AWARD NUMBER: W81XWH-15-1-0144

TITLE: New Strategies in Targeted Interventions for Posttraumatic Osteoarthritis (PT-OA)

PRINCIPAL INVESTIGATOR: Caroline Dealy, PhD

RECIPIENT: University of Connecticut
Farmington, CT 06032

REPORT DATE: August 2016

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2016	Annual	1 Jul 2015 - 30 Jun 2016

4. TITLE AND SUBTITLE

New Strategies in Targeted Interventions for Posttraumatic Osteoarthritis (PT-OA)

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0144

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Caroline Dealy, PI

E-Mail:dealy@uchc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Connecticut
Health Center
263 Farmington Avenue
Farmington CT 06030-5335

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Materiel 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

The subject of this project is Post-Traumatic Osteoarthritis (PT-OA), which is a form of Osteoarthritis (OA) with particularly rapid onset that leads to painful and disabling disease in otherwise healthy and relatively young adults, including military service members who have sustained traumatic joint injuries. This project will provide mechanistic proof-of-concept for our hypothesis that stimulating anabolic cartilage responses, and inhibiting catabolic ones, will slow the onset or progression of PT-OA following traumatic joint injury. The project will investigate the mechanistic involvement of EGFR growth factor signaling in mediating anabolic and catabolic responses in articular cartilage, and will develop strategies for modulating EGFR signaling in the joint as a means to control EGFR-mediated anabolic and catabolic responses. The impact of the project is to provide proof-of-concept support for a new mechanistically-based therapeutic intervention for PT-OA.

15. SUBJECT TERMS

Post-traumatic osteoarthritis, osteoarthritis, articular cartilage, anabolic, catabolic, mechanism, EGFR

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
U	U	U	UU	66	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

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

The subject of this project is Post-Traumatic Osteoarthritis (PT-OA), which is a form of Osteoarthritis (OA) with particularly rapid onset that leads to painful and disabling disease in otherwise healthy and relatively young adults, including military service members who have sustained traumatic joint injuries. This project will provide mechanistic proof-of-concept for our hypothesis that stimulating anabolic cartilage responses, and inhibiting catabolic ones, will slow the onset or progression of PT-OA following traumatic joint injury. The project will investigate the mechanistic involvement of EGFR growth factor signaling in mediating anabolic and catabolic responses in articular cartilage, and will develop strategies for modulating EGFR signaling in the joint as a means to control EGFR-mediated anabolic and catabolic responses. The impact of the project is to provide proof-of-concept support for a new mechanistically-based therapeutic intervention for PT-OA.

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

Post-traumatic osteoarthritis, osteoarthritis, articular cartilage, anabolic, catabolic, mechanism, EGFR

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.

Goal 1: Determine if growth-factor mediated activation of EGFR signaling stimulates a progenitor response in the mouse joint, and if this progenitor response slows surgically-induced PT-OA progression in mouse models.

Target date for completion was 12 months (10 months + 2 months to compile data and publish results). Significant progress was made during the reporting period (see Accomplishments, below). Anticipated target for completion is 6-8 months + 2 months to compile data and publish results.

Major Goal 2: Determine if inhibition of EGFR signaling inhibits catabolic responses that slow surgically-induced PT-OA progression in mouse models.

Target date for completion is 18 months (12/30/16).

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.

Accomplishments relevant to Goal 1: To determine if growth-factor mediated activation of EGFR signaling stimulates an anabolic progenitor response in the mouse joint, and if this progenitor response slows surgically-induced PT-OA progression in mouse models.

1). Goal 1 - Major activity #1

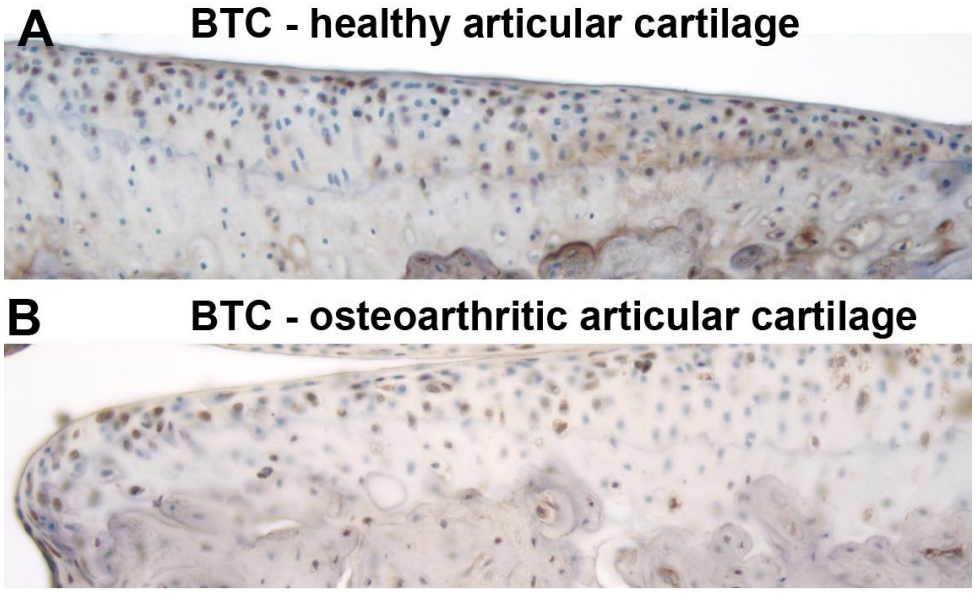
The role of the EGFR family of growth factor signaling molecules in mediating anabolic progenitor cell responses in articular cartilage has only recently been revealed (by the Dealy lab), and the mechanism by which it does so is unknown. An important activity we have completed during this period is to confirm localization of betacellulin, the growth factor we have proposed for use in this project as an EGFR agonist, in the articular cartilage of mouse knee joints.

2) Objective

Immunohistochemistry was used to examine the localization of betacellulin in samples of murine knee articular cartilage, to validate the mechanistic relevance of betacellulin to our hypothesis, and to validate our choice of betacellulin as is the optimal EGFR agonist.

3) Results

We confirmed presence of abundant betacellulin in healthy articular cartilage (brown stained cells in A below) and also found that lower levels of betacellulin are present in osteoarthritic articular cartilage (see B below). This confirms the mechanistic relevance of betacellulin to our hypothesis, and validates our choice of betacellulin as is the optimal EGFR agonist in articular cartilage.



CONTINUED:

Accomplishments relevant to Goal 1: To determine if growth-factor mediated activation of EGFR signaling stimulates an anabolic progenitor response in the mouse joint, and if this progenitor response slows surgically-induced PT-OA progression in mouse models.

1. Goal 1 - Major activity #2

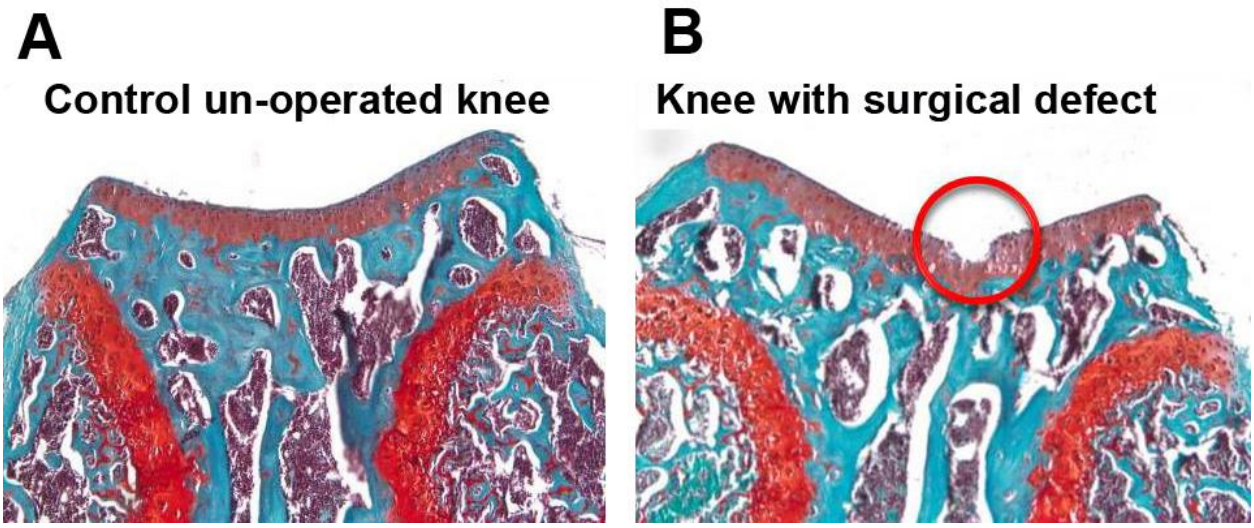
Post-traumatic osteoarthritis occurs following injury to the joint, and is characterized by rapid onset of disease signs which lead to disabling disease in typically healthy and often young individuals. The classic small animal model of PT-OA is to surgically cut the internal ligaments of the joint that hold the ends of the femur and tibia together. When these ligaments are cut, the joint becomes unstable, eventually leading to secondary articular cartilage damage due to uneven loading. This is a well-established model and we and others use it extensively for PT-OA studies (see also Goal 2 progress, below). However, a deficiency of this model is that it cannot be used to assess the ability of a test agent to prevent PT-OA, since PT-OA will occur as long as the ligaments are cut whether a test agent is present or not. To address this deficiency we developed a complementary mouse PT-OA model in which a discrete defect is made directly in the articular cartilage using a fine microsurgical tool, leaving the joint ligaments intact.

2) Objective

As a complementary approach to PT-OA induction via ligament transection, we used surgery to directly create a defined articular cartilage defect as a means to induce PT-OA. A custom-made device was used to create reproducible defects of consistent size and depth in knee articular cartilage. Joints were harvested at various times later and PT-OA progression assessed.

3) Results

Fig A below shows the articular cartilage of the normal non-operated knee (transactional view). Fig B shows the articular cartilage defect created by our defect surgery. These results demonstrate the feasibility of this approach for generating reproducible, critical size discrete defects in the articular cartilage. Critical size defects do not self-repair on their own and lead to PT-OA. In the context of another project, studies are in progress using this model to test the hypothesis that genetic EGFR signal activation promotes a progenitor cell response in articular cartilage that slows PT-OA progression. This complementary work is important progress relevant to completion of Goal 1.



CONTINUED:

Accomplishments relevant to Goal 1: To determine if growth-factor mediated activation of EGFR signaling stimulates an anabolic progenitor response in the mouse joint, and if this progenitor response slows surgically-induced PT-OA progression in mouse models.

1) Major Activity #3

Intra-articular injection offers a means to deliver bioactive agents like growth factors directly into the joint. However a means to prolong the stability and release of the growth factor is needed to make this a clinically feasible approach. Growth factor delivery via microspheres injected into the joint offers a solution to this problem. An important activity relevant to Goal 1 which is currently underway is to generate microspheres of a bio-compatible material that will be used to deliver the betacellulin growth factor. The material we have chosen is PLGA, a FDA approved material.

2) Objective

In order to safely deliver the growth factor into the joint and provide prolonged release, we are currently generating PLGA-microspheres which will encapsulate the EGFR agonist, betacellulin.

3) Results

We believe that generating the PLGA spheres we need for this project ourselves is far preferable to outsourcing them commercially, as it gives us control over the quality and quantity of the final product, increasing efficiency and reducing wastage. Accordingly, during the period of reporting, Dr Dealy established a collaboration with Dr Syam Nukavarapu, a materials scientist and tissue engineer at UConn, who is both very skilled and very familiar with generating microspheres of biomaterials including PLGA for growth factor delivery. Dr. Nukavarapu is assisting us (at no cost) in generating the spheres for this project. As in the approved proposal, a double emulsion step is used to encapsulate the growth factor, and in vitro release kinetics will be assessed prior to use. Making the spheres ourselves is not only better in terms of quality but also is cost-effective as students who are funded from other programs (for example, campus diversity programs) who are available to do this work at no cost as a research project. This also adds to the multiple training opportunities that this project has already provided to our academic community (see also Training Opportunities, below). As making our own spheres will improve the quality of the research results and hence increase the impact of the project overall, our progress in making the spheres ourselves is an important accomplishment relevant to Goal 1.

Please see Appendix for a Letter of Support from Dr Nukavarapu and his CV

Accomplishments relevant to Goal 2: To determine if inhibition of EGFR signaling inhibits catabolic responses that slow surgically-induced PT-OA progression in mouse models.

