AWARD NUMBER: W81XWH-20-1-0481

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

PRINCIPAL INVESTIGATOR: Paula T. Hammond

CONTRACTING ORGANIZATION: Massachusetts Institute of Technology, Cambridge, MA

REPORT DATE: July 2021

TYPE OF REPORT: Annual

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 2021	Annual	01Jul2020-30Jun2021

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-20-1-0481

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Paula T. Hammond

E-Mail: hammond@mit.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts Institute of Technology
77 Massachusetts Ave
Cambridge MA 02139

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Material Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

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			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	17	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

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:

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:

What were the major goals of the project?

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?

Protein Conjugation

Aim 1 of the grant focuses on exploring the disease-modifying properties of dendrimer-conjugated anti-catabolic biologics, specifically interleukin 1 receptor antagonist (IL-1RA), versus anabolic biologics, insulin growth factor-1 (IGF-1). This aim requires us to adopt our current synthetic protocol of biologic-dendrimer conjugation to IL-1RA. With the unfortunate discontinuation of the manual separation columns previously used in this protocol, we have been working to translate our manual protocol to a fast-protein liquid chromatography (FPLC) system. This requires us to not only learn the FPLC system and develop methods to produce reproducible and pure bioconjugation products, but also to ensure the dendrimer-tethered biologics remain bioactive.

IGF-1 N-terminus conjugation of a protected thiol and subsequent thiol deprotection, as well as the conjugation of AlexaFluor-647 for ease of *in vivo* visualization, has been successfully translated to the FPLC using a desalting column. A single, 5-mL Cytiva HiTrap desalting column was unable to provide sufficient separation between desired product and byproduct, but two columns set in parallel resulted in adequate separation (Figure 1). This is due to the increased column retention time, allowing for more prolonged interaction with the stationary phase resulting in increased separation. After the reaction between dendrimer-bound maleimide and protein-bound thiol, separation between bound and unbound protein was performed using a 1-mL Cytiva HiTrap SP HP cation exchange column. After unbound protein removal, protein-dendrimer conjugates were eluted from the column using a high salt concentration, pooled together from multiple fractions, desalted using centrifugal filters, and sterile filtered. Unfortunately, though the resulting dendrimer-IGF-1 product showed bioactivity (Figure 2), only one protein was bound for every two dendrimers, which is similar to the conjugation efficiency when performed manually (Figure 2). Moving forward, we hope to revise the bioconjugation chemistry in order to increase bioconjugation efficiency and create a more robust synthetic scheme.

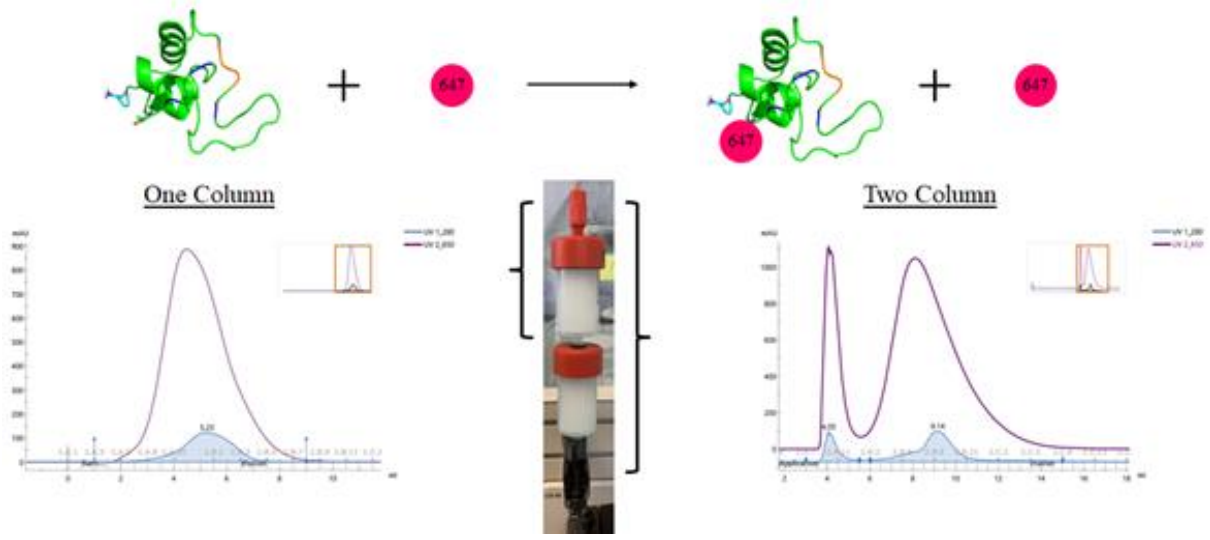


Figure 1: Protein conjugation using FPLC with two desalting columns in parallel to purify protein modifications (AlexaFluor 647 and thiol conjugation) provides increased product purity.

