AWARD NUMBER: W81XWH-20-1-0481

TITLE: Cartilage-Penetrating Nanocarrier-Drug Conjugate for Disease-Modifying Intervention in Post-Traumatic Osteoarthritis

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REPORT DATE: JULY 2023

TYPE OF REPORT: Annual Technical Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2023	Annual Technical Progress Report	1JUL2022 - 30JUN2023

4. TITLE AND SUBTITLE
Cartilage-Penetrating Nanocarrier-Drug Conjugate for Disease-Modifying Intervention in Post-Traumatic Osteoarthritis

5a. CONTRACT NUMBER
W81XWH-20-1-0481

5b. GRANT NUMBER
PR191750

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
191750

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Massachusetts Institute of Technology

Massachusetts Institute of Technology
77 Massachusetts Avenue
Cambridge, MA 02138

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRDC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Post traumatic osteoarthritis (PTOA) is a debilitating disease that causes the breakdown of cartilage in articulating joints when triggered by an injury to the cartilage. It is a condition that represents 12% of all osteoarthritis (OA) cases, and it has a significant impact on soldiers and civilians who suffer from extensive pain and gradual degradative loss of joint function. There have been a number of attempts to create a biologic disease-modifying osteoarthritis drug (DMOAD) to either stop OA progression or reverse the disease entirely, but these drug candidates have failed in clinical trials due to poor delivery to cartilage and lowered access to cartilage matrix producing cells, which are critical for regeneration and recovery in cartilage. In order to achieve clinical success, the drug delivery challenges that caused the proposed drugs to fail must be resolved. Our labs demonstrated that biologic drugs can be directly conjugated to a positively charged, multivalent dendrimer nanocarrier that has been modified with biocompatible polymeric groups to yield a nanocarrier for biologic proteins without loss of bioactivity. The nanocarrier is successful in addressing the drug delivery challenges that caused OA biologic drugs to fail in clinical trials. These dendrimer-drug conjugates have been shown to create a tenfold increase in joint residence time compared to the free drug, from about 3 days to 30 days, and we have shown promising cartilage regeneration results in rat studies. This PRMRP grant will move this technology toward clinical translation by improving the procedure of attaching proteins to polymeric nanocarriers and investigating the best biologic for therapeutic efficacy using tissue regeneration as a primary and pain as a secondary endpoint. Work under this grant will also determine biodistribution, dosing, and ultimately efficacy in a large animal PTOA dog model to establish the promise of this approach and establish the data needed to move it forward for IND applications for clinical trials and ultimately to the clinic. The goal of the proposed research is to conduct essential translational research on this technology to evaluate and further develop the technology as a potential disease-modifying therapy for human PTOA. Our focus so far has been on the improvement of bioconjugation chemistry. Previous versions of our polymeric nanocarrier have utilized maleimide-thiol chemistry to attach bioactive proteins, namely insulin-like growth factor 1 (IGF-1), onto the carrier, resulting in only about 1 protein on every other carrier. This ratio of drug to nanocarrier indicates that there will be free cationic polymer without any therapeutic character in our mixture. To prevent this, we have implemented the use of azide-DBCO chemistry, allowing us to conjugate three proteins onto each nanocarrier, greatly reducing the likelihood of free polymers within the therapeutic mixture. In addition, this bioconjugation protocol has been adapted to an automated purification system, allowing for more uniform and replicable purifications of future conjugates. These results will help streamline the preclinical studies as well as the translational process. Once the retention of IGF-1 anabolic bioactivity is proven, the bioconjugation protocol will be applied to anti-catabolic interleukin-1 receptor antagonist. We anticipate the bioconjugation of this anti-catabolic protein to be similar in success to IGF-1. This improved bioconjugation protocol will then be implemented for all pre-clinical studies moving forward. We anticipate that as a result of this work, a minimally invasive intra-articular injection therapy for preventative and early stage OA will be advanced toward clinical translation that will prevent and ultimately reverse OA progression by promoting growth of healthy cartilage tissue to repair the joint. The potential impact of advancement of this technology on the outcomes of military patients includes increased productivity for much longer periods of their lives, and decreased military health care costs as a result of an accessible intra-articular treatment.