1) Major Activity #1

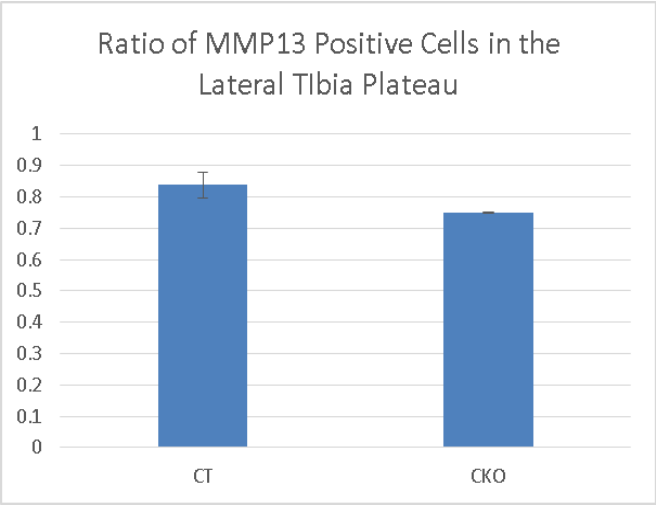
Catabolic responses to articular cartilage injury play a major role in the degradative changes that occur in damaged articular cartilage in PT-OA disease. EGFR signal activation is a known trigger for induction of these catabolic responses, which include reduced synthesis of cartilage matrix proteins and increased synthesis of matrix degradative enzymes. Accordingly, we propose that inhibiting EGFR signal activity will slow PT-OA progression. Studies to test this hypothesis using chemical EGFR inhibitors, injected into the joints of osteoarthritic mice, are proposed in Goal 2, which has an original target completion of 18 months, which will likely be extended as a result of our anticipated no-cost extension request. Importantly, however, we have tested our hypothesis in a complementary fashion in the context of a different project, and our results are directly relevant to this Goal (see below).

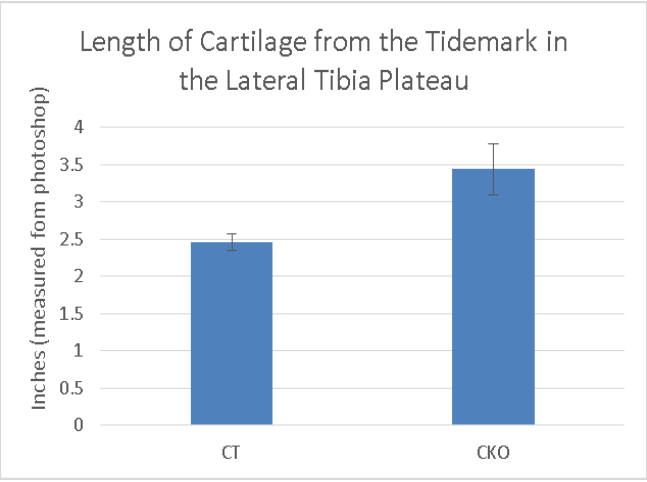
2) Objective

In a complementary test of the hypothesis that inhibiting EGFR signal activity will slow PT-OA progression, in the context of another project, we examined the progression of surgically induced PT-OA in transgenic mice with cartilage-specific EGFR loss. We predicted that PT-OA progression would be slower in the mice without EGFR signal activity.

3) Results

Indeed, we found that PT-OA disease signs were less severe in mice without EGFR signaling than in normal mice. Our quantitative summaries are shown below. We found that the levels of the degradative enzyme MMP13 are lower in EGFR-deficient mice after PT-OA induction surgery compared to normal mice. We also found that the articular cartilage was thicker in EGFR-deficient mice after PT-OA surgery compared to wildtype mice after PT-OA surgery. These results confirm our hypothesis that absence of EGFR signaling slows PT-OA disease progression. This is important proof-of-concept to support the chemical inhibitor experiments that will be carried out in Goal 2. These results were compiled into a student research paper (see Appendix). Ultimately, we anticipate that these results will be combined with data from Goal 2 of this project to generate a substantial research paper.





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.

This project provided training opportunity for the following individuals:

1. A Master's candidate in UConn's Professional Science Masters' program, who did a semester-long mentored independent study on this project.

2. A post-baccalaureate student who desired additional research skills and experience to prepare for PhD-Medical School and a future career as an Academic Scientist-Clinician.

3. An undergraduate honors student who did a summer internship on this project and presented her research in an oral talk at a University student symposia. This student will also be carrying out her Honor's thesis research on this project.

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.

The research results were disseminated in two ways:
1. A research paper (see Appendix) written by the Masters' student as a component of his requirement for his independent study course, which will ultimately be expanded into a full-length publication.

2. An oral presentation was given by the summer research honors student at a University-wide student symposium.

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

A a no-cost extension request is being prepared to extend the duration of the project by 8-12 months. A no-cost extension is possible because we have been extremely careful with spending, and because Dr Dealy is able to leverage personnel and resources at no cost towards completion of this project. This includes a student who will work on this project at no cost. The no-cost extension will enable us to accomplish all of the goals of this project.

4. IMPACT: Describe 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).

We have recently obtained complementary evidence to support our hypothesis that reducing EGFR activity in late-stage PT-OA will slow disease progression (see page 7, Accomplishments relevant to Goal 2). As this is one of the major hypotheses in this project, this is an important accomplishment. Moreover, this evidence provides important proof-of-concept to support development of future therapeutics that act to block EGFR signaling in late stage PT-OA.

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:

The only change to report is that a no-cost extension request is being prepared to extend the duration of the project by 8-12 months. A no-cost extension is possible because we have been extremely careful with spending, and because Dr Dealy is able to leverage personnel and resources at no cost towards completion of this project. This includes a student who will work on this project at no cost. The no-cost extension will enable us to accomplish all of the goals of this project.

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.

We encountered delays in generating the PLGA spheres via commercial sources, which we have resolved by recruiting the assistance of a skilled materials scientist tissue engineer, Dr Nukavarapu, who is now teaching us how to make the spheres ourselves, at no cost. Making the spheres ourselves is far preferable to outsourcing them, because it gives us control of the quality and quantity of the final product, which will improve research results and hence overall impact of the project. It is also time and cost effective, as we can leverage student personnel to make the spheres at no cost. The delay in generating the spheres will not prevent us from accomplishing all of the goals of the project, as we will complete the project through a no-cost extension of 8-12 months which is currently being prepared.

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.

We have been careful in spending and have leveraged resources and personnel where possible to make progress on this project while conserving funds. This will ensure good progress going forward, and into the anticipated no-cost extension which will be requested.

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.

Significant changes in use or care of human subjects

NA

Significant changes in use or care of vertebrate animals

No changes

Significant changes in use of biohazards and/or select agents

No changes

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).*

Fisher, M., Sonokawa, M., Conroy, S., Shepard, J., Dealy, N. Reducing EGFR signal activity slows progression of post-traumatic osteoarthritis in a mouse model. In preparation:

  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).

Dissertation in partial fulfilment of Master's Degree: "Effect of EGFR Loss of Function on Osteoarthritis Disease Progression in a Transgenic Mouse Model". by Scott Conroy, M.S. Candidate, Applied Genomics, Professional Science Masters Program. Research paper submitted to fulfil requirements of Independent Study and Masters degree requirements. Please see Appendix.

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.*

Oral Presentation at UConn Biomedical Sciences Summer Research Symposium:

"A Critical Defect Model to Analyze the Role of EGFR Signaling in the Repair of Murine Osteoarthritic Cartilage", by Bridget Oei, Molecular Cell Biology Honors Program and Biomedical Sciences Summer Internship Program. Oral presentation. Please see Appendix.

- **Website(s) or other Internet site(s)**
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.

No web links to report

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

In complementary but relevant studies, a new mouse model of PT-OA induced by surgical generation of critical articular cartilage defects was generated. This model is complementary to the ligament transection model used in this project. In the context of other studies, the new model will be fully characterized and published as a resource to other investigators.

-
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.

No inventions 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.*

In complementary but relevant studies, a new mouse model of PT-OA induced by surgical generation of critical articular cartilage defects was generated. This model is complementary to the ligament transection model used in this project. An oral presentation on the model is in the Appendix. In the context of other studies, the new model will be fully characterized and published as a resource to other investigators. In the context of this project, this model will be a useful complement to the ligament transection model.

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".

Note: New Collaborator on the Project:

Dr Syam Nukavarapu, PhD (Assistant Professor of Biomedical Engineering, UConn) (0% effort) is a new collaborator on this project who is assisting us at no cost with making growth factor delivery spheres. Please see the Appendix for Dr Nukavarapu's Letter of Support and CV.

SEE NEXT PAGE FOR THE TABLE of OTHER PARTICIPANTS-

Name:	Caroline Dealy, PhD
Project Role:	PI
Nearest person month worked:	0.6 calendar months (5%)
Contribution to Project:	As PI, Dr Dealy directs the project and all of its activities.
Funding Support:	0.6 calendar months (5%) on this project
Name:	Melanie Fisher, PhD
Project Role:	Res Associate
Nearest person month worked:	12 calendar months (80%)
Contribution to Project:	Dr Fisher carries out the murine surgeries and conducts and/or oversees phenotypic analyses and statistical analyses of data
Funding Support:	12 calendar months (80%)
Name:	Mayuko Sonokawa, B.S
Project Role:	Res Technician
Nearest person month worked:	12 calendar months (20%), but no effort charged to this project
Contribution to Project:	Ms Sonokawa is assisting with phenotypic analyses
Funding Support:	Health Center Research Advisory Fund
Name:	Scott Conroy, M.S.
Project Role:	Student Independent Study Researcher
Nearest person month worked:	12 calendar months (20%)
Contribution to Project:	Mr Conroy assisted in complementary studies examining effects of EGFR loss on PT-OA progression in transgenic mice.
Funding Support:	Professional Science Masters Program
Name:	Bridget Oei, B.S candidate
Project Role:	Student Honors Researcher
Nearest person month worked:	12 calendar months (20%)
Contribution to Project:	Ms. Oei is carrying out her honors thesis research on this project and is assisting with phenotyping.
Funding Support:	Stamps Scholars Program, UConn

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.

A previously active grant, Development of a Therapy for Osteoarthritic Cartilage Damage using hESC-derived Chondrogenic Cells, is now closed.

What other organizations were involved as partners?
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.

Name:	Caroline Dealy, PhD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.6 calendar months (5%)
Contribution to Project:	As PI, Dr Dealy directs the project and all of its activities.
Funding Support:	0.6 calendar months (5%) on this project
Name:	Melanie Fisher, PhD
Project Role:	Res Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12 calendar months (80%)
Contribution to Project:	Dr Fisher carries out the murine surgeries and conducts and/or oversees phenotypic analyses and statistical analyses of data
Funding Support:	12 calendar months (80%)
Name:	Mayuko Sonokawa, B.S
Project Role:	Res Technician
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	12 calendar months (20%), but no effort charged to this project
Contribution to Project:	Ms Sonokawa is assisting with phenotypic analyses
Funding Support:	Health Center Research Advisory Fund
Name:	Scott Conroy, M.S.
Project Role:	Student Independent Study Researcher
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	12 calendar months (20%)
Contribution to Project:	Mr Conroy assisted in complementary studies examining effects of EGFR loss on PT-OA progression in transgenic mice.
Funding Support:	Professional Science Masters Program
Name:	Bridget Oei, B.S candidate
Project Role:	Student Honors Researcher
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	12 calendar months (20%)
Contribution to Project:	Ms. Oei is carrying out her honors thesis research on this project and is assisting with phenotyping.
Funding Support:	Stamps Scholars Program, UConn

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://ers.amedd.army.mil** for each unique award.

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

NOT APPLICABLE

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.

Material in the Appendix:

1. Letter of Support from Dr Syam Nukavarpu, collaborator
2. CV of Dr Syam Nukavarapu
3. Research Dissertation of Scott Conroy in partial fulfilment of Masters Degree and Independent Study Requirements
4. Oral presentation by Bridget Oei given at UConn Student Summer Research Symposium

University of Connecticut Health Center
Orthopedic Surgery, and Biomedical Engineering

August 30, 2016

Dear Dr. Dealy,
I am pleased to provide this letter of support for your DOD – supported project "New Strategies in Targeted Interventions for Post-Traumatic Osteoarthritis" which is ongoing in your laboratory. I am impressed by your progress on this project examining the therapeutic potential for EGFR signal modulation as an intervention for osteoarthritis. I am happy to assist you to help you develop PLGA-spheres for your project, which will be used to deliver EGFR growth factor ligand into the joints of mice with post-traumatic osteoarthritis.

My own area of research is in biomaterials and tissue engineering, and a focus in my own laboratory is development of novel scaffold materials including those containing PLGA, for the purpose of bone fracture repair and articular cartilage defect repair. As such, I share your interest in post-traumatic osteoarthritis, and am very excited by the novel ideas you have proposed to use PLGA spheres to deliver EGFR ligands into the joint, which I believe has significant therapeutic potential.

I am very experienced with working with PLGA, and I am particularly experienced in making spheres of various types in the micro and nano size range. I am also familiar with growth factor encapsulation and delivery using PLGA materials. I am happy to offer my assistance to provide training to your personnel to create the growth-factor-PLGA spheres and to carry out the release and delivery kinetics in your lab.

I have enjoyed our frequent discussions about the scientific and clinical challenges of osteoarthritis. Our research programs both benefit from these exchanges, and I look forward to our continued interactions on this and other projects of mutual interest.

Sincerely,

Syam Nukavarapu, Ph.D.
Assistant Professor
Orthopaedic Surgery
Biomedical Engineering
Materials Science & Engineering

University of Connecticut Health Center
263 Farmington Avenue
Farmington, CT 06030-3711
Ph: (860)-679-4183, Fax: (860)-679-1553
Email: syam@uchc.edu

CURRICULUM VITAE

Name Syam P. Nukavarapu, Ph.D.
Department of Primary Appointment Department of Orthopaedic Surgery
Institute for Regenerative Engineering
UCONN Health
263 Farmington Ave.
Farmington CT-06030-3711
Work Phone: (860)-679-4183
Cell- 484-767-5831
Fax- 860-679-1553
Email- syam@uchc.edu

Academic Appointments, including Joint Appointments at UConn Health.