Unfortunately, not long after our last update, our FPLC refrigerator, set at 4°C, malfunctioned in the middle of the night, increasing the temperature to almost 40°C. Not only did we need to fix the refrigerator, but we also decided to request a preventative maintenance of the FPLC to ensure nothing was damaged during the period of increased temperature. With the precautions put in place by the university to ensure student safety during the pandemic, we did not have access to the FPLC for a few months, forcing us to focus on other aspects of the project.

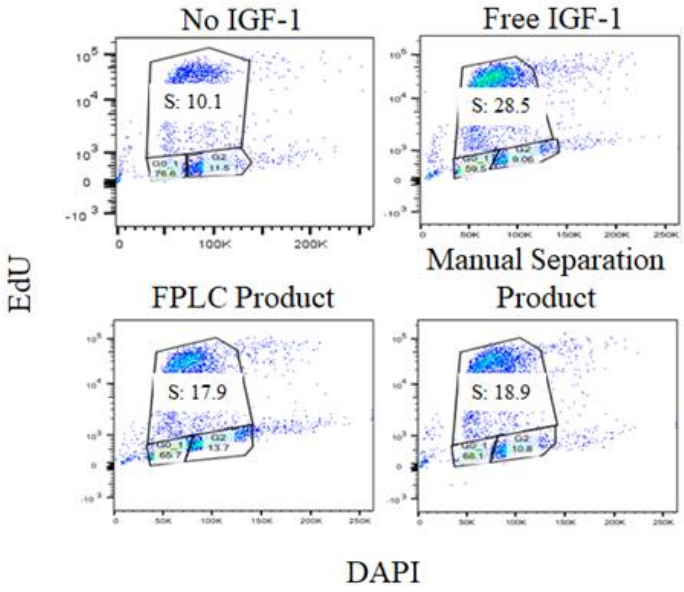
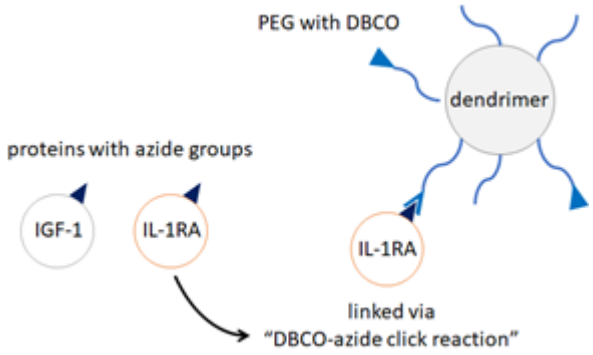


Figure 2: Representative protein bioactivity analysis. The percent of cells in the S-phase of the cell cycle increases when Dendrimer-IGF-1 conjugates are used as treatment, though not as much as free IGF-1 treatment. These experiments were performed using the original thiol-maleimide bioconjugation.

Linker Chemistry

To develop a more robust bioconjugation chemistry, we attempted to create a partially-azide functionalized PAMAM surface for click chemistry reactions. To create this surface, we decided to first make a partially-bromine functionalized surface, and use SN2 substitution techniques to convert the bromines to azide groups. In this manner, we could continue to use NHS-amine chemistry to convert primary amines to amide bonds, and allow for other desired nucleophilic attacks of the bromine to occur if the azide-functionalization proved to be non-ideal. However, due to issues associated with primary amine-bromide byproducts and subsequent gelation, the process of attaching primary halides to the dendrimer surface followed by nucleophilic attack is no longer feasible. Instead, we worked to develop a method for dibenzocyclooctene (DBCO) - azide click reaction between azide-containing proteins and DBCO-containing dendrimers (Figures 3).

