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TITLE: Synovial Macrophage Targeting Immunomodulatory Therapies for Post-Traumatic Osteoarthritis

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14. ABSTRACT

The primary hypothesis of the project is that direct delivery into the joints of a Folate Receptor-2 (FR-2) targeting drug carrier loaded with zoledronate (ZA), a bisphosphonate (BP) can selectively eliminate a subset of activated synovial macrophages, modulate joint inflammation, and modify disease outcomes of PTOA. The objectives of this project will be realized by two specific aims:

Aim 1: Develop and evaluate folic acid functionalized microparticle for targeted and sustained delivery of bisphosphonate to FR-2+ activated macrophages. The goal of aim 1 is to develop polymer microparticle drug carrier which will selectively target activated macrophages expressing FR-2. The influence of selective removal of activated macrophages will be assessed by comparing inflammation state of the macrophages pre- and post- treatment.

Aim 2: Detect presence of activated macrophages and evaluate therapeutic efficacy of macrophage targeting microparticles in a non-surgical, cyclical loading, clinically relevant mouse model of PTOA. ACL rupture will be induced in mice by **c**yclic mechanical loading of the knee joint. Similar to humans, this mouse model shows signs of rapid PTOA development following ACL rupture. Therapeutic benefits of the targeted drug carrier developed in Aim1 in the mouse model will be determined by comparing disease outcomes and joint health between control (saline treated) and targeted drug carrier treated groups.

15. SUBJECT TERMS None listed.

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1. **INTRODUCTION:**

   The primary hypothesis of the project is that direct delivery into the joints of a Folate Receptor-2 (FR-2) targeting drug carrier loaded with zoledronate (ZA), a bisphosphonate (BP) can selectively eliminate a subset of activated synovial macrophages, modulate joint inflammation, and modify disease outcomes of PTOA. The objectives of this project will be realized by two specific aims:
   Aim 1: Develop and evaluate folic acid functionalized microparticle for targeted and sustained delivery of bisphosphonate to FR-2+ activated macrophages. The goal of aim 1 is to develop polymer microparticle drug carrier which will selectively target activated macrophages expressing FR-2. The influence of selective removal of activated macrophages will be assessed by comparing inflammation state of the macrophages pre- and post- treatment.
   Aim 2: Detect presence of activated macrophages and evaluate therapeutic efficacy of macrophage targeting microparticles in a non-surgical, cyclical loading, clinically relevant mouse model of PTOA. ACL rupture will be induced in mice by **c**yclic mechanical loading of the knee joint. Similar to humans, this mouse model shows signs of rapid PTOA development following ACL rupture. Therapeutic benefits of the targeted drug carrier developed in Aim1 in the mouse model will be determined by comparing disease outcomes and joint health between control (saline treated) and targeted drug carrier treated groups.

2. **KEYWORDS:** Folate receptor 2, microparticles, nanoparticles, zoledronic acid, macrophages, inflammation, osteoarthritis, post-traumatic osteoarthritis (PTOA), bisphosphonates.

3. **ACCOMPLISHMENTS:**

   - **What were the major goals of the project?**

   The major goals as specified in the approved SOW are listed below. For Specific Aim 1 Major task 1 and 2 have been completed while work on Major Task is 3 is ongoing. For Specific Aim 2 Major Task 1 is completed. Accomplishments supporting the advancement of Aims 1 and 2 has been provided in the report further.

Specific Aim 1: Develop and evaluate folic acid functionalized microparticle for targeted and sustained delivery of bisphosphonate to FR-2+ activated macrophages

- **Major Task 1** Fabrication of folic acid conjugated poly (ethylene glycol)-block-poly(lactic-co-glycolic acid) (PEG-PLGA) microparticles for encapsulation of calcium-zoledronate (Ca-ZA) nanoparticles. **Hypothesis/Objective:** Design microparticle with three design features: 1) size below 5 μm; 2) load calcium-zoledronate (Ca-ZA) nanoparticles in microparticles and 3) Functionalize folic acid ligand on microparticle surface. Date of Completion: March 2022; 100% completed.