Assistant Professor (In-Residence Track) **8/2008 – Present**
Department of Orthopaedic Surgery
UCONN Health
Farmington CT-06030

Assistant Professor **8/2009 – Present**
Institute for Regenerative Engineering
UCONN Health
Farmington CT-06030

Assistant Professor (Core Faculty) **9/2009 – Present**
Biomedical Engineering
UCONN School of Engineering
Storrs, CT 06269

Assistant Professor (Joint Appointment) **8/2008 – Present**
Materials Science and Engineering
UCONN School of Engineering
Storrs, CT 06269

Professional Experience prior to appointment at UConn Health

Post-doctoral Fellow **8/2005 – 7/2008**
Department of Orthopaedic Surgery
University of Virginia
Charlottesville, VA-22904

Post-doctoral Fellow **10/2003 – 7/2005**
Department of Materials Science & Engineering
Lehigh University
Bethlehem, PA-18015

Educational Background

Ph.D. **8/2003**
"Multifunctional Nanocomposites"
Materials Research Center
Indian Institute of Science (IISc)
Bangalore, India
Master of Science (M.Sc.) **5/1998**
General Chemistry
University of Hyderabad
Hyderabad, TS, India
Bachelor of Science (B.Sc.) **5/1996**
Physics, Chemistry and Mathematics
Nagarjuna University
Nagarjuna Nagar, AP, India

Print and Oral Scholarship:

* represents Dr. Nukavarapu as the corresponding author and underlined names are his trainees.

1. a. List of Publications in Peer-Reviewed Journals: Original Research Articles

1. Amini, A.R., Xu, T.O., Chidambaram, R., **Nukavarapu, S.P.*** Oxygen Tension Controlled Matrices with Osteogenic and Vasculogenic Cells for Vascularized Bone Regeneration In Vivo. *Tissue Eng Part A, 2016, 22, 610-620.* ***(Note- featured on cover)*** *Impact Factor-4.448*
2. Majumdar, S., Pothirajan, P., Dorcemus, D., **Nukavarapu, S.P.**, Kotecha, M. High Field Sodium MRI Assessment of Stem Cell Chondrogenesis in a Tissue-Engineered Matrix. *Ann Biomed Eng., 2016, 44, 1120-1127*. ***(Note- accepted in 2015)*** *Impact Factor-3.195*
3. Mikael, P.E., Xin, X., Urso, M., Jiang, X., Wang, L., Barnes, B., Lichtler, A.C., Rowe, D.W., **Nukavarapu S.P.*** A Potential Translational Approach for Bone Tissue Engineering Through Endochondral Ossification. *IEEE Eng Med Biol Soc., 2014*, *3925-3928. Impact Factor-2.057*
4. Mikael, P., Amini, A.R., Basu, J., Arellano-Jimenez, M.J., Laurencin, C.T., Sanders, M., Carter, B.C., **Nukavarapu, S.P**.* Functionalized Carbon Nanotube Reinforced Scaffolds for Bone Regenerative Engineering: Fabrication, In Vitro and In Vivo Evaluation. *Biomedical Materials., 2014, 9, 035001.* ***(Note- Editor's pick, 2014)*** *Impact Factor-3.697*
5. Pothirajan, P., Dorcemus, D., **Nukavarapu, S.P.**, Kotecha, M.* True MRI Assessment of Stem Cell Chondrogenesis in a Tissue Engineered Matrix. *IEEE Eng Med Biol Soc., 2014*, *3933-3936*. *Impact Factor-2.057*
6. Amini, A.R., **Nukavarapu, S.P**.* Oxygen Tension Controlled Matrices for Enhanced Osteogenic Cell Survival and Performance. *Ann Biomed Eng., 2014, 42, 1261-1270. Impact Factor-3.195*
7. Igwe, J., Mikael, P., **Nukavarapu, S.P.*** Design, Fabrication and In Vitro Evaluation of a Novel Polymer-Hydrogel Hybrid Scaffold for Bone Tissue Engineering. *J Tissue Eng Regen Med., 2014, 8, 131–142. Impact Factor-5.199*

8. Amini, A.R., Adams, D., Laurencin, C.T., **Nukavarapu, S.P**.* Optimally Porous and Biomechanically Compatible Scaffolds for Large Area Bone Regeneration. *Tissue Eng Part A., 2012, 18, 1376-1388. Impact Factor-4.448*
9. Amini, A.R., Laurencin, C.T., **Nukavarapu, S.P.*** Differential Analysis of Peripheral Blood- and Bone Marrow-Derived Endothelial Progenitor Cells for Enhanced Vascularization in Bone Tissue Engineering. *J Orthop Res., 2012, 30, 1507-1515. Impact Factor-2.986*
10. **Nukavarapu, S.P.***, Amini, A.R. Optimal Scaffolds and Effective Progenitor Cells for the Regeneration of Vascularized Bone. *IEEE Eng Med Biol Soc., 2011, 2464-2467. Impact Factor-2.057*
11. Taylor, E.D., Nair, L.S., **Nukavarapu, S.P.**, McLaughlin, S., Laurencin, C.T. Novel Nanostructured Scaffolds as Therapeutic Replacement Options for Rotator Cuff Disease. *J Bone Joint Surg Am., 2010, 92, 170-179. Impact Factor-5.280*
12. Deng, M., Nair, L.S., **Nukavarapu, S.P.,** Jiang T., Kanner W.A., Li X., Kumbar, S.G., Weikel, A.L., Krogman, N.R., Allcock, H.R., Laurencin, C.T., Dipeptide-Based Polyphosphazene and Polyester Blends for Bone Tissue Engineering. *Biomaterials*, *2010 31(18), 4898-908*. *Impact Factor- 8.557*
13. Jiang, T., **Nukavarapu, S.P.**, Deng, M., Jabbarzadeh, E., Kofron, M.D., Doty, S.B., Abdel-Fattah, W.I., Laurencin, C.T. Chitosan-Poly(lactide-co-glycolide) Microsphere Based Scaffolds for Bone Tissue Engineering: In Vitro Degradation and In Vivo Bone Regeneration Studies. *Acta Biomaterilia, 2010, 6, 3457-3470. Impact Factor- 6.025*
14. Deng, M., Nair, L.S., **Nukavarapu, S.P.,** Kumbar, S.G., Brown, J.L., Krogman, N.R., Weikel, A.L., Allcock, H.R., Laurencin, C.T. Biomimetic, Bioactive Etheric Polyphosphazene-Poly(lactide-co-glycolide) Blends for Bone Tissue Engineering. *J. Biomed. Mater. Res. A., 2010, 92(1), 114-25. Impact Factor- 3.369*
15. Deng, M., Nair, L.S., **Nukavarapu, S.P.,** Kumbar, S.G., Jiang T, Weikel AL, Krogman NR, Allcock HR, Laurencin, C.T., In situ Porous Structures: A Unique Polymer Erosion Mechanism in Biodegradable Dipeptide-Based Polyphosphazene and Polyester Blends Producing Matrices for Regenerative Engineering. *Advanced Functional Materials, 2010, 20, 2794-2806. Impact Factor-11.805*
16. Krogman, N.R., Weikel, A.L., Kristhart, K.A., **Nukavarapu, S.P.**, Deng, M., Nair, L.S., Laurencin, C.T., Allcock, H.R. The Influence of Side Group Modification in Polyphosphazenes on Hydrolysis and Cell Adhesion of Blends with PLGA. *Biomaterials, 2009, 30, 3035-3041. Impact Factor-8.557*
17. Krogman, N.R., Weikel, A.L., Kristhart, K.A., **Nukavarapu, S.P.**, Nair, L.S., Laurencin, C.T., Allcock, H.R. Hydrogen Bonding in Blends of Polyesters with Dipeptide-Containing Polyphosphazenes. *J. Applied Polymer Science, 2009, 115, 431-437. Impact Factor-1.60*
18. Deng, M., Nair, L.S., **Nukavarapu, S.P.,** Kumbar S.G., Jiang, T., Krogman, N.R., Singh A., Allcock, H.R., Laurencin, C.T., Miscibility and In Vitro Osteocompatibility of Biodegradable Blends of Poly[(ethyl alanato) (p-phenyl phenoxy) phosphazene] and Poly(lactic acid-glycolic acid). *Biomaterials*, *2008, 29, 337-49. Impact Factor-8.557*
19. Kumbar S.G., **Nukavarapu, S.P.,** James, R., Nair, L.S., Laurencin, C.T., Electrospun Poly(lactic acid-co-glycolic acid) Scaffolds for Skin Tissue Engineering. *Biomaterials*, *2008, 29, 4100–4107. Impact Factor-8.577*

20. **Nukavarapu, S.P.,** Kumbar S.G., Brown, J.L., Krogman, N,R., Weikel A.L., Hindenlang, M.D., Nair, L.S., Allcock, H.R., Laurencin, C.T., Polyphosphazene/Nano-hydroxyapatite Composite Microsphere Scaffolds for Bone Tissue Engineering. *Biomacromolecules, 2008, 9(7), 1818-1825. Impact Factor-5.750*
21. **Nukavarapu, S.P.**, Wang, J., Pattnaik, R.K., Jain, H., Toulouse, J. Preform Fabrication and Drawing of $KNbO_3$ Modified Tellurite Glass Fibers. *J. Non-Cryst. Solids, 2006, 352, 519-523. Impact Factor-1.766*
22. Wang, J., **Nukavarapu, S.P.**, Kiang, K., Pattnaik, R.K., Toulouse, J., Jain, H. Source of Optical Loss in Tellurite Glass Fibers. *J. Non-Cryst. Solids, 2006, 352, 510-513. Impact Factor-1.766*
23. **Nukavarapu, S.P.,** Varma, K.B.R. Evolution of Ferroelectric $LiNbO_3$ Phase in a Reactive Glass Matrix ($LiBO_2$ – Nb_2O_5). *J. Non-Cryst. Solids, 2005, 351, 1455-1465. Impact Factor-1.766*
24. **Nukavarapu, S.P.** Varma, K.B.R. Crystallization Kinetics of the $LiBO_2$-Nb_2O_5 Glass Using Differential Thermal Analysis. *J. Am. Ceram. Soc., 2005, 88, 357-361. Impact Factor-2.107*
25. **Nukavarapu, S.P.,** Varma, K.B.R. Crystallization, Dielectric, Polar and Optical Characteristics of $Li_2B_4O_7$-SrO-Bi_2O_3-Nb_2O_5 Glasses and Glass Nanocomposites. *Phys. Chem. Glasses., 2003, 44, 365-375. Impact Factor-0.691*
26. **Nukavarapu, S.P.**, Takahashi, Y., Benino, Y., Fujiwara, T., Komatsu, T., Varma, K.B.R. Evolution and Characterization of Fluorite-like Nano $SrBi_2Nb_2O_9$ Phase in the Glass System SrO-Bi_2O_3-Nb_2O_5-$Li_2B_4O_7$. *J. Solid. State. Chem., 2003, 173, 209-215. Impact Factor-2.133*
27. **Nukavarapu, S.P.,** Varma, K.B.R. Structural and Dielectric Properties of Ferroelectric $Sr_{1-x}Ba_xBi_2(Nb_{0.5}Ta_{0.5})_2O_9$ and $Sr_{0.5}Ba_{0.5}Bi_2(Nb_{1-y}Ta_y)_2O_9$ Ceramics. *Mater. Res. Bull., 2003, 38, 195-206. Impact Factor-2.288*
28. **Nukavarapu, S.P.**, Subbanna, G.N., Varma, K.B.R. Evolution of Ferroelectric $SrBi_2Nb_2O_9$ Phase in the $Li_2B_4O_7$-SrO-Bi_2O_3-Nb_2O_5 Glass System. *Ferroelectrics*, *2002, 281, 135-150. Impact Factor-0.469*
29. **Nukavarapu, S.P.,** Varma, K.B.R. Nanocrystallization of $SrBi_2Nb_2O_9$ From Glasses in the System $Li_2B_4O_7$-SrO-Bi_2O_3-Nb_2O_5. *Mater. Sci. Eng. B., 2001, 90, 246-253. Impact Factor-2.169*
30. **Nukavarapu, S.P.,** Varma, K.B.R. Phase Evolution, Characterization and Impedance Spectroscopic Analysis of Nanocrystalline $SrBi_2Nb_2O_9$ in the Glassy $Li_2B_4O_7$ Matrix. *J. Nanosci. Nanotech., 2001, 1, 425-432. Impact Factor-1.556*
31. **Nukavarapu, S.P.** Varma, K.B.R. Dielectric, Structural and Ferroelectric Properties of Strontium Borate Glasses Containing Nanocrystalline Bismuth Vanadate. *J. Mater. Chem., 2001, 11, 1912-1918. Impact Factor-6.626*
32. **Nukavarapu, S.P.,** Varma, K.B.R., Lang, S.B. Dielectric Anomaly in Strontium Borate - Bismuth Vanadate Glass Nanocomposite. *J. Phys. Chem. Solids*, *2011, 62, 1299-1310. Impact Factor-1.853*
33. **Nukavarapu, S.P.,** Subbanna, G.N., Varma, K.B.R. Glass Nanocomposite of Strontium Bismuth Niobate and Lithium Borate: Structural and Dielectric Investigations. *Materials Letters, 2001, 47, 11-19. Impact Factor-2.489*