15. SUBJECT TERMS
NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	23	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Osteoarthritis following traumatic joint injury is a condition that can debilitate the lives of soldiers and veterans, making it difficult for them to function effectively while serving, and impacting their work and personal lives significantly on return from active duty. There is a need for regenerative therapy that can be administered quickly and early, within hours of an injury. Such therapy would ideally have disease-modifying properties that can arrest further damage of the cartilage and reverse degradation, preventing the onset of OA following trauma. The technology developed by our lab will provide a means of mediating gradual regeneration of healthy cartilage tissue and collagen matrix in the joint. The approach developed is cell-free and offers a unique early stage intervention to rescue damaged cartilage, without the risk of donor site morbidity or transmission of infection. We devised a nanoparticle that can penetrate the cartilage, act over multi-week timescales, and target the cells in the joint directly, stimulating regeneration and growth of chondrocytes and establishing a more rapid generation of new cartilage at the injury site, addressing OA before it has begun to become problematic. Another avenue of intervention is using anti-catabolic proteins, halting joint inflammation and degradation before it has begun. This capability would lead, in the worst case, to less severe OA symptoms appearing at a later point in life, and in the best case, to the full long-term remediation of joint damage and complete recovery, or lack of degradation, of the cartilage. By bringing the joint back to recovery, it is anticipated that OA can be fully eliminated when patients are treated early enough, thus greatly improving the quality of life for the 26% of soldiers who incur OA during service, and their families, and increasing the productivity of the affected military personnel.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

osteoarthritis, growth factor, drug delivery, cartilage repair, intra-articular injection, targeted nanoparticle, IGF, IL-1RA, anabolic, anti-catabolic corticosteroid, controlled release, layer-by-layer assembly, electrostatic complex, cartilage penetrating nanoparticle.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The goal of the proposed research is to conduct essential translational research on this technology to evaluate and further develop the technology as a potential disease-modifying therapy for human post-traumatic osteoarthritis. Thus, the proposed research follows directly from the effort of the prior work and moves it toward potential clinical trials, but also extends the scope of prior work via the exploration of an additional drug candidate to use with our delivery technology. The addition of the second therapeutic candidate will validate our approach as a platform technology, enabling a broader set of potential therapeutic targets, as noted in the following proposed aims:

1) Exploration and comparison of disease-modifying biologics with anabolic and anti-catabolic mechanisms of action in OA Based on our success enhancing delivery and efficacy of IGF-1, a primarily anabolic therapeutic, with optimally PEGylated dendrimers, we seek to investigate if our dendrimer formulation can also improve the efficacy of an anti-catabolic biologic drug, and to determine whether the anabolic or anti-catabolic formulations should be moved forward for pre-clinical studies in Aims 2 and 3 . 1.1 We will first adapt our synthetic protocol to conjugate dendrimers with interleukin 1 receptor antagonist (IL-1RA), a clinical disease-modifying rheumatoid arthritis drug with published delivery challenges in human OA trials (6). 1.2 Once synthesis of a bioactive dendrimer-IL-1RA conjugate is successful, we will test the effects of dendrimer-IL-1RA vs. free IL-1RA and vehicle control in a cytokine injured cartilage model using ex vivo bovine and human cartilage explants. 1.3 We will then evaluate the delivery and efficacy of dendrimer-IL-1RA in the same rodent osteoarthritis model used to test dendrimer-IGF-1. Based on the in vitro and in vivo results, we will move forward with either IGF-1 or IL-1RA as the drug conjugated to our optimally PEGylated dendrimer nanocarrier.

2) Biodistribution, dose finding and immunogenicity studies of dendrimer-drug For the selected dendrimer-drug conjugate, we will identify the maximum tolerated dose in rats and characterize the accompanying toxicology to establish a dosing regimen to guide large animal studies. 2.1 We will first investigate the biodistribution of dendrimer-drug conjugates to identify any potential off-target tissues in which the dendrimer-drug conjugates may accumulate. 2.2 These data will indicate which organs to investigate in toxicology studies using histology and blood chemistry panels. Such toxicology biomarkers will be used to identify a maximum tolerated dose. 2.3 We will perform immunogenicity studies on single and repeat injections of dendrimer-drug conjugate to ascertain the risk of anti-PEG or anti-dendrimer antibody generation over the duration of chronic therapy.

3) Evaluation of improved delivery and efficacy of dendrimer-drug in a canine PTOA model 3.1 We will establish a surgically-induced model of post-traumatic osteoarthritis in canines by transection of the anterior cruciate ligament (ACLT) followed by 1 month of unrestricted movement to induce cartilage lesions. The animals will be administered dendrimer-drug conjugate intra-articularly into the affected knee joint. 3.2 We will evaluate the pharmacokinetics (PK) of dendrimer-drug in the injured canine joint using in vivo imaging of dendrimer-drug labeled with a radioactive or fluorescent tracer. Drug concentrations in synovial fluid aspirates will also be measured. 3.3 Pharmacodynamics (PD) will be observed by biomarker analysis of synovial fluid aspirates from longitudinal timepoints. 3.4 Finally, outcomes related to disease progression will be assessed throughout the study. MRI will be used to measure cartilage volume, which will be compared among treatment groups. We will examine terminal histopathology of cartilage, bone, and synovium at a specified endpoint of the study and score conditions of each tissue based on published research society (OARSI) guidelines. Routine veterinary assessment of joint pain and function will be performed throughout the study.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

3.2 *Major Activities and Significant Results and Outcomes by Task*

Summary: Significant key advances have been made over the past year, which include completion of in vivo pharmacokinetic studies and efficacy study in rats for both conjugated constructs. Additional confocal microscopy images were obtained to confirm successful binding of both dendrimer formulations to their receptors as well as the ex vivo efficacy result of G6P8-IL1RA. CRO has been established for the dog studies and currently the drafting non-disclosure agreements (NDA).