- **Major Task 2** Quantify Ca-Za drug loading in microparticles and assess release rate of drug from PEG-PLGA microparticles. Hypothesis/Objective: To obtain a minimum sustained release rate of 4.5 ug/ml/day (minimum required daily dose of the ZA in mice) for a period of 2 weeks (time point of peak inflammation in PTOA animal model). 90% completed.

- **Major Task 3** Folate Receptor-2 (FR-2) expression on macrophages and selective uptake of folic acid conjugated microparticle by macrophages in vitro. Hypothesis/Objective: Identify expression of FR-2 in cultured macrophages and increase selective uptake of microparticles in FR-2 expressing microparticles. 70% completed.

Specific Aim 2 Detect presence of activated macrophages and evaluate therapeutic efficacy of macrophage targeting microparticles in a non-surgical, cyclical loading, clinically relevant mouse model of PTOA.

- Major Task 1 Establish mouse model of post traumatic osteoarthritis (PTOA) and image activated macrophage in vivo. Hypothesis/Objective: In cyclic mechanical mouse model of PTOA synovitis develops within 3-5 days and macrophage infiltration peaks at 2 weeks which should express FR-2 the marker of activated macrophages. Completed: Nov 2022.

- **What was accomplished under these goals?**

Accomplishments Under These Goals

The work in Year 1 has successfully advanced the goals of Aim 1 and 2 in alignment with the SOW.

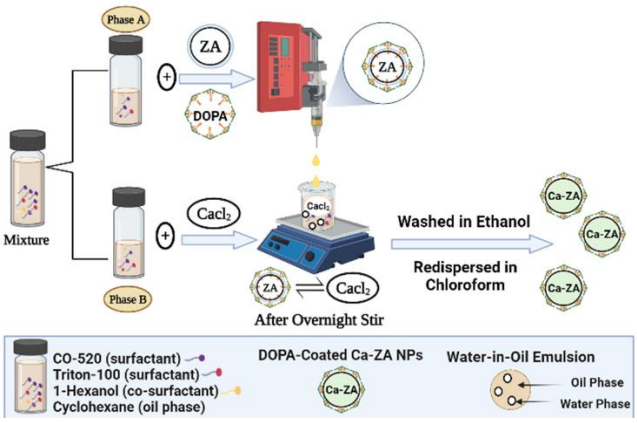

Figure 1: Reverse microemulsion method for making calcium-zoledronic Acid (Ca-ZA)

Design Calcium-Zoledronic acid (Ca-ZA) nanoparticles using reverse emulsion method: We have established a method to get calcium and zoledronic acid nanoparticles reproducibly. The method used to produce the nanoparticles is shown in Figure 1. Major findings: We have modified the method to increase drug loading in nanoparticles while keeping the size of the particle in the same range. The nanoparticles can now be made with zoledronic acid concentration ranging from 27 uM to 1 M. This gives us flexibility to load high doses of drug in the nanoparticle for sustained and controlled release.

Characterization of nanoparticle size and concentration, cell uptake in RAW264.7 murine macrophages and drug release: Ca-ZA nanoparticles sizes were characterized using dynamic light scattering and transmission electron microscope (TEM) (Figure 2). We also studied uptake of the nanoparticles by the RAW macrophage cell line. The nanoparticles were incubated in phosphate buffer saline of pH 7 and pH 5.5 and release rate of ZA from nanoparticles was quantified using ICP-OES for 5 days. Major findings: The Ca-ZA nanoparticles are found to consistently have size of 44 ± 3 nm and a zeta potential of -17.3 ± 1 mV. The nanoparticles are efficiently taken up by the cells within 4 h of incubation showing the feasibility of delivering nanoparticles to the macrophages (Figure 3). The nanoparticles showed a pH sensitive release at pH 5.5 while no release at pH 7.0 (Figure 3). This shows high potential for intracellular release of ZA from nanoparticles taken up targeted populations of macrophages.

