Zoledronate Loaded Nanoparticles in Microparticles: A Potential Macrophage Targeting Delivery System for Osteoarthritis Treatment.

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INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease with no known cure. Mounting evidence indicates that synovial inflammation is linked to OA progression and pain[1]. Thus, modulating inflammation remains a potential disease-modifying strategy for OA therapy. Notably, a sub-population of activated macrophages secretes pro-inflammatory and catabolic mediators that contribute significantly to the inflammatory cascade that drives OA. Given this, targeting these macrophages holds promise for modulating inflammation and, consequently, halting OA progression. Although bisphosphonates (BPs) exhibit strong affinity and toxicity against macrophages[2,3], their rapid clearance from the joint upon injection renders them ineffective for intraarticular delivery. Thus, we developed a novel therapeutic system comprising BP-based nanoparticles loaded into polymeric microparticles functionalized with folic acid for sustain and target delivery of zoledronate (Zol), a potent third-generation BP, to eliminate these disease-driving macrophages involved in OA progression.

METHODS: Microparticles made up of polyethylene glycol- polylactic-co-glycolic acid (PEG-PLGA; Sigma Aldrich) were synthesized via a coaxial flow phase separation method and characterized for size and morphology using an inverted microscope[4]. To form the BP-based nanoparticles, Zol was complexed with calcium via reverse microemulsion method, and the resulting nanoparticles (CaZol NPs) were characterized by dynamic light scattering (DLS) and transmission electron microscope (TEM) for size and zeta potential. The incorporation of nanoparticles into microparticles (CaZol NiM) followed a process similar to the synthesis of unmodified microparticles except by utilizing a doped organic phase consisting of a well-mixed combination of a known mass of CaZol NPs and a predetermined volume of the polymer solution in place of just a pure organic phase made up of only polymer solution. Further characterization including Fourier transform infrared (FTIR), energy-dispersive x-ray (EDAX), and fluorescence microscopy were conducted to confirm the presence of nanoparticles in microparticles. The concentration of Zol released from microparticles was determined using inductively coupled plasma – optical emission spectroscopy (ICP-OES). To investigate uptake mechanism of these particles, CaZol NiM was functionalized with folic acid as a targeting ligand, (CaZol NiM-FA) using folate conjugated PEG-PLGA polymer. Hence using confocal microscopy and flow cytometry, we investigated the uptake of CaZol NPs, CaZol NiM, and CaZol NiM-FA by activated Raw 264.7 macrophage cells stimulated with lipopolysaccharides (LPS; Invitrogen).

RESULTS: PEG-PLGA microparticles had an average diameter of 5.7 ± 1.5 um while the average size and zeta potential of CaZol nanoparticles were determined to be 44.6 ± 3.1 nm and -17.3±1 mV respectively (Fig. 1A, B). Fluorescence detection of coumarin6 labelled CaZol NPs in CaZol NiM confirmed Zol incorporation into CaZol-NiM (Fig. 1C, D). Cell uptake was confirmed by the internalization of coumarin6-stained particles in the cytoplasm of the cells (Fig. 1E, F). Moreover, FTIR peaks at 1574cm-1 corresponding to N-H bending confirmed the successful incorporation of folic acid to CaZol-NiM (Fig. 1G). Preferential uptake of CaZol-NiM-FA by macrophages was achieved as compared to CaZol-NiM (Fig. 1H).

DISCUSSION: The results indicate that not only do PEG-PLGA microparticles ensure sustained release of CaZol nanoparticles and minimize burst release of Zol as demonstrated by our uptake studies (Fig. 1E, F) and ICP-OES release studies (data not shown), but also show that these microparticles can additionally serve as suitable platform for incorporating ligands necessary for target delivery. Notably, the observed increase of microparticles uptake by CaZol-NiM-FA is evidently justified by the strong affinity between folate derivatives and folate receptor (FR-2) which is highly expressed by activated macrophages[5]. To further elucidate the therapeutic potential of this platform, future studies will focus on testing our novel system in clinically relevant osteoarthritis mouse model.

CLINICAL RELEVANCE: Nanoparticles in microparticles technology may be exploited as a promising platform to revamp the pharmacokinetics properties of Zol into a potential therapeutic agent for sustain and target delivery application for osteoarthritis treatment.

REFERENCES: (1) Kraus et al., *Osteoarthritis and Cartilage* (2016); (2) Murat, et al. *Knee Surgery, Sports Traumatology, Arthroscopy* (2015); (3) Thea and Holen., *Journal of translational medicine* (2011); (4) Sagoe, et al. *Molecules* (2023); (5) Yang, et al. *Biomaterials* (2021).

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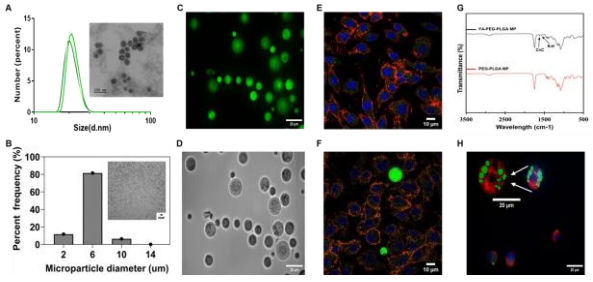

Figure 1: (A) Representative DLS (left) and TEM (right) images of CaZol NPs. (B) Size distribution and brightfield of PEG-PLGA microparticles. (C, D) Fluorescent and brightfield images of coumarin 6 CaZol NPs in PEG-PLGA microparticles. (E) Confocal image of CaZol NPs uptake studies (F) Confocal image of CaZol NiM uptake studies. (G) FTIR spectrum of folic acid conjugated microparticles. (H) Confocal image of CaZol NiM-FA uptake studies. Panels: Green: Coumarin6 (particles), Blue: DAPI (nucleus), Red (E and F): Phalloidin(actin), Red(H): Cell mask deep red (plasma membrane).