Confirmation of binding

In vitro bioactivities of both anabolic (IGF-1) and anti-catabolic (IL-1RA) nanoformulations (G6P8-IGF1 and G6P8-IL1RA respectively) have been explored in the last annual report as a part of aim 1.2 of the grant. In order to further confirm that the bioactivity is the result of the therapeutic proteins binding to their respective receptors, we have utilized confocal microscopy to visualize their bindings. The confocal images confirmed that both IGF-1 and IL-1RA, whether they are conjugated to the dendrimer or not, are able to bind to their respective receptors (Figure 1). This confirms that the subsequent bioactivities are indeed due to the therapeutic proteins binding to the receptors. The nuclei of the cells are stained with Hoechst33342 (blue), the receptors are immunostained with AF488 (green), and the therapeutics are tagged with AF647 (red). Colocalization of green and red signals indicates successful binding of therapeutics to their receptors.

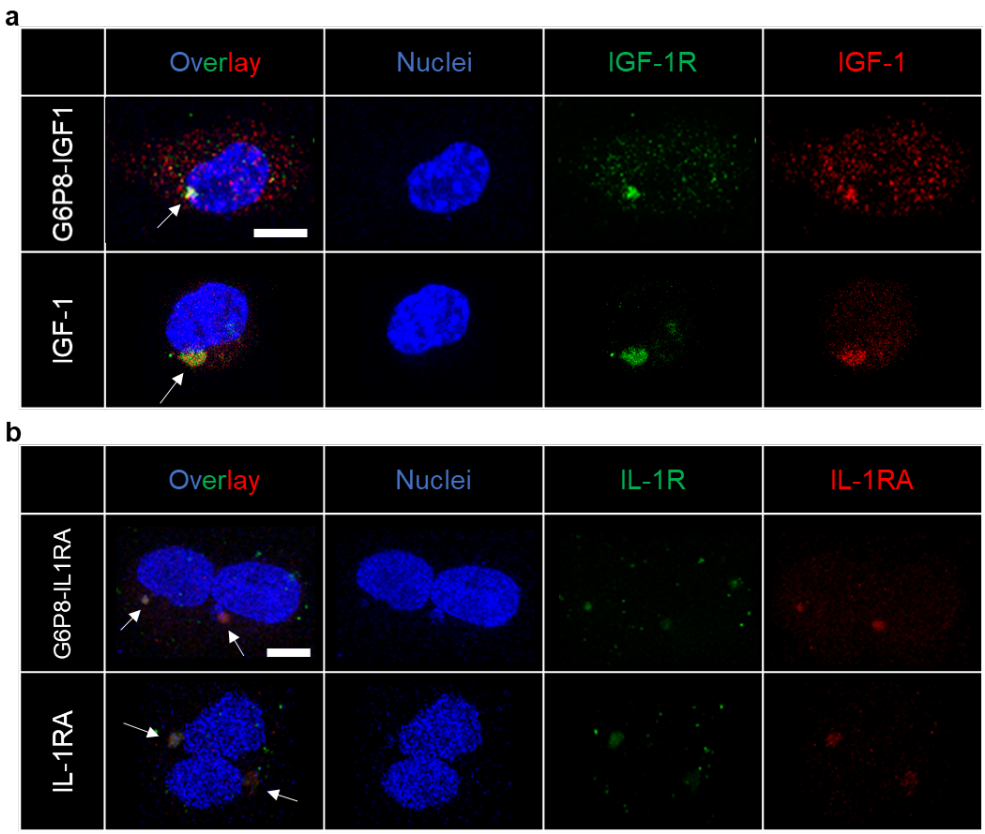

Figure 1. Confocal microscopy of G6P8-IGF1 (**a**) and G6P8-IL1RA (**b**) binding to their receptors. Colocalization of the receptor signals (green) and the protein signals (red) is indicated with white arrows.