1.a. Peer Reviewed-Invited Review Articles

1. Dorcemus, D., **Nukavarapu S.P.*,** Tissue Engineering of Skeletal Tissues. Reference Module in Biomedical Sciences. Elsevier. *2014*, 10.1016/B978-0-12-801238-3.00027-1. (Note- Biomedical Science Encyclopedia) *Impact Factor-not available*
2. **Nukavarapu, S.P.***, Dorcemus, D. Osteochondral Tissue Engineering: Current Strategies and Challenges. *Biotechnology Advances*, *2013, 31, 706-721*. *Impact Factor-9.599*
3. Amini, A.R., Laurencin, C.T., **Nukavarapu, S.P**.* Bone Tissue Engineering: Recent Advances and Challenges. *Crit Rev Biomed Eng. 2012, 40, 363-408. Impact Factor-not available*
4. Amini, A.R., Wallace, J., **Nukavarapu, S.P.*,** Short-Term and Long-Term Effects of Orthopedic Biodegradable Implants. *Journal of Long-Term Effects of Medical Implants*, *2011, 21, 93-122. Impact Factor-0.00*
5. Mikael, P., and **Nukavarapu, S.P.*** Functionalized Carbon Nanotube Composite Scaffolds for Bone Tissue Engineering: Prospects and Progress. *Journal of Biomaterials and Tissue Eng. 2011, 1, 76-85*. *Impact Factor-2.066*
6. Nanotechnology and Orthopedics: a Personal Perspective. Laurencin, C.T., Kumbar, S.G., **Nukavarapu, S.P.,** *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 2009, 1(1), 6-10. Impact Factor- 4.494*
7. Kumbar, S.G., **Nukavarapu, S.P.,** James, R., Hogan, M.V., Laurencin, C.T. Recent Patents on Electrospun Biomedical Nanostructures: An Overview, *Recent Patents on Biomedical Engineering*, 2008, *1, 68-78. Impact Factor- 2.575*
8. Kumbar S.G., **Nukavarapu, S.P.,** James, R., Laurencin, C.T. Electrospun Nanofiber Scaffolds: Engineering Soft Tissues. *Biomed. Materials, 2008, 3(3), 34002, 1-15. Impact Factor- 3.697*
9. Kumbar, S.G., Bhattacharyya, S., **Nukavarapu, S.P.,** Khan, Y., Nair, L.S., Laurencin, C.T. *In Vitro* and *In Vivo* Characterization of Poly(organophosphazenes) for Biomedical Applications. *J. Inorg. Organomet. Polym. Mater., 2006, 16(4), 365-385*. *Impact Factor-1.160*

1. b. Peer Reviewed-Book Chapters

1. Stahl, T., Anslip, A., Lei. L., Santos, N.D., Nwachuku, E., DeBerardino, T., **Nukavarapu, S.P.*** "Osteochondral Tissue Engineering: Non-invasive Assessment of Tissue Regeneration" in "Magnetic Resonance Imaging in Tissue Engineering" Eds. Kotecha, Magin, Mao 2016, Wiley (Accepted-in press).
2. Joshi, S.U., Barbu, R.O., Carr-Reynolds, M., Barnes, B., **Nukavarapu, S.P.*** "Patient-derived and Intra-operatively formed Biomaterial for Tissue Engineering" in "Adult Stem Cells: Methods and Protocols" Eds. Di Nardo, Dhingra, Singla, Human Press/Springer, 2016. (Accepted-In press)
3. Francois, E., Dorcemus, D., **Nukavarapu, S.P.*** "Biomaterials and Scaffolds for Musculoskeletal Tissue Engineering" in "Engineering Musculoskeletal Tissues and Interfaces" Eds. Nukavarapu, Laurencin, Freeman, Woodhead Publishers, 2015, 3-23.
4. **Nukavarapu, S.P.***, Casettari L, Almobarak A, Luzzi A. "Hydrogels: Cell Delivery and Tissue Regeneration" in "Encyclopedia of Biomedical Polymers and Polymeric Biomaterials" Eds. Mishra, CRC Press, 2015, 3841-3852.

5. Mikael, P., **Nukavarapu, S.P**.* "Cell-Based Approaches for Bone Regeneration" in "Bone Graft Substitutes and Bone Regenerative Engineering" Eds. Laurencin, Jiang, ASTM, 2015, 97-116.
6. Mikael, P., Wallace, J., **Nukavarapu, S.P.*** "Nanotubes for Tissue Engineering" in "Nanomedicine: Technologies and Applications" Eds. Webster, Woodhead Publishing Ltd., 2012, 460-489.
7. Igwe, J., Amini, A., Mikael, P., Laurencin, C., **Nukavarapu, S.P.*** "Nanostructured Scaffolds for Bone Tissue Engineering" in "Active Implants and Scaffolds for Tissue Engineering" Eds. Zilberman, Springer, 2011, 169-192.
8. **Nukavarapu, S.P.**, Wallace, J., Elgendy, H., Lieberman, J., Laurencin, C. "Bone and Biomaterials" in "An Introduction to Biomaterials" Eds. Hollinger, CRC Press, 2011, 571-593.
9. **Nukavarapu, S.P.,** Kumbar, S.G., Nair, L.S., and Laurencin, C.T. "Nanostructures for Tissue Engineering/Regenerative Medicine" in "Biomedical Nanostructures, Eds. Gonsalves", K.E., Laurencin, C.T., Halberstadt, C., and Nair, L. S., John Wiley & Sons, Inc., 2008, 377-407.
10. **Nukavarapu, S.P.,** Kumbar, S.G., Merrell, J.G., and Laurencin, C.T. "Electrospun polymeric nanofiber scaffolds for tissue regeneration." in "Nanotechnology and Tissue Engineering: The Scaffold", Eds. Laurencin, C.T., and Nair, L.S., Taylor & Francis Group 2008, 199-219.
11. **Nukavarapu, S.P.,** Kumbar, S.G., and Laurencin, C.T. "Polyphosphazene scaffolds for tissue engineering" in "Polyphosphazenes for Biomedical Applications", Eds. Andrianov, Taylor & Francis Group 2008, 119-138.

1.c. Books:

1. **Nukavarapu, S.P.,** Liu, H., Deng, T., Oyen, M., Tamerler, C., "Advances in Structures, Properties and Applications of Biological and Bioinspired Materials", MRS F13 Symposium Proceedings, Cambridge Press, 2014-ISBN: 978-1-60511-598-6.
2. **Nukavarapu, S.P.,** Freeman. J., Laurencin, C.T., "Regenerative Engineering of Musculoskeletal Tissues and Interfaces", Woodhead Publishing, 2015-ISBN: 978-1-78242-301-0.

1. d. Patents:

1. **Nukavarapu, S.P.**, Laurencin, C.T., Amini, A., Dorcemus, D. "Gradient Porous Scaffolds". US Non-provisional patent application US20140178455 A1, Published 2013. ***(Note- 24 out of 30 claims are from Dr. Nukavarapu)***
2. Laurencin, C.T., **Nukavarapu S.P.,** Kumbar S.G., "Carbon Nanotube Composite Scaffolds for Bone Tissue Engineering". U.S. Patent 8614189, Issued 2014. **(*Note- 6 out of 16 claims are from Dr. Nukavarapu)***
3. Laurencin, C.T., Kumbar, S.G. **Nukavarapu, S.P.,** James, R., "Mechanically Competent Natural Polymer Based Bone Grafts for Bone Repair and Regeneration". US non-provisional patent application US2010/0249931A1, World patent application WO/2010/096199, Published 2010. ***(Note- 6 out of 22 claims are from Dr. Nukavarapu)***

2. a. Peer Reviewed Published Abstracts/Posters:

Note that presentation of selected abstracts is detailed below where the underlined names are his trainees.

1. Paiyz M, **Nukavarapu, S.P.*** In Vitro and In Vivo Evaluation of a Hybrid Matrix System for Bone Regeneration Through Endochondral Ossification. 10th World Biomaterials Congress Meeting, May 17- 22, 2016, Montreal, Canada.
2. Dorcemus D, **Nukavarapu, S.P.*** Formulation of a Co-differentiation Media for Osteochondral Tissue Engineering. BMES Annual Meeting, Oct 7-10, 2015, Tampa, FL.
3. Mikael, P., Dorcemus, D., Barnes, B., **Nukavarapu, S.P.***. Autologous Progenitor Cells for Bone Tissue Engineering, StemConn 2015, May 11, Hartford, CT.
4. Majumdar, S., Dorcemus, D., **Nukavarapu, S.P.,** Kotecha, M. Differentiating Engineered Bone from Cartilage Using Diffusion Tensor MRI for Osteochondral Tissue Engineering, TERMIS-AM Annual Meeting, Dec 13-16, 2013, Washington D.C.
5. Pothirajan, P., Dorcemus, D., **Nukavarapu, S.P.,** Kotecha M. High Field Sodium MRI for Early Stage In Vitro Assessment of GAG in Engineered Cartilage, TERMIS-AM Annual Meeting, Dec 13-16, 2014, Washington D.C.
6. Dorcemus D, Stephens CJ, Mikael P, Igew J, Amini A, **Nukavarapu, S.P.*** Clinically Translatable Strategies for Tissue Repair and Regeneration. EU-US Frontiers of Engineering (FOE) Meeting, National Academy of Engineering, Nov 10-12, Seattle, WA.
7. Stephens CJ, **Nukavarapu, S.P.*** Pateint-derived Biomaterial for Intra-operative Bone Tissue Engineering. Emerging Researchers National Conference in STEM, 2015 Washington D.C. (undergraduate student poster).
8. Mikael P, **Nukavarapu, S.P.*** Engineered Hydrogel System for Bone Regeneration Through Endochondral Ossification. Biomedical Engineering Society (BMES) Annual Meeting, 2014, San Antonio, TX.
9. Stephens CJ, Mikael P, **Nukavarapu, S.P.*** Patient-derived Biomaterial for Bone Regeneration. Biomedical Engineering Society (BMES) Annual Meeting, 2014, San Antonio, TX. (undergraduate student poster).
10. Aekins R, Dorcemus D, **Nukavarapu, S.P.*** Biodegradable Polyurethanes and Its Application in Tissue Engineering. Biomedical Engineering Society (BMES) Annual Meeting, 2014, San Antonio, TX. (undergraduate student poster).
11. Xin X, Jiang X, Wang L, Mikael P, Shin K, **Nukavarapu, S.P.,** Rowe D, Lichtler A. Culture of Human BM-MSC in Physiological O2 Improves Robustness of Bone Formation in a Mouse Calvarial Defect Model. American Society for Bone and Mineral Research (ASMBR) Annual Meeting, 2014, Houston, TX.
12. Dorcemus D, Mikael P, Stephens C, Lichtler A, Rowe D, **Nukavarapu, S.P.*** Completely Intra-operative Tissue Engineering Strategy for Tissue Repair and Regeneration. Gordon Research Conference on Musculoskeletal Biology and Bioengineering, 2014, Andover, NH.
13. Mikael P, Barnes B, **Nukavarapu, S.P.*** Autologusly Enriched Human Bone Marrow Aspirate for Bone Tissue Engineering. Society for Biomaterials 2014 Annual Meeting, Denver, CO.