Short PEG linkers containing terminal DBCO functionalities have been successfully conjugated onto PAMAM dendrimers through NHS-amine chemistry. The quantification of DBCO incorporation can easily be assessed using proton NMR. Similarly, short PEG linkers containing terminal azide functionalities have been conjugated onto IGF-1 proteins using NHS-amine chemistry targeting the N-terminus by modulating pH. In addition to the PEG linkers, AlexaFluor 568 and AlexaFluor 647 have been conjugated to PAMAM and IGF-1, respectively, with removal of unreacted AlexaFluor verified through thin layer chromatography and gel electrophoresis. The click chemistry reaction between DBCO and azide is a well studied, robust reaction that takes place under physiological conditions without the need for copper. Thus, by combining the azide-containing IGF-1 and DBCO-containing PAMAM in phosphate buffered saline, we were able to covalently bind IGF-1 to PAMAM dendrimers after 48 hours of mixing at 4°C.



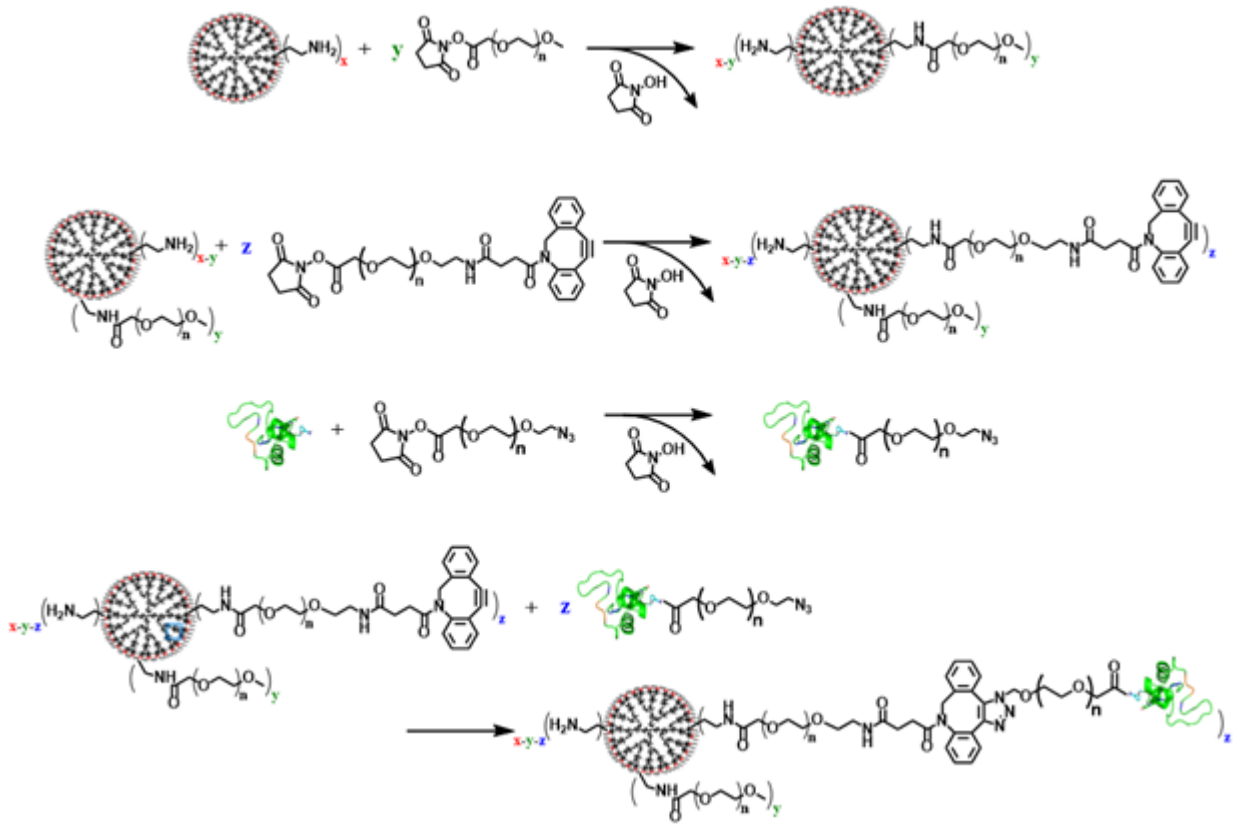


Figure 3: Schematic of PEGylated dendrimer-drug conjugate dibenzocyclooctene (DBCO) - azide click reaction. Reactions schemes showing Dendrimer-PEG reaction, Dendrimer-PEG-DBCO reaction, Protein-PEG-Azide reaction, and finally Dendrimer-PEG-Protein DBCO-Azide reaction.