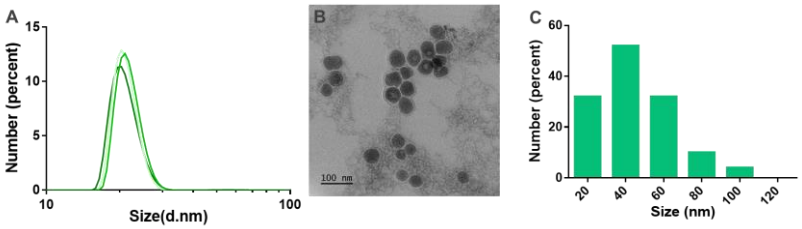

Figure 2. Characterization of Calcium-Zoledronic acid nanoparticles. Ca-ZA nanoparticles size measured using A) DLS B) TEM image of the nanoparticles. C)percent size distribution of nanoparticles counted using ImageJ using TEM images.

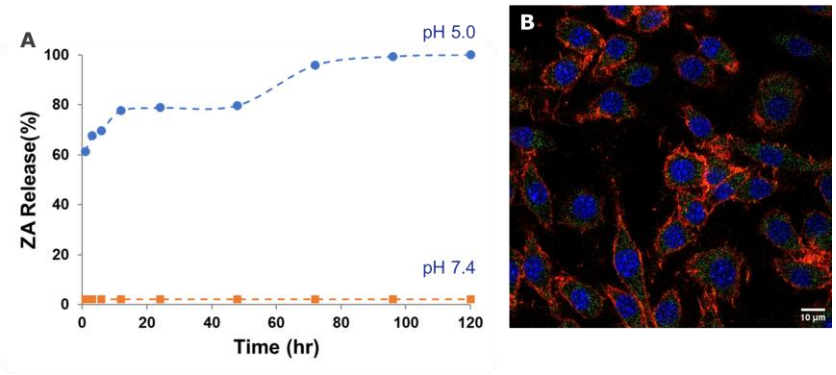

Figure 3. A) pH sensitive release of ZA from the nanoparticles. B) Cellular uptake of coumarin stained Za-CA nanoparticles by RAW macrophages after 4 h of incubation.

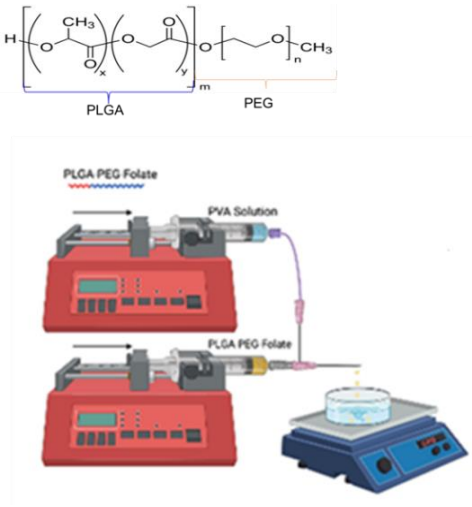

Figure 4: Coaxial phase separation method for making microparticles. DCM: Dichloromethane, PEG-PLGA: polyethylene glycol- poly(lactic-co-glycolic) acid, PVA: Polyvinyl Alcohol

Fabrication of PEG-PLGA microparticles: We have established a coaxial flow and phase separation method to make polymeric microparticles composed of diblock copolymer of polyethylene glycol- poly(lactic-co-glycolic) acid (PEG-PLGA) (Figure 4). Major findings: Changing different fabrications parameters we are able to reproducibly make particles of size range 4 – 40 µm with a narrow polydispersity of 0.2 to 0.3. Particularly we can make ~5 µm particle using various polymer concentration of 0.1 – 5 mg/ml. This is a critical finding and novelty of the process. Higher polymer concentration of the polymer will allow us to load higher amounts of the nanoparticles in the microparticles while still having particles of desired size range of 5-7 µm. Using model hydrophilic dye rhodamine (similar in hydrophilicity to Ca-ZA nanoparticles) we have verified higher encapsulation efficiency of 30, 57 and 70 % with increasing polymer concentration of 0.1, 1 and 5% w/v respectively and similar size range of 5-7 µm (Figure 5). Microparticles of 5 -7 µm are shown to be preferentially taken up by macrophages.