14. Mikael P, **Nukavarapu, S.P.*** Hyaluronan-Fibrin Gel System for Cartilage-Mediated Bone Regeneration. Society for Biomaterials 2014 Annual Meeting, Denver, CO.
15. Pothirajan P, Dorcemus D, **Nukavarapu, S.P.,** Kotecha M. Identifying Contributions from Scaffolds, Cells and Extracellular Matrix in MRI of Polymer-Hydrogel-based Engineered Cartilage. Society for Biomaterials 2014 Annual Meeting, Denver, CO.
16. Dorcemus D, Mikael P, Bezwada RS, **Nukavarapu, S.P.*** Novel Absorbable Polyurethane Biomaterials and Scaffolds for Tissue Engineering. Materials Research Society Fall Meeting, 2013, Boston, MA.
17. Pothirajan P, Dorcemus D, **Nukavarapu, S.P.,** Kotecha M, Standardization of Parametric MRI Quantification for Stem Cell Based Tissue Engineered Cartilage, Stem Cell and Regenerative Medicine, Sept, 2013, University of Illinois at Chicago (UIC), Chicago, IL.
18. **Nukavarapu, S.P.*** Tissue Engineered Matrices for Large Area Bone Regeneration, Gordon Research Conference on Musculoskeletal Biology & Bioengineering, 2012, Andover, NH.
19. Arellano-Jiménez JM, Mikael P, Bogart J, **Nukavarapu, S.P.,** Laurencin CT, Carter BC. Microtomy of Reinforced Polymer Scaffolds. Microscopy Society of America Meeting, 2012.
20. Igwe J, Amini A, **Nukavarapu, S.P.*** Fabrication and Evaluation of a Novel Scaffold System with High-Density Cell Seeding for Bone Regeneration: An Investigation of Cell Density Enhanced Osteogenic Expression. Orthopaedic Research Society Annual Meeting, 2012.
21. Amini A, **Nukavarapu, S.P.*** Comparative Analysis of Endothelial Progenitor Cells Isolated from Peripheral Blood and Bone Marrow for Enhanced Vascularization in Bone Tissue Engineering Applications. Tissue Engineering and Regenerative Medicine International Society, 2011.
22. Mikale P, Amini A, Igwe J, **Nukavarapu, S.P.*** Carbon Nanotubes composite scaffolds for Bone Tissue Engineering: Mechanical and *In Vivo* Investigation. Biomedical Engineering Society (BMES) Annual Meeting, 2011, Hartford, CT.
23. **Nukavarapu, S.P.*** Two Pronged Approach for Engineering Vascularized Bone. Gordon Research Conference on Biomaterials & Tissue Engineering, 2011, Holderness, NH.
24. Amini A, Mikael P, Adams D, Laurencin CT, **Nukavarapu, S.P.*** Fully Osteoconductive and Mechanically Compatible Scaffolds for Effective Bone Regeneration. Orthopaedic Research Society Annual Meeting, 2011.
25. Igwe J, Laurencin CT and **Nukavarapu, S.P.*** Osteogenic and Mechanically Compatible Hybrid Grafts for *in situ* Bone Regeneration. Tissue Engineering and Regenerative Medicine International Society, 2010.
26. Wallace J, Mikael P, **Nukavarapu, S.P.*** Novel Biodegradable Composite Scaffolds for Bone Tissue Engineering. New England Science Symposium, 2010, Harvard Medical School, Boston, MA. (Medical student poster).
27. Mikael P, Basu J, **Nukavarapu, S.P.,** Laurencin CT and Carter B. Characterization of Carbon Nanotube Reinforced Polymer Scaffold for Bone Tissue Engineering. Microscopy Society of America Meeting, 2010.
28. **Nukavarapu, S.P.**, Kumbar SG and Laurencin CT. Novel Water Dispersible Carbon Nanotube Composite Scaffolds for Bone Tissue Engineering. Tissue Engineering and Regenerative Medicine International Society, December 7-10, 2008.

29. Harmon MD, Kumbar SG, James R, **Nukavarapu, S.P.**, Oredein OY, Burns C and Laurencin CT. Infection Resistant Polyphosphazene-PLGA Blend Electrospun Nanofiber Matrices for Wound Healing Applications. American Academy of Orthopaedic Surgeons Annual Meeting, 2008.
30. **Nukavarapu, S.P.**, Kumbar SG, Krogman N, Nair LS, Allcock HR and Laurencin CT. Fabrication and In-Vitro Evaluation of Novel Bone Regeneration Scaffolds Based on Polyphosphazene-Nano Hydroxyapatite Composites. Orthopedic Research Society Meeting, 2008.
31. **Nukavarapu, S.P.,** Krogman NR, Kumbar SG, Brown JL, Nair LS, Allcock HR and Laurencin CT. Novel Biodegradable Polyphosphazene-Nanohydroxyapatite Microsphere Scaffolds for Bone Tissue Engineering. Society for Biomaterials Meeting, 2007.

2. b. Invited presentations

i. At Professional Conferences:

Podium Presentation of Peer Reviewed Abstracts where the underlined names are his trainees:

1. Dorcemus D, **Nukavarapu, S.P.***. Inverse Gradient Matrix System for Osteochondral Tissue Engineering, 10th World Biomaterials Congress Meeting, May 17- 22, 2016, Montreal, Canada.
2. Mikale P, Xin X, Urso M, Jiang X, Wang L, Barnes B, Lichtler A, Rowe D, **Nukavarapu, S.P.*** A Potential Translational Approach for Bone Tissue Engineering Through Endochondral Ossification. IEEE Engineering in Medicine and Biology Meeting, 2014, Chicago, IL.
3. Pothirajan P, Dorcemus D, **Nukavarapu, S.P.,** Kotecha M. True MRI Assessment of Stem Cell Chondrogenesis in a Tissue Engineered Matrix. IEEE Engineering in Medicine and Biology Meeting, 2014, Chicago IL.
4. Dorcemus D, **Nukavarapu, S.P.*** Development of a Unique Scaffold System for Osteochondral Tissue Engineering. New England Science Symposium, 2014, Harvard Medical School, Boston, MA.
5. Dorcemus D, **Nukavarapu, S.P.*** Development and Investigation of a New Generation Matrix for Osteochondral Tissue Engineering. Society for Biomaterials 2014 Annual Meeting, 2014, Denver, CO.
6. Dorcemus D, **Nukavarapu, S.P.*** Unique Scaffold System for Osteochondral Tissue Engineering. Materials Research Society Fall Meeting, 2013, Boston, MA.
7. Mikael P, **Nukavarapu, S.P.*** Advanced Scaffold Design for Cartilage Mediated Bone Tissue Engineering. Materials Research Society Fall Meeting, 2013, Boston, MA.
8. Varma KBR, **Nukavarapu, S.P.,** Murugan GS, Paramesh G. Multifarious Glass Nanocrystal Composites. Materials Research Society of Singapore, ICMAT 2013, Singapore.
9. Amini A, **Nukavarapu, S.P.*** Peripheral Blood-Derived Endothelial Progenitor Cells for Vascularized Bone Tissue Engineering. Tissue Engineering and Regenerative Medicine International Society, 2013, TERMIS-EU, Istanbul, Turkey.

10. Amini A, **Nukavarapu, S.P.*** Scaffold Optimization and Pre-Vascularization Strategies for Large-Area Bone Regeneration. SFB Annual Meeting, 2013, Boston, MA.
11. Amini A, Laurencin CT, **Nukavarapu, S.P.*** Effective Progenitor Cell Populations for Vascularized Bone Tissue Engineering. IADR/AADR Annual Meeting, 2013, Seattle, WA.
12. Amini A, Laurencin CT, **Nukavarapu, S.P.*** Oxygen Tension Controlled Matrices for Enhanced Bone Tissue Engineering. Biomedical Engineering Society, 2012, Atlanta, GA.
13. Amini A, Laurencin CT, **Nukavarapu, S.P.*** Pre-Vascularization of Optimally-Porous Scaffolds for Enhanced Vascularization and Bone Formation In Vivo. Society for Biomaterials Annual meeting, 2012, New Orleans, LA.
14. Amini A, Laurencin CT, **Nukavarapu, S.P.*** Development and Evaluation of Optimized Scaffolds Pre-seeded with Effective Progenitor Combination for Vascularized Bone Regeneration. Orthopaedic Research Society annual meeting, 2012.
15. Amini A, Laurencin CT, **Nukavarapu, S.P.*** Design and Characterization of Fully Osteoconductive Scaffolds for Homogeneous and Enhanced Bone Regeneration. Society for Biomaterials Annual Meeting, 2011.
16. Wallace J, Mikael P and **Nukavarapu, S.P.*** Biodegradable Polymer-Magnesium Composite scaffolds for Bone Tissue Engineering: Effect of Magnesium on Osteoblast Proliferation, Maturation and Mineralization, Society for Biomaterials Meeting, 2010. (medical student presentation)
17. Jiang T, **Nukavarapu, S.P.,** Deng M. Kofron M, Doty S and Laurencin CT. In Vivo Bone Formation Using Chitosan/PLAGA Based Scaffolds in A Rabbit Ulnar Non-Union Defect Model. Orthopaedic Research Society Meeting, 2010.
18. Taylor ED, **Nukavarapu, S.P.,** Nair LS and Laurencin CT. Novel Nanostructured Scaffolds as Therapeutic Replacement Options for Rotator Cuff Disease. American Academy of Orthopaedic Surgeons Annual Meeting, 2009.
19. Kumbar SG, **Nukavarapu, S.P.**, James R, Nair LS and Laurencin CT. Nanobased Fiber Matrices for Wound Repair: Optimization for Human Skin Fibroblast Growth, Society for Biomaterials Meeting, 2007.
20. Deng M, Nair LS, **Nukavarapu, S.P.,** Kumbar SG, Jiang T, Krogman NR, Allcock HR and Laurencin CT. Biodegradable Poly[(ethyl alanato)(phenyl phenoxy)phosphazene] – Poly(lactide-co-glycolide) Blends: Miscibility and Osteocompatibility Evaluations, Society for Biomaterials Meeting, 2007.

ii. Invited Podium Presentations at National Professional Conferences:

1. **Nukavarapu SP,** "Tissue Engineering and Non-invasive Monitoring of Tissue Regeneration", 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Chicago, IL, Aug 26-30, 2014.
2. **Nukavarapu SP,** "Nanostructured Scaffolds for Bone Tissue Engineering", 7th World Congress of Biomechanics, Boston, MA, July 6-11, 2014.
3. **Nukavarapu SP.** "Scaffold Design for Large-Area and Vascularized Bone Regeneration", International conference of design of Biomaterials, Bangalore, India, Dec 9-11, 2012.

4. **Nukavarapu SP.** "Polymer-Hydrogel Hybrid Scaffolds: An Effective Graft System for Bone Tissue Engineering" 33rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Boston, MA, Aug 30-Sept 03, 2011.

ii. At Another College or University or Scholarly Institution:

Invited Presentations:

1. Nukavarapu SP, "Bone-Cartilage Interfacial Tissue Engineering" Rutgers University, BME Graduate Seminar, Piscataway, NJ, September 12, 2016 (scheduled)
2. **Nukavarapu SP, "**Bone and Bone-Cartilage Interface Tissue Engineering" Worcester Polytechnic Institute (WPI), BME Graduate Seminar, Worcester, MA, Apr 6, 2016.
3. **Nukavarapu SP,** "Tissue Engineering and Regenerative Medicine: From Concepts to Reality", BITS Pilani, Hyderabad, India, February 12, 2015.
4. **Nukavarapu SP,** "Advanced Strategies for Bone Tissue Engineering", University of Illinois at Chicago (UIC), BME Graduate Seminar, Chicago, IL, March 7, 2014.
5. **Nukavarapu SP, "**Scaffold Based Bone Tissue Engineering: Advances and Challenges", State University of New York (SUNY), Downstate Medical Center, NY, November 4, 2010.

Invited Moderator for Podium Presentations at National Meetings:

2016	Three-dimensional Fabrication 10th World Biomaterials Congress, Montreal, Canada.
2016	Biomaterials Cartilage I Orthopedic Research Society (ORS), Orlando, FL.
2015	Tissue Engineering Society for Biomaterials, Charlotte, NC.
2014	Bone and Cartilage Tissue Engineering II Biomedical Engineering Society (BMES), San Antonio, TX.
2014	Bone Tissue Engineering IEEE EMBS, Chicago, IL.
2014	Cardiovascular Biomaterials and Blood Biocompatibility II Society for Biomaterials, Denver, CO.
2014	Advances in Programmable Biomaterials Society for Biomaterials, Denver, CO.
2014	Orthopaedic Polymers Society for Biomaterials, Denver, CO.
2014	Bone Tissue Engineering Orthopedic Research Society, New Orleans, LA.
2012	Tissue Engineering Orthopedic Research Society, San Antonio, TX.
2012	Orthopedic Biomechanics: Vertebrate & Discs Biomedical Engineering Society, Atlanta, GA.
2011	Vascular Tissue Engineering IEE EMBS, Boston, MA.

2010	Orthopaedic Biomaterials Society for Biomaterials, Seattle, WA.

Session Organizer

2015	Biomaterials and Matrices for Osteochondral Tissue Engineering Society for Biomaterials, Charlotte, NC.
2013	Advanced Composites and Structures: Micro/Nano/Pico-Technology and Applications Materials Research Society, Boston, MA.
2013	Advanced Composites and Structures: Advanced Scaffolds for Tissue Engineering II Materials Research Society, Boston, MA.
2013	Biomaterials Strategies for Large-area Bone Regeneration Society for Biomaterials, Boston, Boston, MA.
2012	Hierarchical Three-dimensional Structures for Tissue Regeneration World Biomaterials Congress, Chengdu, China.
2011	Scaffold Assisted Bone Defect Repair/Regeneration Society for Biomaterials, Orlando, FL.
2010	Biomaterial technologies for Treating Non-union bone defects: Research developments and Clinical applications Society for Biomaterials, Seattle, WA.