G6P8-IL1RA Ex Vivo Bioactivity

Ex vivo bioactivity G6P8-IGF1 was confirmed in the last annual report as a part of aim 1.2 and was beginning to test the ex vivo bioactivity of G6P8-IL1RA at the end of last annual update. We have used the similar setup as G6P8-IGF1 ex vivo bioactivity study where we extracted bovine cartilage explants to test ex vivo bioactivity. Instead of looking at the increase in glycosaminoglycan (GAG) synthesis rate like we examined with G6P8-IGF1, here, we focused on the reduction of GAG loss rate to test anti-catabolic activity. The cartilage explants were treated with IL-1 to trigger GAG loss and G6P8-IL1RA was added to the media to prevent catabolic activity of IL-1 which would result in reduction of GAG loss rate. Both free IL-1RA and G6P8-IL1RA were able to significantly reduce the rate of GAG loss when challenged with IL-1 (Figure 2). Slight decrease in bioactivity of G6P8-IL1RA compared to free IL-1RA is expected due to charge-charge interaction of the cationic dendrimer with the surrounding anionic extracellular matrix which hinders G6P8-IL1RA's capacity to compete with IL-1. However, in the in vivo setting, the extended retention time in the joint space is expected to outweigh this disadvantage, since free IL-1RA will be completely removed from the joint space within 4 days. As long as G6P8-IL1RA retains some level of bioactivity, the extended retention time will allow sustained effect in vivo.

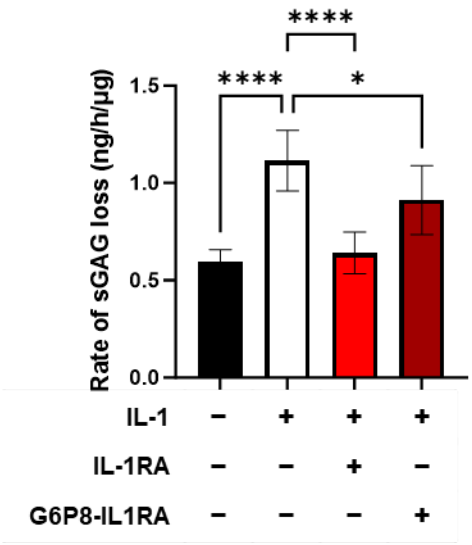

Figure 2. Ex vivo bioactivity of G6P8-IL1RA. IL-1RA is able to significantly reduce the rate of GAG loss in the cartilage explant even after dendrimer conjugation.

Pharmacokinetic Study

As a part of aim 1 of the grant, the pharmacokinetics of the dendrimer formulations were investigated. Through aim 1.1 and aim 1.2, the dendrimer-protein conjugates were already optimized and showed significant in vitro and ex vivo bioactivity. Thus, as a part of aim 1.3, we sought to confirm the ability of the dendrimer formulations to attach to the negatively charged cartilage matrix and prolong the residence time in the joint space in vivo compared to their free-protein counterparts. Upon intra-articularly injecting 40ul of fluorophore-concentration matched free proteins or dendrimer formulations (7.5µM AF647 conjugated to proteins) in both of their hind limb joints of male Sprague-Dawley rats, the fluorescence signals were monitored using IVIS for 8 weeks (Figure 3a). The signals from the dendrimer formulations were able to be detected even after 8 weeks post-injection while the free proteins were cleared from the joint space below the detection limit as early as day 4. Pharmacokinetic profiles of the fluorescence intensity quantified from IVIS images show rapid clearance of free proteins from the joint space while the dendrimer formulations showed enhanced retention (Figure 3b). Half-lives calculated from the pharmacokinetic profiles show that the half-lives of the dendrimer formulations were almost 8-fold longer compared to their free protein counterparts (Figure 3c).

