Folic acid conjugated and zoledronic acid loaded microparticles for targeted drug delivery to activated macrophages and treatment of osteoarthritis

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Statement of Purpose: Osteoarthritis (OA), a disease caused by chronic inflammation and wearing of articular cartilage, affects over 150 million people worldwide **(Thompson 2021)**. Synovial inflammation is now increasingly being recognized as a major contributor to osteoarthritis (OA) progression and pain. Activated synovial macrophages in an OA joint have been shown to overexpress folate receptor-2 (FR-2). Restricted expression of FR-2 in activated macrophages makes it an excellent molecular target for OA drug therapies **(Chen 2020)**. In this study we have identified a population of activated macrophages specifically expressing FR-2 and designed folic acid conjugated and zoledronic acid loaded microparticles for targeted drug delivery to activated macrophages. Bisphosphonates such as zoledronic acid (ZA) are known to slow-down/stop metabolic diseases such as OA. However, ZA is rapidly cleared from the body before it can exert its therapeutic effects. We hypothesize that encapsulating bisphosphonate nanoparticles in folic acid conjugated microparticles will allow for the drug to remain in the body longer, target the macrophages, and help slow-down and/or stop the progression of OA.

Methods: *Identification of macrophage surface markers:* Macrophages obtained from peripheral blood mononuclear cells (PBMCs) were maintained in folic acid free medium and differentiated with monocyte colony stimulating factor (MCSF) to obtain activated macrophages. Macrophages surface markers were identified using flow cytometry. *Microparticle synthesis and characterization.* Polyethylene glycol – poly (lactic-co-glycolic acid) (PEG-PLGA) microparticles were synthesized through coaxial microfluidic phase separation technique. Needle size, flow rate and concentration of outer fluid polyvinyl alcohol and inner fluid PEG-PLGA dissolved in dichloromethane (DCM) was varied. The microparticles were imaged and sized with ImageJ. Folic acid was conjugated to microparticle surface using amine functionalized PEG-PLGA (NH2-PEG-PLGA) via standard carbodiimide chemistry. Nanoparticle synthesis. Calcium-zoledronic (Ca-Zol) acid nanoparticles were synthesized with reverse microemulsion method and characterized with dynamic light scattering (DLS) transmission electron microscope (TEM). Nanoparticle encapsulation in microparticles. Ca-Zol nanoparticle loaded microparticles were obtained by adding the drug to the DCM used for dissolving PEG-PLGA during microparticle preparation. *In-vitro cellular uptake.* The microparticles were incubated with RAW264.7 macrophage cells and cellular uptake was observed by confocal microscope. *In-vitro cellular release.* The nanoparticle loaded microparticles were placed in a dialysis bag within 0.1M Phosphate Buffered Saline (PBS) buffer and aliquots were collected at different time points. The concentration of ZA released was determined by using inductively coupled plasma – optical emission spectroscopy.

Results: Macrophages cultured in presence of MCSF expressed CD14+ and CD163+ surface markers indicating a M2 macrophage phenotype and most of them (~ 94%) showed co-expression of FR-2 with a mean fluorescent intensity (MFI) of 1124. Size of PEG-PLGA microparticles could be controlled between 2 to 40 µm depending on inner needle gauge and flow rate of the PEG-PLGA phase (**Figure 1A**). The average size of Ca-ZA nanoparticles was 170 nm (**Figure 1B**). Folic acid conjugated and Ca-ZA nanoparticles loaded PEG-PLGA microparticles sustainably released the encapsulated drug over 14 days**.**

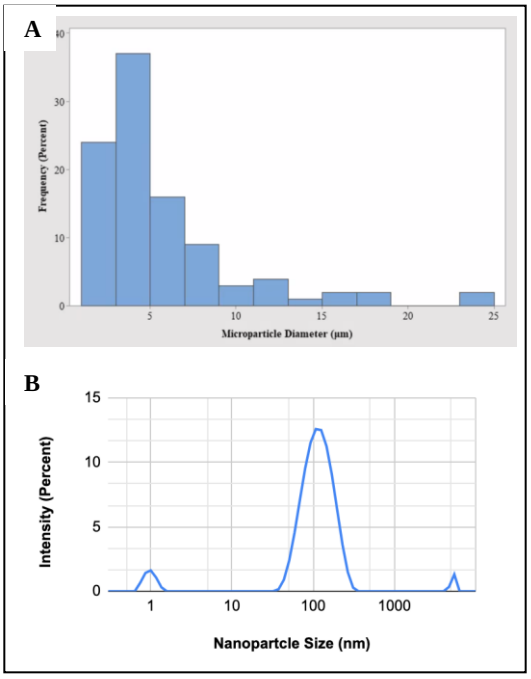

Figure 1. A) A histogram displaying the frequency of microparticle diameters. Microparticles were made using 5% PVA with 10ml/hr flow rate, a 30G needle gauge and 1% PEG-PLGA with 0.1ml/hr flow rate microparticles. **B)** The size distributions of the nanoparticles as obtained by DLS results.

Conclusions: Activated macrophages in the M2 phenotype overexpress FR-2 which can be used for targeted drug delivery. Folic acid conjugated PEG-PLGA microparticles can potentially be used for targeted delivery of encapsulated drugs to synovial macrophages.

References: Chen Y Am J Transl Res. 2020; 12:261-268.; Thomson A. Frontiers in Immunology 2021; 12:1831.