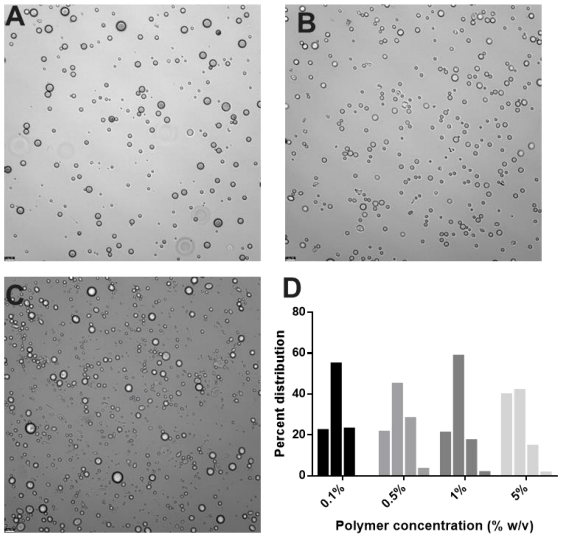

Figure 5: PEG-PLGA microparticles made using coaxial flow-phase separation. Microparticles made with A) 0.1% w/v polymer; B) 1% w/v polymer and C) 5% w/v polymer. D) Percent size distribution of the microparticles made using different polymer concentration. Scale bars are 20 µm.

Loading of calcium-zoledronic acid (Ca-ZA) nanoparticles in PEG-PLGA microparticles: We loaded Ca-ZA nanoparticles by adding to the polymer/solvent phase during PEG-PLGA microparticle synthesis. Using fluorescently stained microparticles and nanoparticles, EDAX analysis and FTIR (Figure 6). Major findings: We observed controlled release of nanoparticles from microparticles and their

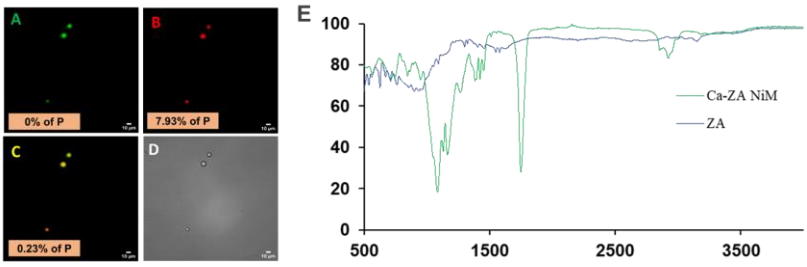

Figure 6. *Encapsulation of nanoparticles in microparticles(A) Coumarin stained microparticles, (B) Rhodamine-stained nanoparticles, (C) Merged, (D) Brightfield. Values at right had corner show P = Phosphorus content as measured by EDAX. Presence of phosphorus indicated nanoparticles were present with microparticles. E) FTIR analysis of the nanoparticles in microparticles shows characteristic peaks for presence of ZA inside microparticles.*

uptake by RAW macrophages.

Detection of folate receptor 2 in primary macrophages isolated from human blood: Peripheral blood derived monocytes were differentiated to macrophages by culturing in presence of MCSF for 7 days. MCSF activated macrophages stained positive for CD14, CD163 which expressed FR-2 as detected by flow cytometry analysis.

Mice model of PTOA and assessment of pain: We have successfully established the mice model of PTOA. The mice knee is placed inside custom made cups and positioned between two platens of a Instron. The knee is cyclically loaded with 12 N force for 60 cycles leading to ACL rupture. This leads to increase in join laxity which verifies ACL rupture and establishment of a PTOA model (Figure 7). Major findings: In this model we find that pain in the injured knee remains elevated for the 3 days during acute phase which correlates with similar findings in ACL injuries leading to OA in humans.