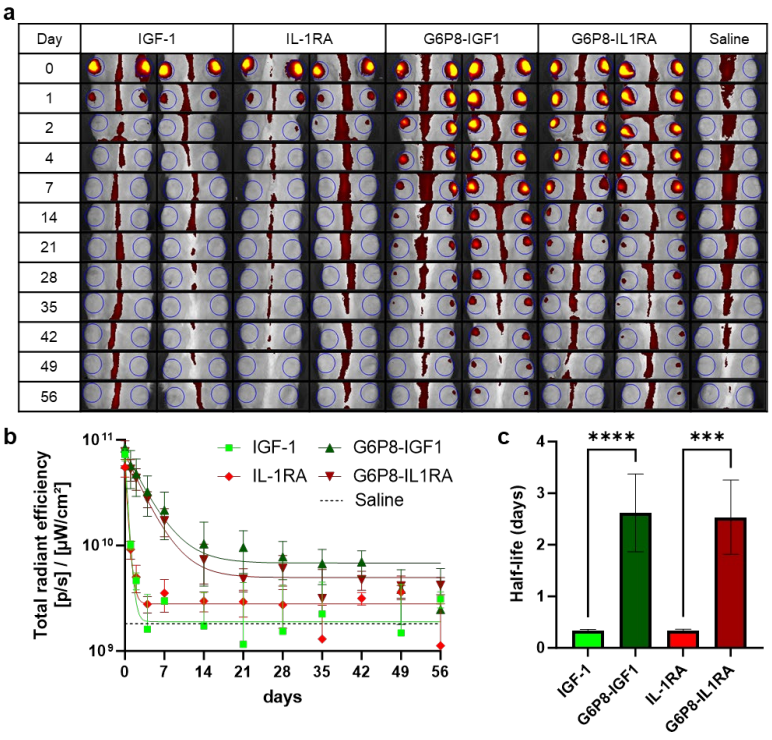

Figure 3. Pharmacokinetics of dendrimer formulations. IVIS imaging (**a**) is quantified and plotted over time (**b**). The half-lives were calculated based on the plot (**c**).

Efficacy study

Final study in aim 1 is to compare the in vivo efficacy of G6P8-IGF1 and G6P8-IL1RA to complete aim 1.3. In order to evaluate the formulations in a disease-relevant setting, anterior cruciate ligament transection and partial medial meniscectomy (ACLT + MMx) surgery model was established on right hindlimb of each rat to surgically induce OA (Figure 4a) and successful removal of medial meniscus was confirmed by microcomputed tomography (µCT; Figure 4b). Two days after surgery, formulations were injected intra-articularly in their respective therapeutic concentrations (40µl of 20µM IGF-1 or 40µl of 9µM IL-1RA) into the injured knee. Pain assessment was done 14 days after surgery and rats were sacrificed for µCT and histology analysis 30 days after surgery (Figure 4c). All of the dendrimer formulations showed prolonged retention compared to their free protein counterparts which is consistent with the pharmacokinetics study (Figure 4d).

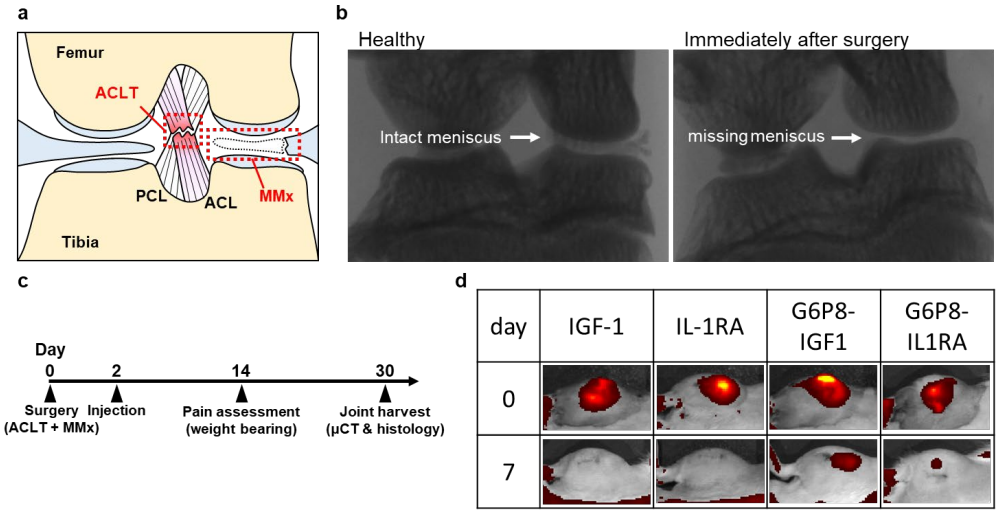

Figure 4. Establishing surgically induced OA model. (**a**) Schematic of anterior cruciate ligament transection and partial medial meniscectomy (ACLT + MMx) surgery model. (**b**) µCT images of healthy and injured knee. (**c**) Timeline of the study. (**d**) Confirmation of extended retention time of dendrimer formulations in the surgery model.

Pain level was assessed two weeks after surgery using an incapacitance meter to measure weight distribution between the healthy hindlimb and the injured hindlimb (Figure 5a). The saline injected control showed significant decrease in weight bearing of the injured leg compared to the even distribution of weight on both hindlimbs of sham surgery control indicating that the OA model does inflict pain to the injured knee (Figure 5b). Among all the treated groups, G6P8-IL1RA showed the best improvement in pain management compared to the saline injected control. The G6P8-IL1RA injected rats recovered to the equal weight distribution observed in the uninjured sham surgery control group. The difference between G6P8-IGF1 and G6P8-IL1RA is potentially due to the difference in their mechanisms of action. While IGF-1 possesses the ability to regrow cartilage matrix, it does not innately have the ability to mitigate pain directly. IL-1RA on the other hand, is an anti-inflammatory drug that inherently has the ability to reduce pain associated with inflammation in the joint.

