INTRODUCTION: Joint injuries are associated with a 3-6x higher risk of developing post-traumatic osteoarthritis (PTOA) (compared to un-injured, age-matched individuals) within 5-10 years. Although current treatment options can provide effective short-term pain relief, there are no FDA approved disease-modifying therapies that either slow the degeneration or promote the regeneration of damaged joints. Intra-articular injections facilitate localized delivery of therapeutics but usually do not target lesions and the joint and often get cleared rapidly through lymphatics due to small size. To address this challenge, we have developed functionalized 4-arm-polyethylene glycol-maleimide microparticles (PEG-4MAL-MP) and demonstrated intra-articular retention for 26 days post-injection. In our PTOA rat model (medial meniscal transection) studies, cartilage degradation with small lesions begins to form by three weeks post-surgery. To improve targeting to the lesion sites, we have recently functionalized PEG-4MAL MP with the mineral-targeting peptide, pVTK. In this in-vitro study, we first validated the attachment of the pVTK-PEG-4MAL-MP to bone-like organoids and ex-vivo bone tissue through confocal and widefield microscopy. Further, to assess attachment stability, we are in the process of varying the incubation time and degree of agitation of the samples. Moving forward, an in-vivo pilot study is being carried out to quantify the retention and specific binding of the PVTK-PEG-4MAL-MP to exposed bone lesions through intra-articular injection at 3 and 6 weeks post-MMT surgery and confirm binding to exposed bone at cartilage lesions towards our long-term goal of developing effective, targeted disease modifying strategies for injured joints and PTOA.

METHODS: Functionalized microparticle fabrication: The functionalized microparticles were formed using a 70 µm channels through a flow-focusing microfluidic device — 5 mM pVTK, scrambled VTK peptide (VTKsc(Genscript)) was conjugated with 6% (w/v) of PEG-4MAL (Laysan Bio) and 0.25 mM Sunfluo488 dye. The attachment of these functionalized, fluorescently tagged microparticles were tested on in-vitro mineralized bone-like organoids (established in our lab) and ex-vivo bone tissue. Preparation of in-vitro and ex-vivo bone samples: (i) Bone-like organoids: hMSC (human mesenchymal stem cell)-laden mineralized collagen was printed (2 µL) using the BioAssembly Bot 8 400 bio-printer (BAB) and further contracted and mineralized to form bone-like organoids. (ii) Ex-vivo samples: Isolated rat tibial bone was tested as: a) small, crushed pieces, b) whole tibial plateau. Incubation and imaging of bone-like organoids: Three types of microparticles (plain, pVTK-functionalized, and VTKscrambled-functionalized) were fabricated. Their attachment to bone-like organoids (n = 5) of three mineral densities (11 day mineralized, 50 day mineralized, and demineralized) was evaluated in a 96-well plate. 100 µL microparticles in 1% BSA solution was added to a single organoid and incubated for 24 hours. Prior to imaging with the Leica Thunder Imager, the organoid samples were rinsed 3x with PBS. Incubation and imaging of ex-vivo samples: Crushed tibial bone mixed with the pVTK-PEG-4MAL-MP overnight prior to imaging with Nikon SoRA spinning disk confocal microscope.

RESULT SECTION: Through qualitative assessment, we found that pVTK functionalization enhanced the attachment of the PEG-4MAL-MP to mineralized surfaces of the organoids when compared to the plain and VTKsc peptide microparticles. As a pilot, we took a single sample from each organoid of different mineral density (that had been incubated with pVTK-PEG-4MAL-MP) and normalized the particle count attached to the surface area taken. The numbers showed that there was a large difference in microparticle attachment between the 50 day mineralized and de-mineralized organoids but not as much between the 50-day and 11-day mineralized organoids. While testing with ex-vivo crushed rat tibial bone pieces, we could observe attachment of the pVTK-PEG-4MAL-MP even with a shorter overnight incubation time.

DISCUSSION: This in-vitro study provides strong proof of concept that pVTK-functionalization improves binding of PEG-4MAL microparticles to highly mineralized surfaces like bone. We are further investigating the particle counts for all the samples for conclusive results on how differences in mineral density would affect the attachment of the functionalized microparticles. Additionally, we are working on testing their attachment stability at different time points and degree of agitation. Experimentation on these different parameters would help us determine ideal injection time points for in-vivo studies. To our knowledge, these are the first in-vitro and in-vivo experiments of mineral-targeting PEG-4MAL microparticles utilized for PTOA treatment. We hypothesize that by targeting the exposed bone areas in vitro, we will better sustain therapeutics to focal damaged regions in the joint and improve therapeutic efficacy. Ongoing work is testing the ability of pVTK-functionalized microparticles to bind to full thickness lesions in PTOA pre-clinical models and extending the duration of therapeutic delivery in vivo.

SIGNIFICANCE/CLINICAL RELEVANCE: This work describes a novel, functionalized hydrogel microparticle that effectively binds to mineralized surfaces in vitro. One potential clinical application is targeted delivery of therapeutics to exposed bone areas in articular cartilage lesions at late stages of post-traumatic osteoarthritis. There is a dire need for effective disease-modifying OA therapies. Targeted intra-articular delivery systems that can retain therapeutics within the joint at focal regions of damage for a longer time period may be an effective strategy to slow degeneration and/or promote regeneration following joint injury.


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