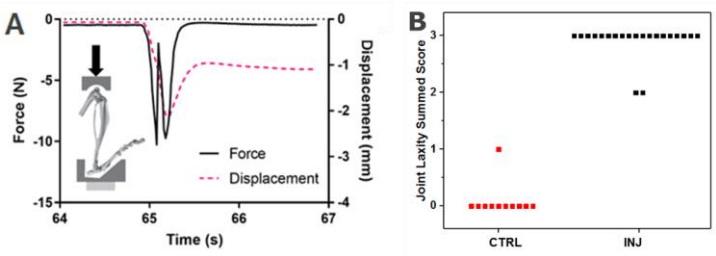

Figure 7. Time versus force and displacement curves showing one cycle of compressive loading with characteristic one-time release in force time curve upon ACL rupture. Inset: I*n vivo* loading setup for cyclic loading to induce non-invasive injury. **B)** Ruptured joints exhibit increased anterior-posterior joint laxity.

- **What opportunities for training and professional development has the project provided?**
  - Nothing to Report
- **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** ?

Unmet Goals to be Pursued in Year 2. No changes are included to the original plans.

1. Assess selective binding of folic acid microparticles to FR-2+ macrophages: fluorescein isothiocyanate (FITC) loaded microparticle uptake in macrophages will images using fluorescent microscopy and compared to controls where FR-2 will be pre-blocked using excess of folic acid.

2. Study presence of FR-2+ macrophages in animal model of OA via live animal imaging.

3. Study biodistribution and retention of folic acid functionalized microparticles using live animal imaging in joint after ACL rupture and injury in mice model of OA.

4. Intraarticular delivery of therapeutics: ZA loaded folic acid functionalized microparticle will be delivered directly to the mice joint and should target FR-2 expressing macrophages in ACL-rupture or injured mice joint to selectively eliminate them while modifying disease course. Longitudinal pain assessments and histology will be done to assess the effectiveness of therapy in modifying the disease outcome.

4. **IMPACT:**

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

        - Nothing to Report

    - **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:**

- **Changes in approach and reasons for change**
  - Nothing to report
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - Nothing to report
- **Changes that had a significant impact on expenditures**
  - Nothing to report
- **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:**

- **Publications, conference papers, and presentations**
  - **Journal publications.** Nothing to report
  - **Books or other non-periodical, one-time publications.** Nothing to report
- **Other publications, conference papers, and presentations.**

1. Sagoe, P., Espiritusanto Y., Zhang K., Gilbert A., Jain E. "Folic acid conjugated and zoledronic acid loaded microparticles for targeted drug delivery to activated macrophages and treatment of osteoarthritis" Invited Talk , ACS Northeast Regional Annual Meeting Rochester, NY Oct 02-05, 2022.

2. Sagoe, P., Espiritusanto Y., Zhang K., Gilbert A., Jain E. "Folic acid conjugated and zoledronic acid loaded microparticles for targeted drug delivery to activated macrophages and treatment of osteoarthritis" Poster Presentation, Controlled Release Society Annual meeting Montreal, Canada July 11-15, 2022.

3. Espiritusanto Y., Zhang K., Gilbert A., Jain E. "Folic acid conjugated and zoledronic acid loaded microparticles for targeted drug delivery to activated

macrophages and treatment of osteoarthritis" Poster Presentation, Society for Biomaterials Annual meeting Baltimore Marriott Waterfront April 27-30, 2022.

- **Website(s) or other Internet site(s)**
  Nothing to report

- **Technologies or techniques**
  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	*Era Jain*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Jain has worked on all aspects of the project. She supervises data collection and analysis, submitted abstracts and manuscripts and mentored graduate students .*
Funding Support:	

Name:	*Paul Sagoe*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Mr Sagoe has worked on all aspects of the project and generated all*

	the data included in the report including the microparticles, nanoparticles.
Funding Support:	

Name:	*Kaixiang Zhang*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Mr. Zhang has worked on establishing protocols for the animal model of osteoarthritis and characterizing pain in the animal model.*
Funding Support:	