Following the FPLC protocol we developed, as previously described, we were able to separate unreacted IGF-1 from PAMAM-protein complexes, as verified by non-reducing SDS-PAGE (Figure 4). Free IGF-1 tagged with AlexaFluor 647 (lane 2, Figure 4-top) migrates to the bottom of the gel, as IGF-1 has a molecular weight of about 7.5 kDa. Dendrimer-AlexaFluor 568 only migrates a small distance within gel (lane 3, Figure 4-bottom). When the protein is bound to dendrimer (lanes 6-8 and 12-15), the protein can only migrate a fraction, thus verifying a successful conjugation of protein to dendrimer. In addition, using a UV-vis spectrophotometer to quantify the absorbance of our pure product at 578 nm and 650 nm, and comparing those values to the pure protein-AlexaFluor 647 complex and PAMAM-AlexaFluor 568 complex, we

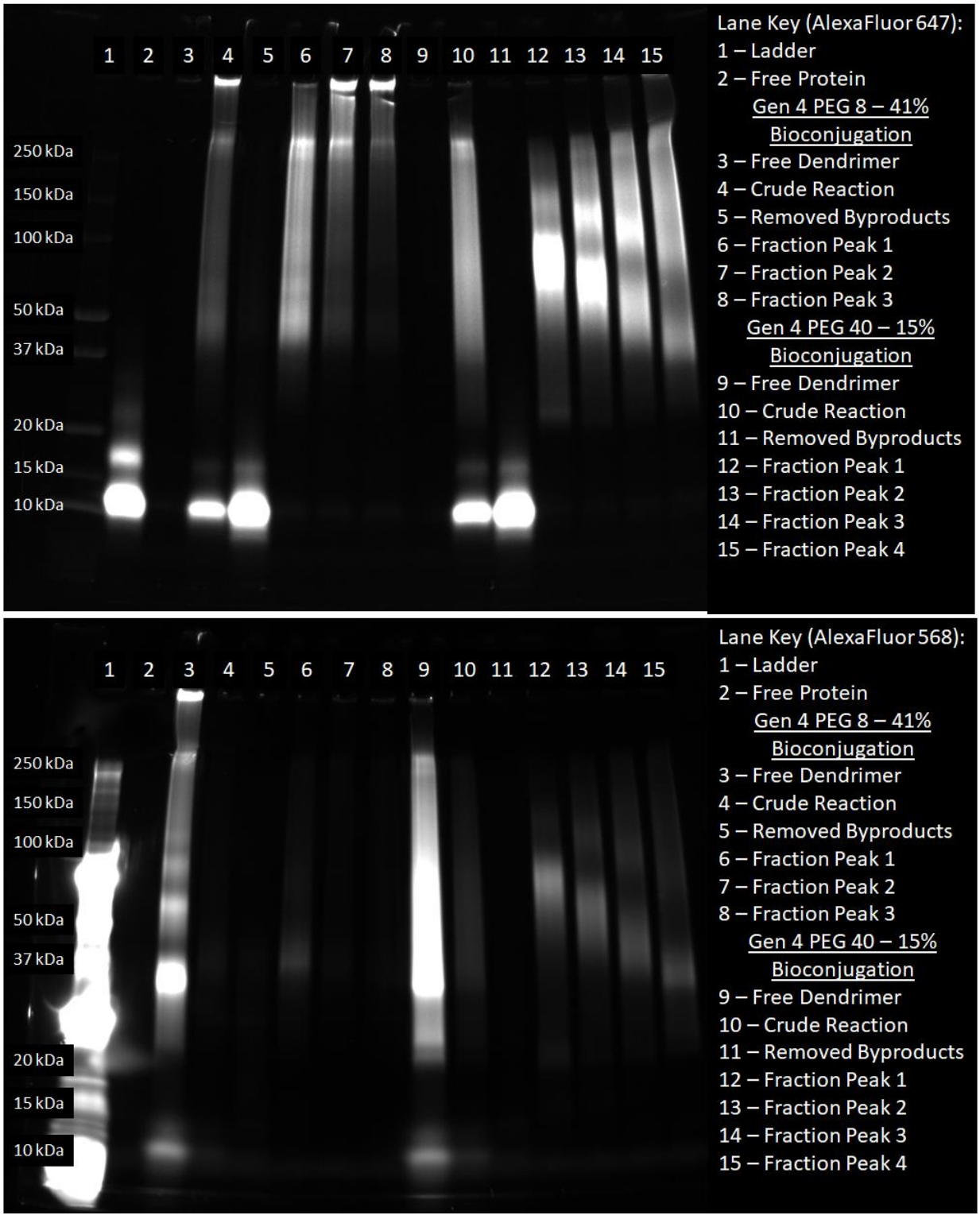


Figure 4: Non-reducing SDS-PAGE to assess conjugation of protein-bound AlexaFluor 647 (top) and dendrimer-bound AlexaFluor 568 (bottom). Lanes were loaded with an equal amount of AlexaFluor 647. Free proteins migrated to the bottom of the gel, but dendrimer-bound proteins are closer to the top of the gel.

were able to quantify the concentration of AlexaFluor 568 and AlexaFluor 647, and ultimately the concentration of PAMAM and protein, respectively, in our purified product. Our new protocol has been able to covalently bind 3 to 4 proteins per dendrimer with a final yield of 30-35% based on absorbance of our purified fractions. This new protocol significantly increases our protein-PAMAM ratio from 0.6 to 3, though further analysis must be conducted to increase final yields. Additionally, we have successfully increased bioconjugation of IGF-1 onto PAMAM-PEG complexes with both short (PEG 8) and long (PEG 40) chain lengths, ensuring that this protocol allows us to retain control of the system's tunable handles.

Our next step in verifying the success of the DBCO-azide reaction will be to confirm the retained bioactivity of the IGF-1 proteins. This will be done by incubating NIH/3T3 cells in a low serum environment, followed by treatment with PAMAM-IGF-1 complexes and subsequent evaluation of the cell proliferation. Finally, the same reaction will be performed with an interleukin-1 receptor antagonist (IL-1RA) to confirm success conjugation, followed by bioactivity evaluation using a reporter cell line. Success will result in enhanced bioconjugation efficiency while maintaining protein bioactivity.

Animal Studies