Current Research Funding:

1. **Title:** Biomedical Science and Engineering Summer School
   Time Commitment: 1.2 calendar months (10% effort)
   Role: PI
   Supporting Agency: National Science Foundation
   Performance Period: 5/15/2016 – 4/31/2017
   Goals/Aims: The goal of this project is to provide research training to the undergraduate and medical students in biomedical science and engineering areas.
   Total direct costs $ 40,130

2. **Title:** Microsphere-cell Constructs for Breast Tissue Engineering
   Time Commitment: 1.2 calendar months (10% effort)
   Role: PI
   Supporting Agency: Biomedical Trust Fund, UConn
   Performance Period: 11/1/2015 – 10/31/2016
   Goals/Aims: The goal of this project is to develop injectable biomaterial and cell combinations for breast tissue augmentation/regeneration.
   Total direct costs $ 85,000

3. **Title:** Rapid and Effective Revitalization of Bone Allografts at the Point of Care
   Time Commitment: 1.2 calendar months (10% effort)

Role: PI
Supporting Agency: Musculoskeletal Transplant Foundation (MTF)
Performance Period: 2/1/2015 – 1/31/2017
Goals/Aims: The goal of this project is to develop an intra-operative method to effectively cellularize bone allografts next to the bedside
Total direct costs $83,823

4. **Title:** Design and evaluation of a matrix system with rapid and efficient cell loading characteristics for segmental bone defect repair
Time Commitment: 1.2 calendar months (10% effort)
Role: PI
Supporting Agency: AO Foundation
Performance Period: 4/1/2013 – 12/31/2016
Goals/Aims: The goal of this project is to implement a Completely Intra-operative Tissue Engineering Strategy (CITES) for a bone defect repair
Total direct costs $120,054

5. **Title:** Research Mentoring Award
Time Commitment: 3 calendar months (25% effort)
Role: PI
Supporting Agency: Connecticut Institute for Clinical and Translational Science
Performance Period: 4/1/2014 – 7/31/2017
Goals/Aims: The goal of this award is to mentor underrepresented minority students from Young Innovative Investigator Program (YIIP) to successfully complete Maters in Biomedical Sciences.
Total direct costs $150,000

6. **Title:** Research Experience and Mentoring Award (supplement to EFRI parent award)
Time Commitment: 1.8 calendar months (15% effort)
Role: Co-PI
Supporting Agency: National Science Foundation
Performance Period: 8/1/2016 – 7/31/2017
Goals/Aims: The goal of this award is to train underrepresented minority students in STEM careers.
Total direct costs $76,046

7. **Title:** Research Experience and Mentoring Award (supplement to EFRI parent award)
Time Commitment: 1.2 calendar months (10% effort)
Role: Co-PI
Supporting Agency: National Science Foundation
Performance Period: 8/1/2015 – 7/31/2016
Goals/Aims: The goal of this award is to train underrepresented minority students in STEM careers.
Total direct costs $76,046

8. **Title:** Electrically Stimulated Complex Tissue Regeneration

Time Commitment: 0.12 calendar months (2% effort)
Role: Co-I
Supporting Agency: National Science Foundation- EFRI- 1332329
Performance Period: 4/1/2013-3/31/2017
Goals/Aims: The goal of this project is to develop strategies to regenerate torn complex tissue systems such as muscle-tendon mediated through the mechanical support of an implanted degradable scaffold and the electrical stimulation. My role in this grant is to direct research training and mentoring activities outlined in the proposal.
Total direct costs $1,521,099

9. **Title:** Building Infrastructure Leading to Diversity (BUILD)
Time Commitment: 0.18 calendar months (3% effort)
Role: Co-I
Supporting Agency: National Institutes of Health
Performance Period: 8/1/2015 – 7/31/2019
Goals/Aims: This is a center award to train the next generation of underserved and under-represented biomedical scientists and engineers
Total direct costs $219,791

Past Research Funding:

1. **Title:** Developing Animal Models for Optimizing the Musculoskeletal Repair Potential of Emerging Human Progenitor Cell Therapies
Time Commitment: 2.4 months (20% effort)
Role: Co-PI
Supporting Agency: US army
Performance Period: 2011-2014
Goals/Aims: The goal of this project is to develop bio-engineered grafts and the relevant animal models for testing the grafts for bone defect repair
Total direct costs $560,000

2. **Title:** AIR Option 2: Research Alliance Polymer, Polymer-Ceramic and Natural Polymer Systems for Soft Tissue and Bone Repair and Regeneration.
Time Commitment: 0.6 calendar months (5% effort)
Role: Co-I
Supporting Agency: National Science Foundation-IIP-1311907
Performance Period: 5/01/2013 – 11/31/2015
Goals/Aims: The goal of this project is to collaborate with other interested entities to accelerate the translation of NSF funded discoveries and innovative technologies to commercial realities in the area of hard and soft tissue regeneration using biomaterial-based approaches
Total direct costs $307,359

3. **Title:** Mechanically and Biologically Compatible Novel Biodegradable Polymer-Carbon Nanotube Scaffolds for Bone Tissue Engineering
Time Commitment: 1.2 calendar months (10% effort)

Role: PI
Supporting Agency: Campus Incentive Grant, The University of Connecticut
Performance Period: 2009-2012
Goals/Aims: The goal of this project is to develop Ultra-strong composite scaffolds and evaluate their suitability for bone tissue engineering
Total direct costs $100,000

4. **Title:** Enhanced Osteo- and Angio-Induction via Polymer/CaO2 Bone Tissue Engineering Scaffolds.
   Time Commitment: 0.6 months (5% effort)
   Role: Co-I
   Supporting Agency: National Institutes of Health
   Performance Period: 2012-2016
   Goals/Aims: The goal of this project is to develop engineered matrices vascularized bone regeneration
   Total direct costs $270,000

5. **Title:** Research Experience and Mentoring
   Time Commitment: 1.2 months (10% effort)
   Role: Co-I
   Supporting Agency: National Science Foundation, EFRI Supplement
   Performance Period: 8/1/2014-7/31/2015
   Goals/Aims: The goal of this award is to train underrepresented minority students in STEM careers.
   Total direct costs $83,000

6. **Title:** Musculoskeletal Bioengineering: A New Focus Group Development for Research Translation and Training
   Time Commitment: 0.6 months (5% effort)
   Role: PI
   Supporting Agency: Connecticut Institute for Clinical Translational Science
   Performance Period: 1/1/2014-12/31/2014
   Goals/Aims: The goal of this project is to build collaborations between the Musculoskeletal and Bioengineering research groups to promote interdisciplinary research, research translation, and research training.
   Total direct costs $25,820

Pending Research Grants:

1. **Title:** Unique Matrix Platform for an Integrated Bone-Cartilage Interface Regeneration
   Time Commitment: 3 months (25% effort)
   Role: PI
   Supporting Agency: National Institute of Health-R01
   Performance Period: 2016 - 2020

Goals/Aims: The goal of this project is to develop a gradient and integrated graft system for bone-cartilage interface (osteochondral) tissue engineering
Total direct costs $1,250,000

2. **Title:** Autologous Biomaterial and Stem Cells for Bedside Tissue Engineering
Time Commitment: 2.4 months (20% effort)
Role: PI
Supporting Agency: National Institute of Health-R21
Performance Period: 2016-2018
Goals/Aims: The goal of this project is to use autologous biomaterial and stem cells to develop a Completely Intra-operative Tissue Engineering Strategy (CITES) for bone defect repair
Total direct costs $275,000

3. **Title:** A novel matrix system for Bedside Tissue Engineering using patient-derived biomaterial and stem cells
Time Commitment: 2.4 months (20% effort)
Role: PI
Supporting Agency: State of Connecticut Regenerative Medicine Research Fund
Performance Period: 2016-2019
Goals/Aims: The goal of this project is to develop a matrix system for bedside tissue engineering
Total direct costs $599,846

4. **Title:** Engineered Osteochondral Plug
Time Commitment: 0.18 months (3% effort)
Role: PI
Supporting Agency: Connecticut Innovations and the Bioscience Innovation Fund
Performance Period: 2016-2017
Goals/Aims: The goal of this project is to design and develop an engineered graft for osteochondral defect repair
Total direct costs $29,769

5. **Title:** Development of a Novel Biodegradable Amorphous Fused Fiber Silica Dioxide Osteochondral Matrix
Time Commitment: 3 months (25% effort)
Role: PI
Supporting Agency: Department of Defense (DOD)
Performance Period: 2017-2018
Goals/Aims: The goal of this project is to design an autologous osteochondral graft and develop a method to pre-culture the graft for implantation
Total direct costs $199,961

Professional Societies:

2006-Current	Society for Biomaterials, USA	Active Member

2007-Current	Orthopaedic Research Society, USA	Active Member
2009-Current	Biomedical Engineering Society, USA	Active Member
2010-Current	Materials Research Society, USA	Active Member
2010-Current	Controlled Release Society, USA	Active Member
2011-Current	IEEE Engineering in Medicine and Biology Society, USA	Active Member

National Committee Service:

2015-Current	Vice-Chair	Tissue Engineering Special Interest Group, Society for Biomaterials
2013-2015	Program Chair	Tissue Engineering Special Interest Group, Society for Biomaterials
2013-Current	Member	Education and Professional Development Committee, Society for Biomaterials
2013-Current	Member	Membership Committee, Biomedical Engineering Society
2009-2011	General Secretary/ Treasurer	Orthopedic Biomaterials Special Interest Group, Society for Biomaterials

Local Committee Service:

2016-Current	Member	Committee on Undergraduate Medical Education (CUME)
2015-Current	Co-Leader	Biomedical Engineering Core Interest Group, Connecticut Institute for Clinical and Translational Science
2014-Current	Department of Biomedical Engineering	Accreditation Board for Engineering and Technology Couse Material Evaluation Committee member, Biomaterials Track.
2014-Current	Department of Biomedical Engineering	Course and Curriculum Evaluation committee – graduate level – Biomaterials track
2013-Current	Co-Leader	Musculoskeletal Research Core Interest Group, Connecticut Institute for Clinical and Translational Science
2012-2016	Skeletal Biology and Regeneration Program (formerly SCOB)	Recruitment committee member

Grant Review Activities:

2015	Peer Reviewed Medical Research Program	Pre-proposal Reviewer, Nanomaterials & Bone Regeneration, Department of
2015	Research Grant Program	Musculoskeletal Transplant Foundation (MTF)

2015	Peer Reviewed Medical Research Program	Teleconference Reviewer, Focused Program-segmental bone defects, Department of Defense, US
2014	Peer Reviewed Orthopaedic Research Program	Review Panel Member, Clinical Trails Program, Department of Defense, US
2014	Orthopedic and Skeletal Biology Study Section	Review Panel Member, Small Business Program, National Institutes of Health
2014	Department of Scientific Research	Main-in Reviewer, Dutch Arthritis Foundation, Netherlands
2013	Health Research	International Reviewer, The Netherlands Organization for Health Research and Development
2009	Challenge Grants	Mail-in Reviewer, National Institutes of Health

Reviewer for:

ACS Biomaterials Science and Engineering, Artificial Organs, Acta Biomaterialia, Biomaterials, Biomedical Materials, BioMed Research International, Journal of Biomedical Materials Research: Part A, Nanotechnology, RSC Advances. Tissue Engineering-A.

Editorial Boards:

2014	Academic Editor	PLoS One
2013	International Advisory Panel Member	Materials Research Express
2013	Editorial Board	Critical Reviews in Biomedical Engineering
2012	Editorial Board	Encyclopedia of Biomedical Polymers and Polymeric Biomaterials, Taylor & Francis Group
2011	Editorial Board	Journal of Biomaterials and Tissue Engineering
2009	Editorial Board	Artificial Organs

Research Supervisory and Training Responsibilities:

High School Students:

2016 Summer	Wesia Malik	Magnet school, New Britain, CT (through HCOP)
2015-2016	Rohit Makol	South Windsor High School, CT
2013-2014	Robert Armentano	Glastonbury High School, CT
2012 Summer	Glademyr Reme	West Hartford High School, CT (through HCOP)

Undergraduate Students:

2016 Summer	Marisa Boch	University of Connecticut, Storrs, CT (through Undergraduate Summer Research Internship in Biomedical Sciences at UConn Health)
2015 Summer	Abeid Anslip	University of Connecticut, Storrs, CT (through HCOP)
2015-	Thomas Xu	University of Connecticut, Storrs, CT
2015-	Shalmli Joshi	University of Connecticut, Storrs, CT (independent study)
2015 Summer	Rares Barbu	University of Connecticut, Storrs, CT (independent study)
2015 Summer	Eve George	Georgia Tech, GA (through Undergraduate Summer Research Internship in Biomedical Sciences at UConn Health)
2014 Summer	Rachelle Aekins	University of Connecticut, Storrs, CT (through NSF-REM)
2014 Summer	Chelsea Stephens	University of Connecticut, Storrs, CT (through NSF-REM)
2014	Dillon Florence	University of Connecticut, CT (independent study)
2013 Summer	Andrew Luzzi	Cornell University, NY (through Undergraduate Summer Research Internship in Biomedical Sciences at UConn Health)
2012 Summer	Nick Lemme	University of Rhode Island, RI (through HCOP)
2011-2012	Deborah Dorcemus	University of Connecticut, Storrs, CT (independent study)

BME Senior Design Students:

Note: Details of the project can be located at http://www.bme.uconn.edu/completed-senior-design-projects.php

2016-2017	Lorenzo Maltes Victoria Spencer Erica Illescas	Next Generation Osteochondral Graft (Team number to be announced)
2016-2017	Jennifer Mcnamara Kalpana Betageri Shalmli Joshi	Bioreactor design for bone-cartilage interface graft culture (Team number to be announced)
2015-2016	Michel Messina Alexandra Riddle David Slattery	Team 9- Osteochondral tissue graft fabrication device phase II
2014-2015	Christopher Ackell Nick O'Leary Brandon Mehnert	Team 9- Designing a completely automated system for the concentration of bone marrow aspirate

	Justin Fleischacker	
2013-2014	Shruti Kuzhippat Smit Patel Alison Welch	Team 1- Osteochondral tissue graft fabrication device (won first place)

Medical Students/ Dental Students/ Masters Students:

2015-2016	Ling Lei	UConn Health (Medical)
2014-2016	Emmanuel Nwachuku	Quinnipiac University, CT (Medical)
2014-2015	Elvis Francois	Meharry Medical College, TN (Medical)
2009-2011	James Wallace	UConn Health (Medical)
2012-2013	Yau Robert	UConn Health (Dental)
2016	Shadaab Kazi	University of Connecticut, CT
2016-	Divya Kamireddi	University of Connecticut, CT (Masters)
2016-	Gowtham Yenduri	University of Connecticut, CT (Masters)
2016-	Katherine Quinones	University of Connecticut, CT (Masters)
Graduated 2016	Melissa Carr-Reynolds	UConn Health (Masters)
Graduated 2016	Nilse Das Santos	UConn Health (Masters)
Graduated 2016	Tyler Stahl	University of Connecticut, CT (Masters)
2015	Brian Walter	University of Connecticut, CT (Masters)
2015	Wesley Sutton	University of Connecticut, CT (Masters)
2015	Caren Necio	University of Connecticut, CT (Masters)
2010 graduated	Paiyz Mikael	University of Connecticut, CT (Masters)

PhD Thesis students:

2016-Current	Hyun Kim	Department of Biomedical Engineering
2012-Current	Deborah Dorcemus	Department of Biomedical Engineering
2010-2015	Paiyz Mikael	Department of Materials Science and Engineering
2009-2012	Ami Amini	Skeletal Biology and Regeneration Program (formerly SCOB)

Postdoctoral Fellows:

2009-2011	John Igwe	**Current Position:** Doing Osteopathic Medicine, Rhode Island College of Osteopathic Medicine, RI

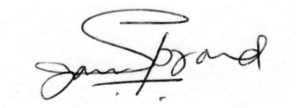
Undergraduate/Graduate Program Teaching Responsibilities:

BME-4701/6086- Advanced Biomaterials	Revived an existing course with advanced topics. A total of 12 lectures covering	Fall 2014, Fall 2015, and offering Fall 2016

	contemporary biomaterials science and engineering principles with the relevant biomedical device examples. Learning objectives were evaluated through 2 exams, 3 home works, 3 quizzes, and debate & class presentation. Had an enrollment of about 30 students	
BME-4910-004- Biomedical Engineering Design II	Senior Design Students, a yearlong commitment with approximately 60 contact hours. Thus far, I have sponsored and successfully advised 3 senior design projects involving a total of 10 students. Currently I am advising two teams involving 6 students	2013-2014, 2014-2015, 2015-2016, and currently advising 2016-2017 student group
BME-4999/BME-5099/ MEDS 6495 "Independent Study" for 1-2 credits	Each semester, one to two undergraduate/ graduate students enroll and gain research experience and scientific writing. To date, I have trained 14 independent-study undergraduates	Offered since Fall 2011
BME-4701/6086	Four Guest Lectures (1.5 h each) covering the basic elements of tissue engineering: Scaffolds, cells, and growth factors	Fall 2010, and Fall 2012

I attest that the information provided in this document is complete, up-to-date and accurate.

Sincerely



Syam P. Nukavarapu
Farmington
7/27/2016

Effect of EGFR Loss of Function on Osteoarthritis Disease Progression in a Transgenic Mouse Model

INTRODUCTION

Osteoarthritis is a debilitating disease that affects 27 million Americans today and is fast becoming a global epidemic (Lawrence et al). Osteoarthritis occurs when the articular cartilage that covers the ends of the long bones is lost due to injury or age. As the normal function of the articular cartilage is to facilitate smooth motion, absorb shock, and protect the ends of the long bones, patients with Osteoarthritis suffer from impaired mobility and severe pain as the ends of the long bones begin to rub against one another (Busija et al). There is no cure for the widespread articular cartilage loss that characterizes severe Osteoarthritis. Total joint replacement, where the joint ends are cut off and replaced with an artificial joint, is usually ultimately required. Accordingly, the goal of current treatments is to prevent or slow Osteoarthritis disease progression by repairing defects in the articular cartilage that are caused by traumatic injury, as these defects typically progress to widespread Osteoarthritis later on in life (Makris et al).

Current cartilage repair approaches generally rely on cells, and include Autologous Chondrocyte Implantation, in which replacement chondrocytes obtained from the patient are implanted into the articular cartilage defect (Brittberg et al); as well as Microfracture in which holes punched into the affected area allow cells from the bone marrow to flood the defect region (Makris et al). However, in both of these approaches, the cartilage formed by the new cells tends to be fibrocartilage, rather than the true hyaline cartilage of the articular surfaces. Fibrocartilage is biochemically and biomechanically inferior when compared to hyaline cartilage (Markis et al). As the wrong kind of cartilage is formed in these approaches, these therapies are not a long -term solution to the problem of Osteoarthritis. The side effects, long recovery times, and lack of adequate repair of these current therapies show that there is an unmet need for better approaches to repair articular cartilage damage, in order to slow the progression of articular cartilage injury to overt Osteoarthritis disease.

Post-traumatic Osteoarthritis develops in three stages after injury to the articular cartilage occurs. First, there is a transient and early catabolic phase characterized by breakdown of the articular cartilage matrices due to increased expression of degradative enzymes such as matrix metalloproteinases (MMPs). The second phase is anabolic, and is characterized by increased

chondrocyte proliferation and increased cartilage matrix production. Lastly there is a prolonged catabolic stage that leads to irreversible destruction of the cartilage and inevitable progression to overt osteoarthritis (Anderson et al). Our overall hypothesis is that by stimulating the anabolic phase and/or inhibiting the progressive catabolic phase we can reduce the incidence and severity of post-traumatic Osteoarthritis.

The Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase present in articular cartilage that has been implicated in articular cartilage homeostasis as well as arthritic disease. Previous studies from the Dealy lab have shown that EGFR signaling can have both anabolic as well as catabolic activities in articular cartilage. For example, the articular cartilage of transgenic mice in which EGFR signaling is experimentally activated is dramatically thickened, demonstrating an anabolic effect of EGFR signaling in articular cartilage proliferation and matrix synthesis (Shepard et al 2013). However, prolonged EGFR signaling in the same transgenic model suppresses matrix synthesis and leads to cartilage breakdown, demonstrating catabolic effects of EGFR signaling (Shepard et al 2013). The goal of this study was to determine which of these two roles (anabolic or catabolic) is the primary function of EGFR signaling during progression of post-traumatic Osteoarthritis. For this purpose, we used transgenic mice in which EGFR signaling in articular cartilage is impaired by genetic EGFR loss. The mice were subjected to surgery to cut the knee ligaments, which destabilizes the joint, leads to injury to the articular cartilage, and eventually causes post-traumatic Osteoarthritis. The knee joints of the mice were harvested at various times after surgery and Osteoarthritis progression was assessed histologically. Our hypothesis was that EGFR knockdown in articular cartilage will slow progression of post-traumatic Osteoarthritis, because the catabolic activities of the EGFR in mediating cartilage matrix degradation will be impaired.

METHODS

Transgenic mice: Conditional EGFR knockouts specific to cartilage were generated using the CreLoxP system. In this system, the bacterial Cre recombinase enzyme recognizes LoxP sites and causes a recombination event to occur. Since LoxP sites are directional we put our gene of interest between two forward facing LoxP sites. Upon induction of Cre recombinase expression, a deletion of the selected gene would occur. To create cartilage specific gene knockout, Cre expression was controlled by the Collagen Type II promoter, which is expressed exclusively in cartilage. To generate EGFR-conditional

knock out (EGFR-cko) mice, EGFR-flox mice were crossed with mice in which cre recombinase expression was controlled by the Collagen type II promoter, which is active only in cartilage tissue. The resultant "EGFR-double-cko" animals, which possess two EGFR-deficient alleles, were then further crossed with heterozygous EGFR null animals in order to generate "EGFR-triple-cko" animals, which possess three EGFR-deficient alleles.

Surgery: At 10-12 weeks of age the mice were subjected to knee ligament transection surgery in order to destabilize the joint and induce post-traumatic osteoarthritis. PMX-ACLT surgery was performed to cut the medial meniscal tibial ligament, the medial collateral ligament, the anterior cruciate ligament, and to remove the anterior portion of the medial meniscus.

Histology: Mice were sacrificed at 6 weeks post-surgery and the left knee joints were fixed in 4% paraformaldehyde and processed for paraffin embedding. 7 um sections were cut on a rotary microtome and mounted on ProbeOnPlus slides. Safranin-O was used to detect proteoglycan (an indicator of cartilage) and slides were then counterstained with Fast Green. Immunohistochemistry was used to quantify cell proliferation and to detect localization of cartilage matrix proteins. Antibodies used were rabbit anti-KI-67 (Abcam, Cambridge, MA, US), 1:100 a marker for proliferation; and, rabbit anti-Aggrecan Neoepitope (Pierce, Rockford, IL, USA), (1:100), and MMP13 (1:100), both markers of cartilage degradation. The slides were washed with a TBS solution with 0.1% Tween 20 and then incubated at room temperature with 1:200 biotinylated goat anti-rabbit IgG (Vector Laboratories). After another washing, the slides were incubated in Vectastain Elite ABC Reagent (Vector Laboratories) and developed using DAB (Vector Laboratories) and then counterstained with hematoxylin. Negative controls but followed the same protocol but were not subjected to a primary antibody. There were no positive reactions in any of the control slides.

Analysis: Digital images of the sections were captured using SPOT software and imaged using Photoshop. The extent of the articular cartilage damage was measured in Safranin-O stained sections using guidelines established by the OARSI (Osteoarthritis Research Society Intl) as outlined by Glasson et al. The scale goes from 0 (no damage) to 1 (loss of proteoglycan staining) to 2-4 (cracks, fibrillation and cartilage erosion) to 5 (complete loss of the articular cartilage surface). Each quadrant (medial femoral, lateral femoral, medial tibial and lateral tibial) of the articular cartilage was scored independently and scores were averaged. To quantify protein expression, positively labeled cells in immunohistochemical assays were counted in a defined area of the lateral tibial articular cartilage using Photoshop and expressed relative to the total number of cells present in the same area, to generate a labeling index.

All analysis was averaged between at least three samples per animal. Data were subjected to student's T test (Stat Pac). Our results came from a litter containing 2 EGFR-triple-cko mice and 2 control wildtype mice.

RESULTS

EGFR loss does not alter gross articular cartilage damage following knee ligament transection surgery.

As shown in Figure 1 below, articular cartilage damage scores according to the OARSI scale (Glasson et al) were not statistically different between EGFR-triple-cko mice and their littermate control wildtype mice 6 weeks after PMX-ACLT surgery.

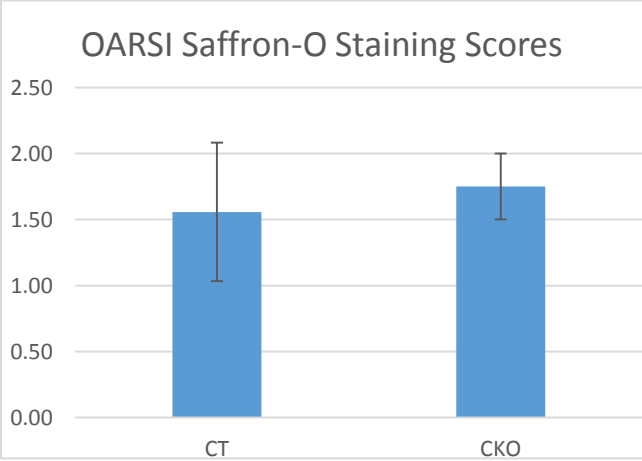


Figure 1: Comparison of articular cartilage damage as assessed by OARSI scoring in the articular cartilage of control wildtype mice (n=2) and littermate EGFR-triple-cko mice (n=2) 6 weeks after PMX-ACLT surgery. P=0.7748

Cell proliferation is increased in the injured articular cartilage of mice with EGFR loss.

As shown in Figures 2 and 3, below, cell proliferation, as assessed by counting Ki-67-positive cells, is significantly increased in the articular cartilage of EGFR-triple-cko mice 6 weeks after ligament transection surgery compared to littermate wildtype control mice.

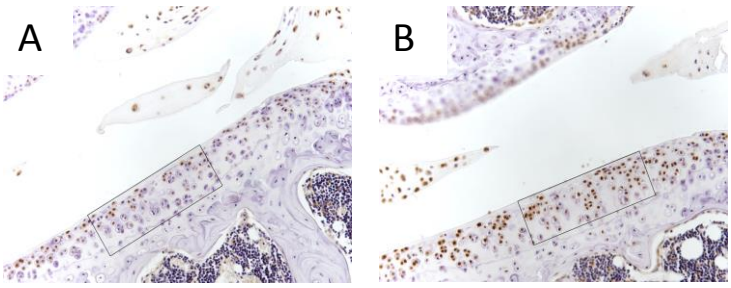


Figure 2: Comparison of cell proliferation as assessed by counting Ki67-positive cells in the boxed area of the lateral tibial articular cartilage of A) control wildtype mice (n=2) and B) littermate EGFR-triple-cko mice (n=2) 6 weeks after PMX-ACLT surgery. Note the presence of numerous Ki67-positive cells present in the EGFR-triple cko cartilage in B.