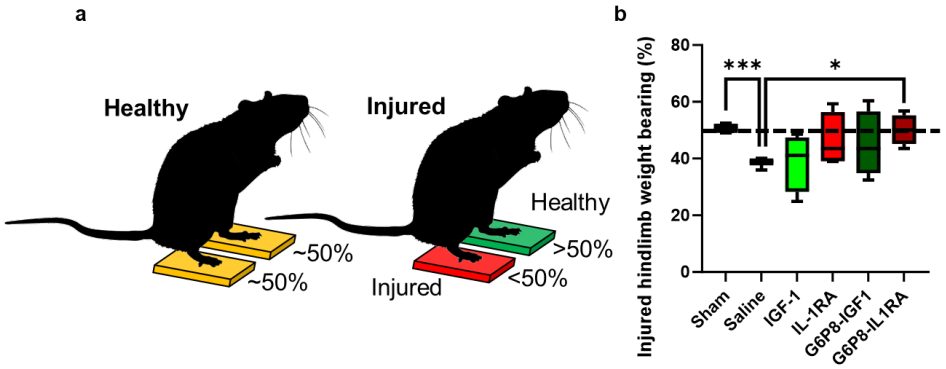

Figure 5. Pain assessment. (**a**) Schematic of measuring hindlimb weight distribution using incapacitance meter. (**b**) Percentage weight bearing of the injured hindlimb reveals G6P8-IL1RA is the best at mitigating pain in the surgically induced OA model.

At the end of the study, the rats were sacrificed and the joint tissues were collected for further analysis. Osteophytes, which are OA-induced bone spurs protruding from the normal bone contour around the joint with reduced bone mineral density, were examined via µCT analysis (Figure 6a, white arrows). The total volume of osteophytes was significantly higher in the saline treated group compared to the sham control (Figure 6b). The dendrimer nanoformulation treated groups (G6P8-IGF1 and G6P8-IL1RA) showed significant decreases in osteophyte volume compared the saline treated group. Also, the osteophyte volumes of both G6P8-IGF1 and G6P8-IL1RA treated groups were comparable to that of the sham control. Although the free protein treated groups (IGF-1 and IL-1RA) showed moderate amount of decrease in volume compared to the saline treated group, the improvement was insignificant.

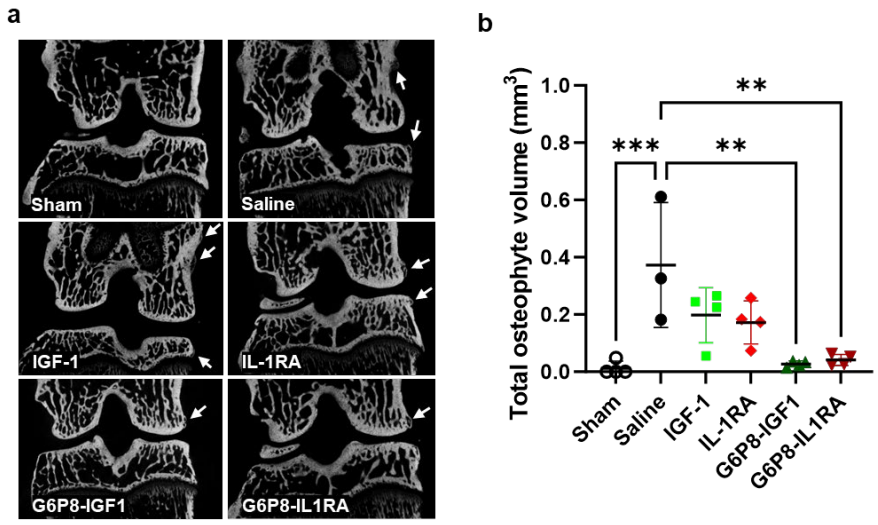

Figure 6. Osteophyte formation in the knee joints of OA burdened rats. (**a**) Representative µCT images showing osteophytes (white arrows). (**b**) Quantification of total osteophyte volumes reveal that both G6P8-IGF1 and G6P8-IL1RA can significantly reduce the osteophyte formation.

After the µCT images were taken, the joint tissues were processed for histopathology analysis. The tissue sections were stained using hematoxylin/eosin (H&E) to assess inflammation in the synovium or toluidine blue/fast green to visualize cartilage degradation. H&E staining revealed that G6P8-IL1RA was able to reduce inflammation in synovium (Figure 7a). Next, for each joint, the section with the most lesion in the medial tibial articular cartilage (Figure 7b, black dashed box) was inspected (Figure 7c). For each section, the total width of degradation, significant width of degradation, and area of degradation were measured (Figure 7b, red line, yellow line, and white box respectively). Quantification of those metrics revealed that in all measurements, saline and the free protein treated rats but not the dendrimer formulation treated rats showed significant deterioration of medial tibial articular cartilage compared to the sham control (Figure d-f). Furthermore, for the dendrimer formulation treated groups, at least one animal from each group showed no detectable degradation of cartilage. This trend is relatively consistent with the previous study done in our lab using G6P8-IGF1.