Name:	*Qiu Wang*
Project Role:	*Consultant (Associate Professor, Measurement and Research Methodology, Department of Higher Education, Syracuse University)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0.5*
Contribution to Project:	*Biostatistician; Dr. Wang assists with determination of sample size, power calculations and statistical analysis of data.*
Funding Support:	

- **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**

    - **COLLABORATIVE AWARDS:**

9. **APPENDICES:** 3 conference abstracts as listed in products are attached as appendices

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

Yohely Espiritusanto[1], Kaixiang Zhang[1], Amelia Gilbert[2], *Era Jain[1]
1.Department of Biomedical & Chemical Engineering and BioInspired Institute, Syracuse University, Syracuse, NY
2. Department of Biomedical Engineering, Rochester Institute of Technology, Rochester, NY

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**.**

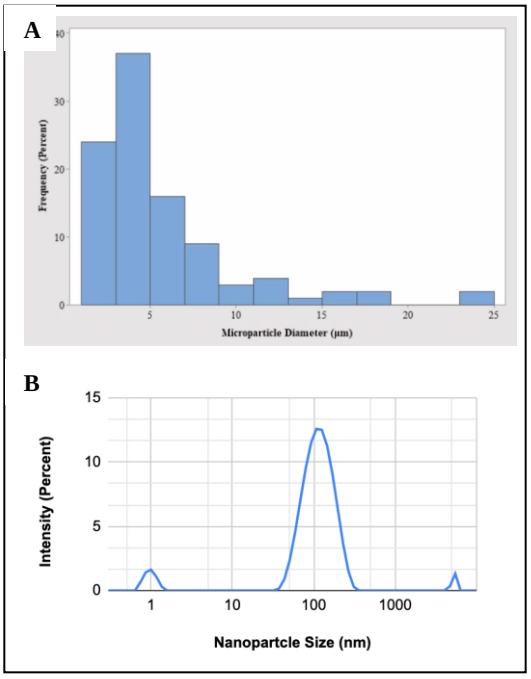

Figure 1. A) A histogram displaying the frequency of microparticle diameters. Microparticles were made using 5% PVA with 10ml/hr flow rate, a 30G needle gauge and 1% PEG-PLGA with 0.1ml/hr flow rate microparticles. **B)** The size distributions of the nanoparticles as obtained by DLS results.

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.

View Abstract

CONTROL ID: 3761582

TITLE: Synovial Macrophage Targeting Microparticles for Treatment of Post-Traumatic Osteoarthritis

PRESENTATION TYPE: Poster

CURRENT CATEGORY: Delivery Vehicle | Route/Target of Delivery | Type of Delivery Agent

AUTHORS (FIRST NAME, LAST NAME): Paul N. Sago[1], Yohely Espiritusanto[1], Kaixiang Zhang[1], Era Jain[1]

INSTITUTIONS (ALL): 1. Biomedical and Chemical Engineering, Syracuse University, Syracuse, NY, United States.

ABSTRACT BODY:

Biography: Era Jain is an Assistant Professor in BMCE at Syracuse University. Her research is focused on developing immunomodulatory drug delivery systems for the treatment of OA.

Introduction: Synovial macrophages are now increasingly being recognized as a major contributor to osteoarthritis (OA) progression and pain [1,2]. In this study we have identified a population of activated macrophages expressing folate receptor-2 (FR-2) and designed folic acid conjugated and zoledronic acid (ZA) nanoparticles, loaded microparticles for targeted drug delivery to activated macrophages for OA treatment.

