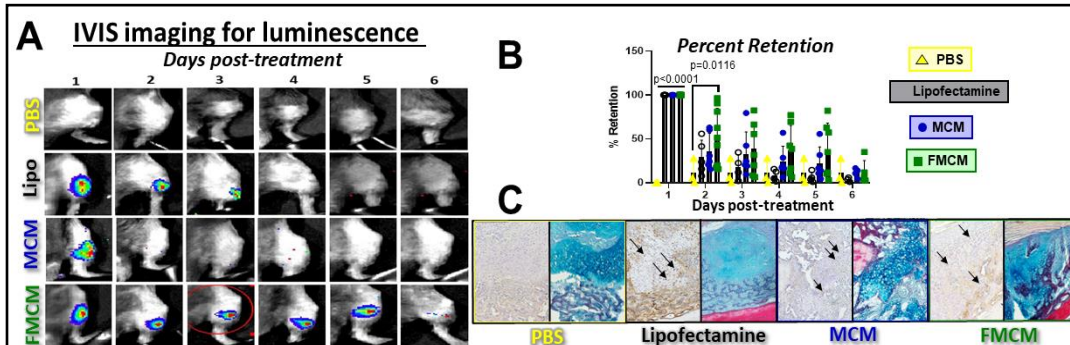
## IMAGES AND TABLES:



**Figure 1.** RNA expression within the murine fracture callus following treatments for inflammatory markers (A-B), and osteogenic markers (D-H). CRP was measured to analyze systemic inflammatory responses (C).



**Figure 2.** Following treatments, fractured limbs were harvested for histological analyses and (A) stained with Hall Brundt's Quadruple (HBQ) Stain, where bone is stained red and cartilage tissue blue. Regions of bone and cartilage composition were quantified (B) 2 and (C) 8 days following treatments.



**Figure 3.** Firefly luciferase was used as a reporter gene to track and measure expression using (A) IVIS imaging. FMCM was shown to have prolonged signal as compared to other treatment groups (B). Firefly IHC and adjacent HBQ slides showed that cells within cartilage and mesenchyme expressed the mRNA (C).