Due to Covid-19 we experienced significant delays in completing training requirements for CAC approval and subsequent in-person animal handling trainings. With campus restrictions easing up, we are moving as swiftly as possible to complete final trainings and start experiments. Both postdocs have been approved by the Committee on Animal Care (CAC) and have been added to the grant's animal protocol. In August, all personnel (graduate student and 2 postdocs) have received training for intra-articular injections; injections are projected to begin in early November. Training sessions for conducting anterior cruciate ligament transection and medial meniscus resection surgery are planned for late December. In preparation, we have made large batches of varying formulations.

Our new postdoc is expected to start on December 1st. The current members of the team will have a comprehensive list of the Institutional Animal Care and Use Committee (IACUC) and The Division of Comparative Medicine (DCM) training requirements to be completed (i.e.: DCM facility orientation, imaging facility orientation, mice and rat handling, surgical wet lab orientation and DCM cage cards, as well as IACUC training on working with mice and rats). We will also contact the DCM staff to arrange special training sessions to ensure the new postdoc will be fully onboarded and approved by the end of December. This postdoc will be taking lead on animal studies, which will help push our work forward.

What opportunities for training and professional development has the project provided?

Currently, there is one fourth-year graduate student and two first-year postdocs working on this project, all focused on translating their *in vitro* discoveries to an *in vivo* model. All three personnel have become competent in synthesizing and characterizing the dendrimer-PEG conjugates *in vitro,* completed all online and classroom training requirements set forth by the MIT animal facility and have begun planning advanced translational *in vivo* experiments. Given the lack of experience in rat handling and experimentation, all three personnel are in the process of obtaining additional training

regarding rat handling and intra-articular injections into the knee joints in preparation for the studies set-forth in this grant proposal. In addition, we have been looking for a new postdoc over the last year to take the lead on our *in vivo* studies. Despite a limited pool of applicants, we are glad to have hired a postdoc coming to us with advanced animal experience, with a start date of December 1st, 2021. After joining our group, they will focus on completing all animal training and requirements in order to begin working on the aims of this grant proposal as soon as possible. In the interim, with support from animal technicians, our graduate student and two postdocs will begin animal experiments once they are fully trained on all the techniques required to meet the aims of this grant proposal.