Methods: Polyethylene glycol – poly (lactic-co-glycolic acid) (PEG-PLGA) microparticles were synthesized through the coaxial microfluidic phase separation technique. Folic acid was conjugated to the microparticle surface via standard carbodiimide chemistry. Calcium-zoledronic (Ca-ZA) acid nanoparticles were synthesized with the reverse microemulsion method and characterized with dynamic light scattering and transmission electron microscope. Ca-ZA nanoparticle loaded microparticles were obtained by adding the nanoparticles to the DCM used for dissolving PEG-PLGA during microparticle preparation. The concentration of ZA released from microparticles was determined by using inductively coupled plasma – optical emission spectroscopy. Mice model of OA and FR-2+ macrophages. Anterior cruciate ligament (ACL) rupture was induced in mice by cyclic mechanical loading of the knee joint to simulate post-traumatic OA development in humans following ACL rupture [3]. At 2 weeks mice were sacrificed and knee tissue was processed for immunostaining.

Results: The size of PEG-PLGA microparticles could be controlled between 7 to 40 μm depending on the inner needle gauge and flow rate of the inner and outer phases. The average size of Ca-ZA nanoparticles was 170 nm. Folic acid conjugated and Ca-ZA nanoparticles loaded PEG-PLGA microparticles sustainably released the encapsulated drug over 14 days. Immunostaining of knee tissue from mice showed the presence of FR-2+ macrophages at 2 weeks post-induction of ACL rupture in the mice model.

Conclusions/Implications: Folic acid conjugated PEG-PLGA microparticles can potentially be used for targeted delivery to activated synovial macrophages expressing FR-2 in mice model of PTOA.

Learning Objective 1: Evaluation of macrophage targeting as potential therapeutics for OA

Activated macrophage targeting nanoparticles-in-microparticles for treatment of post-traumatic osteoarthritis

Presenting author: Era Jain
Co-authors: Paul Nana Kwame Sago, Yohely Espiritusanto
Department of Biomedical & Chemical Engineering and BioInspired Institute, Syracuse University, Syracuse, NY

Introduction: Synovial macrophages are now increasingly being recognized as a major contributor to osteoarthritis (OA) progression and pain [1,2]. In this study we have identified a population of activated macrophages expressing folate receptor-2 (FR-2) and designed folic acid conjugated and zoledronic acid (ZA) nanoparticles, loaded microparticles for targeted drug delivery to activated macrophages for OA treatment.

Methods: Polyethylene glycol – poly (lactic-co-glycolic acid) (PEG-PLGA) microparticles were synthesized through coaxial microfluidic phase separation technique. Folic acid was conjugated to microparticle surface via standard carbodiimide chemistry. Calcium-zoledronic (Ca-ZA) acid nanoparticles were synthesized with reverse microemulsion method and characterized with dynamic light scattering and transmission electron microscope. Ca-ZA nanoparticle loaded microparticles were obtained by adding the nanoparticles to the DCM used for dissolving PEG-PLGA during microparticle preparation. The concentration of ZA released from microparticles was determined by using inductively coupled plasma – optical emission spectroscopy. Nanoparticle loaded microparticles were incubated with polarized macrophages expressing FR-2+. Uptake if particles in to the macrophages was observed using confocal imaging.

Results: Size of PEG-PLGA microparticles could be controlled between 7 to 40 µm depending on inner needle gauge and flow rate of the inner and outer phase. The average size of Ca-ZA nanoparticles was 170 nm. Folic acid conjugated and Ca-ZA nanoparticles loaded PEG-PLGA microparticles sustainably released the encapsulated drug over 14 days**.** The particles could selectively target FR-2+ activated macrophages and deliver the drug.

Conclusion: Folic acid conjugated PEG-PLGA microparticles can potentially be used for targeted delivery to activated synovial macrophages expressing FR-2 in mice model of PTOA.

Acknowledgments: This work was supported by DoD Discovery Award W81XWH-22-1-0020

References: [1] Sellam J and Berenbaum F, Nature Reviews Rheumatology 2010: 625-635. [2] Kraus V B, et al., Osteoarthritis and Cartilage 2016: 1613-162. [3] Berke I, et al., Osteoarthritis and Cartilage 2021: 248-256.

Presenter Biography: Era Jain is an Assistant Professor in BMCE at Syracuse University. Here research is focused at developing immunomodulatory drug delivery systems for treatment of OA.

Learning Objective: Evaluation of macrophage targeting as potential therapeutics for OA