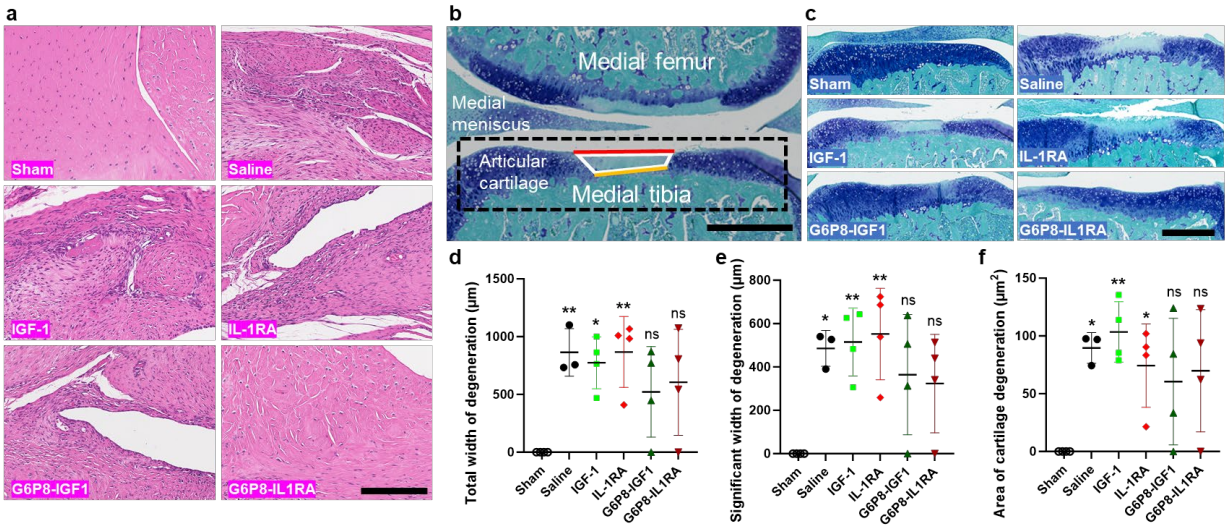

Figure 7. Histology analysis of the cartilages of OA burdened rats. (**a**) Representative H&E images showing reduced synovitis in G6P8-IL1RA treated synovium. Scale bar, 200 μm. (**b**) The total width of degeneration (red line), significant width of degeneration (yellow line), and the area of degeneration (white box) of the medial tibial articular cartilage (dashed box) is measured for evaluating the severity of the damaged as the result of OA progression. Scale bar, 500 μm. (**c**) Representative toluidine blue/fast green images of medial tibial articular cartilage. The free protein treated groups showed severe damage to the cartilage. Scale bar, 500 μm. (**d**) Total width of degeneration (μm). (**e**) Significant width of degeneration (μm). (**f**) Area of cartilage degeneration (μm^2).

We have collected enough data to demonstrate the efficacy and was able to determine the final formulation to be used for the rest of the aims. According to the osteophyte formation and cartilage degradation, both G6P8-IGF1 and G6P8-IL1RA are equally viable formulation for translation. Therefore, in the context of translation, we have selected G6P8-IGF1 as the final formulation to be used for the canine study since the cost of IGF-1(~$100/mg) is much cheaper than IL-1RA(~1,000/mg). Although G6P8-IL1RA was better than G6P8-IGF1 at mitigating pain, this can be easily remedied by co-delivering anti-inflammatory agent along with G6P8-IGF1 thus the benefit of selecting G6P8-IL1RA was not enough to justify.

Planned Future Work

For the maximum tolerated dose (MTD) and biodistribution (BioD) studies of aim 2, we will be using G6P8-IGF1 as the final formulation as determined in aim 1. Setting the dose used in the efficacy study (0.3mg/kg of G6P8) as the 1x benchmark, 0x, 1x, 5x, 10x dose will be tested on healthy rats (n=4), and another 10x dose will be tested on rats with OA induced by ACLT-MMx surgery (n=4). After 28 days post injection, heart, lung, liver, spleen, kidney, joint will be collected and probe for AF647 signal attached to the formulation. Complete blood count and systemic toxicity analysis will be performed on day 0, 14, and 28. Organs with significant accumulation or rats with abnormal blood panel result will be subjected to further histology analysis. The MTD will be determined on the basis of complete blood count, systemic toxicity, and biodistribution result.