How were the results disseminated to communities of interest?
Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

The new postdoc on the project, who is starting December 1st, will begin trainings for animal studies as soon is cleared to do so. During these trainings, which are anticipated to take a month or two, the team will work to conjugate IL-1RA to the dendrimer nanocarrier in the same fashion that IGF-1 has been, as reported here. In addition to this bioconjugation, the bioactivity of conjugated IGF-1 and IL-1RA will be assessed through *in vitro* experimentation. Once bioactivity has been verified of both conjugates, our first major *in vivo* study will be to assess the efficacy of anti-catabolic vs. anabolic mechanisms of action in mitigating osteoarthritis development in a surgically-induced rat OA model. Once these results have been analyzed, the preferred mechanism of action will be further studied, first in biodistribution studies.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We have substantially improved the efficiency of the synthetic protocol to conjugate proteins onto the surface of cationic nanocarriers. Prior, only every other nanocarrier contained a single protein, resulting in the potential for free cationic nanocarriers being present in the therapeutic cocktail. Now, with the improved protocol, we have been able to conjugate about 3 proteins onto the nanocarrier, greatly reducing the likelihood of a bare nanocarrier being present. This work elucidates the optimal bioorthogonal conjugation chemistry between the two methods.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Delays to launch project were significant in the first few months (July – September) and the hiring of a postdoc with the originally desired animal and potentially orthopedic expertise has been greatly delayed due to a paucity of outstanding candidates. Despite these delays, we have been able to successfully launch the program and will be able to address the mechanism of action efficacy, biodistribution, and other in vivo characterization planned by this summer.

Actual or anticipated problems or delays and actions or plans to resolve them

Delays to launch the project were significant in the first few months (July-September), as graduate students and postdocs were on an extremely limited schedule due to COVID-19 protocols. This included maximum weekly hours of 15 hours as required by the institution. From September-May, workers were able to work more hours per week, but were restricted to 'shifts' so that the capacity of the laboratory would not reach a maximum. As a result, there were significant delays in work. Additionally, these restrictions resulted in significant delays in trainings, such as animal trainings and new personnel training.

Changes that had a significant impact on expenditures

Hiring a postdoc to work on the project with the desired animal and potentially orthopedic expertise was greatly delayed due to the paucity of outstanding candidates as a result of COVID-19. Luckily, we were able to find a candidate with animal experience and expertise and he will be starting on the project in December of 2021. Due to this, there has been a lack of progress on tangible animal results, as no one currently on the project has animal experience. This was exacerbated by the delays in animal training at the institution, preventing the current grad students and postdocs from quickly gaining animal expertise.

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

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: *Nothing to Report*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Paula T. Hammond
Project Role: PI, MIT
Nearest Person-Month Worked: 0.5 month each year (Academic salary covered by MIT)

Name: Alan Grodzinsky
Project Role: Co-I, MIT
Nearest Person-Month Worked: 0.5 month (summer) each year (Academic salary covered by MIT)

Name: Simone Douglas-Green
Project Role: Postdoctoral Associate, MIT
Nearest Person-Month Charged: 2 months
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: Elad Deiss-Yehiely
Project Role: PhD Candidate, Graduate Research Assistant, MIT
Nearest Person Month Charged: 0.25 months
Contribution to Project: Protein purification, conjugation and isolation of IGF-carrier-conjugate.

Name: Brandon Johnston
Project Role: PhD Candidate, Graduate Research Assistant, MIT
Nearest Person-Month Charged: 0 months each year
Contribution to Project: Protein purification, conjugation and isolation of ILRA1 drug carrier-conjugate, completion of necessary trainings prior to commencing animal studies. Note: While Brandon worked considerably on this project this year, his funding came from alternate sources.

Name: Rami Chakroun
Project Role: Postdoctoral Associate, MIT
Nearest Person-Month Charged: 0 months each year

Contribution to Project: Synthesis, bioconjugation, and bioactivity confirmation of dendrimer-IGF-1 conjugates, completion of necessary trainings prior to commencing animal studies. Note: While Rami worked considerably on this project this year, his funding came from alternate sources.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report

9. APPENDICES: *Nothing to Report*