For the canine experiment of aim 3, we have established an AAALAC-accredited CRO, specifically Inotiv, and is in the process of drafting an NDA. The CRO needed to be changed from InterVivo to Inotiv since InterVivo was not AAALAC-accredited. Once the NDA is established, we will finalize the studies to be performed at the CRO. We are expecting to start the canine study in October. We will send our formulation to the CRO at the dose determined in aim 2 and the CRO will perform all

the studies that were agreed upon. The canine efficacy study is a 72-day experiment that includes protein retention via fluorescence imaging, MRI images, plasma and synovial fluid collection, and ultimately the treated joints for histological analysis. However, due to unavailability of IVIS and PET imaging modalities, the CRO will not be able to perform live animal pharmacokinetics study of protein retention via fluorescence imaging. Instead, we will be evaluating the remaining formulation in the joint at the end of the study ex vivo.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to report.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

In vivo efficacy study with rat PTOA model indicates that both G6P8-IGF1 and G6P8-IL1RA are able to significantly inhibit the OA progression, with G6P8-IL1RA potentially being better and consistent in pain mitigation over G6P8-IGF1. To maximize the positive outcome in the clinical settings, combination of pro-anabolic therapeutics with anti-inflammatory agent or combining both pro-anabolic and anti-catabolic therapeutics may be desirable when considering DMOAD for translation. Altogether, the data so far indicate not only the versatility and effectiveness of the nanoformulations, but also provide insights on how these nanoformulations would perform in clinical settings.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

CRO was changed from Canada-based InterVivo to the US-based Inotive due to InterVivo not being an AAALAC-accredited CRO. The CRO had the capability to do in vivo imaging with the dogs as proposed, but the equipment is out of order at the moment and will be unavailable for 15 months with no alternative available. Therefore, unfortunately we will not be able to do live in vivo imaging with the dogs.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Products include the IGF-1 and IL-1RA dendrimer conjugates for treatment of post-traumatic osteoarthritis as outlined above.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Paula T. Hammond

Project Role: PI, MIT

Nearest Person-Month Worked: .5 month(summer) year (Academic salary covered by MIT)

Name: Joon Ho Park

Project Role: Postdoctoral Associate, MIT (replacing Rami Chakroun)

Nearest Person-Month Worked: 12 months this year.
Contribution to Project: synthesis, in vitro, and in vivo testing of G6P8-IL1RA, in vivo studies using rat models, and coordination with CRO for dog model study.

Name: Brandon Johnston

Project Role: PhD Candidate, Graduate Research Assistant, MIT

Nearest Person-Month Worked: 6 months this year (graduated 4/2023)

Contribution to Project: synthesis, in vitro, and in vivo testing of G6P8-IGF1, and in vivo studies using rat models.

Name: Simone Douglas-Green

Project Role: Postdoctoral Associate, MIT
Nearest Person-Month Worked: 1 month this year.

Contribution to Project: Design of cartilage transport measurements and key in vitro experiments to determine efficacy of nanoparticle release and the relationship between size and charge and protein corona in such systems, involved in design and integration of knowledge for in vivo studies. In vivo testing of the dendrimer-IGF-1 conjugates, including pharmacokinetics, surgical model induction.

Name: Hou, Xiuyun

Project Role: Senior Lab manager

Nearest Person-Month Worked: 3.5 months this year

Contribution to Project: TBP

Name: Vo, Chau

Project Role: PhD Candidate, Graduate Research Assistant, MIT

Nearest Person-Month Worked: 1 month this year

Contribution to Project: TBP

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Previously Active Grants that closed:

Title: ISN4 - 1.5 Rapid Hemostasis for the Treatment of Incompressible Wounds
Sponsor: ARO-ISN UARC
Project Period: 1/2018 – 12/2022
Amount:

Title: Targeting and transfection of hematopoietic stem cells
Sponsor: Children's Hospital Boston (Gates Foundation)
Project Period: 8/2020 – 10/2022
Amount:

Title: A novel sshRNA-antimiR combination therapy for accelerating healing of diabetic foot ulcer
Sponsor: Somagenics (NIH)
Project Period: 10/2019 – 08/2022
Amount:

Title: Application of Nanoparticles to Deliver Antibody Therapeutics in Neurological Disease
Sponsor: Alector
Project Period: 7/2021 – 7/2022
Amount:

Previously pending Grants that are now active:

Title: In vivo genetic treatment of Sickle Cell Disease
Sponsor: Children's Hospital Boston (Gates Foundation)
Project Period: 1/2022 – 9/2023
Amount:
Person months: 0.01/year

Title: Acceleration of burn healing through a novel sustained-release smart dressing
Sponsor: Somagenics Inc. (prime: DOD)
Project Period: 9/2022 – 3/2024
Amount:
Person months: 0.01/year

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.