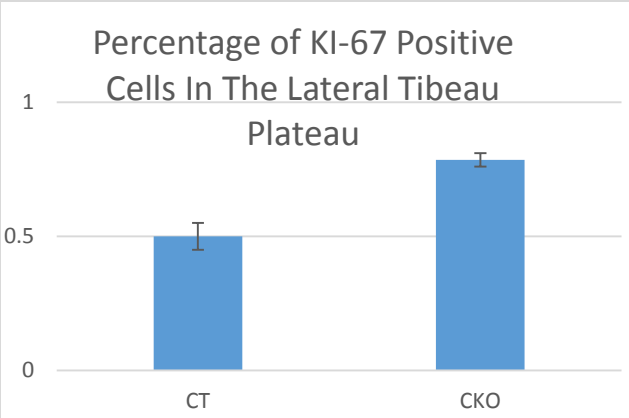


Figure 3: Comparison of cell proliferation as assessed by Ki67-labeling index in the lateral tibial articular cartilage of control wildtype mice (n=2) and littermate EGFR-triple-cko mice (n=2) 6 weeks after PMX-ACLT surgery. Note the increased number of proliferating cells in the EGFR-triple-cko articular cartilage. P=0.0332

Matrix degradation in injured articular cartilage is decreased in mice with EGFR loss

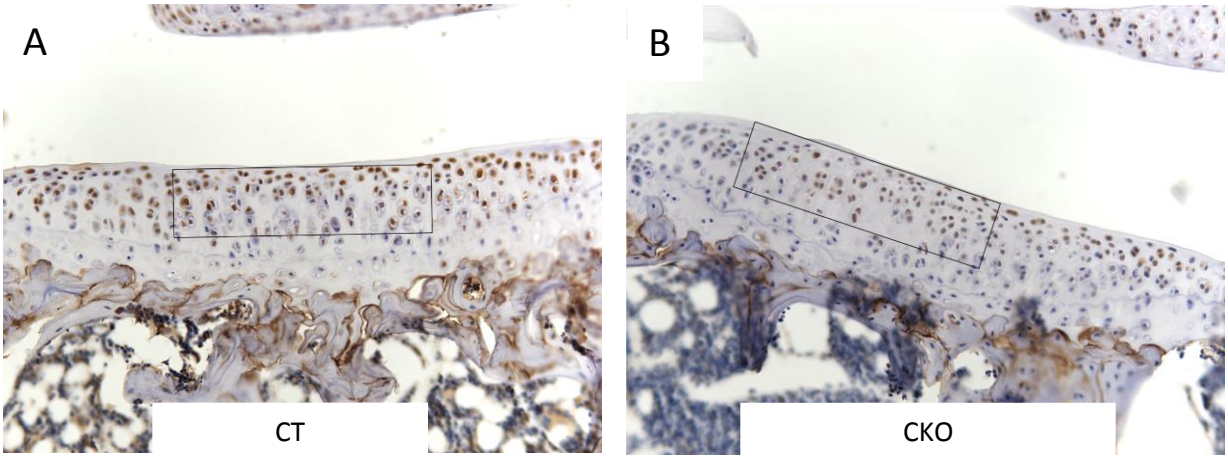


Figure 4 (CT) and 5 (CKO): Comparison of matrix degradation as assessed by MMP13 labeling index in the lateral tibial articular cartilage of control wildtype mice (n=2) and littermate EGFR-triple-cko mice (n=2) 6 weeks.

As shown in Figures 4 and 5, above, matrix degradation, as assessed by counting MMP 13 positive cells, is decreased in the articular cartilage of EGFR-triple-cko mice 6 weeks after ligament transection surgery compared to littermate wildtype control mice.

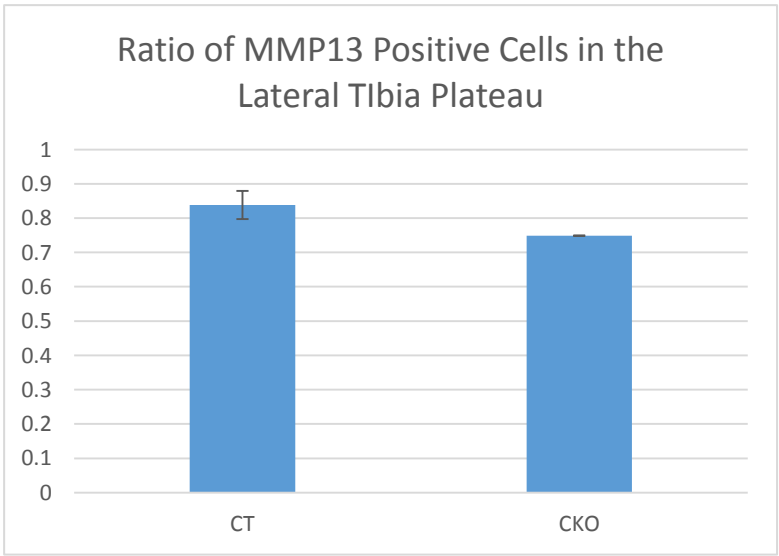


Figure 7: Comparison of matrix degradation as assessed by MMP 13-labeling index in the lateral tibial articular cartilage of control wildtype mice (n=2) and littermate EGFR-triple-cko mice (n=2)

6 weeks after PMX-ACLT surgery. Note the decreased presence of MMP13 staining in the cko as opposed to the control. P=0.1579

Articular cartilage thickness is increased in mice with EGFR loss

As shown in Figure 7, below, an increase in articular cartilage thickness was observed in EGFR-triple-cko mice 6 weeks after ligament transection surgery compared to littermate wildtype control mice.

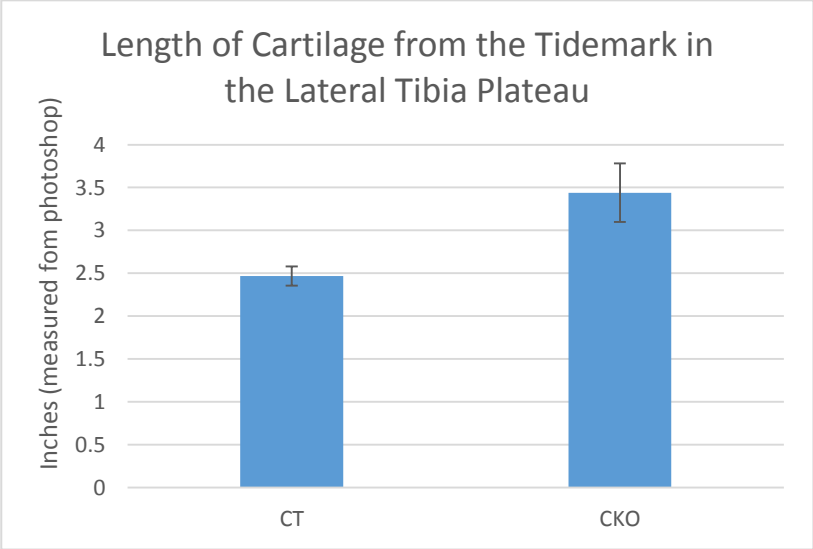


Figure 7: The thickness of the articular cartilage the lateral tibial articular cartilage of control wildtype mice (n=2) and littermate EGFR-triple-cko mice (n=2) 6 weeks after PMX-ACLT surgery. Note the increase in cartilage thickness in the cko. P=0.1144

Discussion

Enomoto et al. reported that EFGR signaling protects chondrocytes from OA-induced apoptosis, increased aggrecan cleavage in models with reduced EFGR expression, as well as higher MMP13 activity. They interpret their results to indicate that a loss of EGFR in the cartilage has a negative effect on the cartilage's ability to repair after joint trauma. Our preliminary studies seem to indicate the exact opposite with a decrease in MMP13 activity in the conditional knockouts as compared to the control and increased thickness of cartilage in the conditional knockouts as compared to the control. We also analyzed KI-67, a proliferative marker in order to determine if there was an increased anabolic response in the ckos. We obtained significant results showing that the proportion of marked cells in the ckos was markedly higher than the control. The OARSI scoring system was used to score the damage to cartilage in Safarin-O stained tissue. The results were not significant and did show a slightly increased score in

the ckos. We believe that this result is due to our low n value, and that further samples are needed for a clear outcome to be determined. While the significance of our results was hampered due to low n values we believed that we have identified several important trends that show an inhibition of EFGR to have a positive anabolic response.

Both non vehicle mouse models used by Enomoto et al used a method of EGFR inhibition that was nonspecific to cartilage and only induced after surgery whereas our model selectively knocked out EGFR in cartilage throughout the entire development of the mouse. We believe that our results could be explained through an unintentional interaction with the nonspecific EFGR inhibition treatments and another member of the EFGR family that may play a role in joint trauma recovery. EFGR or Erb1 is part of a family of four related proteins that crosslink to form heterodimers. If there were two receptor types present, EFGR with its proven strong catabolic response and an unidentified other receptor that provided an anabolic response, then a nonspecific and leaky Erb inhibitor might suppress both receptors. This would allow the dominant catabolic effect to still be active through the incomplete inhibition and may silence the anabolic response. Our method being specific to EFGR would inhibit the catabolic response and allow the anabolic response to continue. Given the disparity between Enomoto et al and our research we believe that more research into EFGR's role in Osteoarthritis is needed. Future studies could examine expression and potential activation of the other EGFR family members in the articular cartilage of normal animals and in the triple CKO animals.

1.

Lawrence, R. C. *et al.* Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum.* **58,** 26–35 (2008).

2.

Yu, X.-M. *et al.* MicroRNAs’ Involvement in Osteoarthritis and the Prospects for Treatments, MicroRNAs’ Involvement in Osteoarthritis and the Prospects for Treatments. *Evidence-Based Complementary and Alternative Medicine, Evidence-Based Complementary and Alternative Medicine* **2015, 2015,** e236179 (2015).

3.

Busija, L. *et al.* Osteoarthritis. *Best Practice & Research Clinical Rheumatology* **24,** 757–768 (2010).

4.

Varady, N. H. & Grodzinsky, A. J. Osteoarthritis year in review 2015: mechanics. *Osteoarthritis and Cartilage* **24,** 27–35 (2016).

5.

Anderson, D. D. *et al.* Post-traumatic osteoarthritis: Improved understanding and opportunities for early intervention. *J. Orthop. Res.* **29,** 802–809 (2011).

6.

Zhang, X. *et al.* Reduced EGFR signaling enhances cartilage destruction in a mouse osteoarthritis model. *Bone Res* **2,** 14015 (2014).

7.

Makris, E. A., Gomoll, A. H., Malizos, K. N., Hu, J. C. & Athanasiou, K. A. Repair and tissue engineering techniques for articular cartilage. *Nature Reviews Rheumatology* **11,** 21–34 (2014).

8.

Shepard, J. B., Jeong, J.-W., Maihle, N. J., O'Brien, S. & Dealy, C. N. Transient anabolic effects accompany epidermal growth factor receptor signal activation in articular cartilage in vivo. *Arthritis Research & Therapy* **15,** R60 (2013).

9.

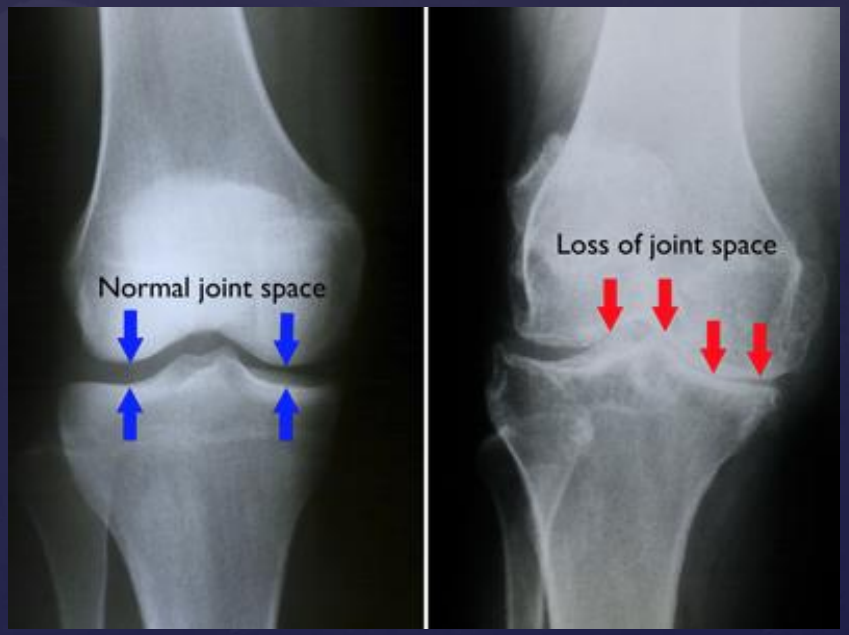
Brittberg, M. *et al.* Treatment of Deep Cartilage Defects in the Knee with Autologous Chondrocyte Transplantation. *New England Journal of Medicine* **331,** 889–895 (1994).

A Critical Defect Model to Analyze the Role of Epidermal Growth Factor Receptor (EGFR) Signaling in the Repair of Murine Osteoarthritic Cartilage

Bridget Oei
UCONN Health Center
Dealy lab: Department of Reconstructive Sciences
Center for Regenerative Medicine and Skeletal Development
Summer 2016

Background

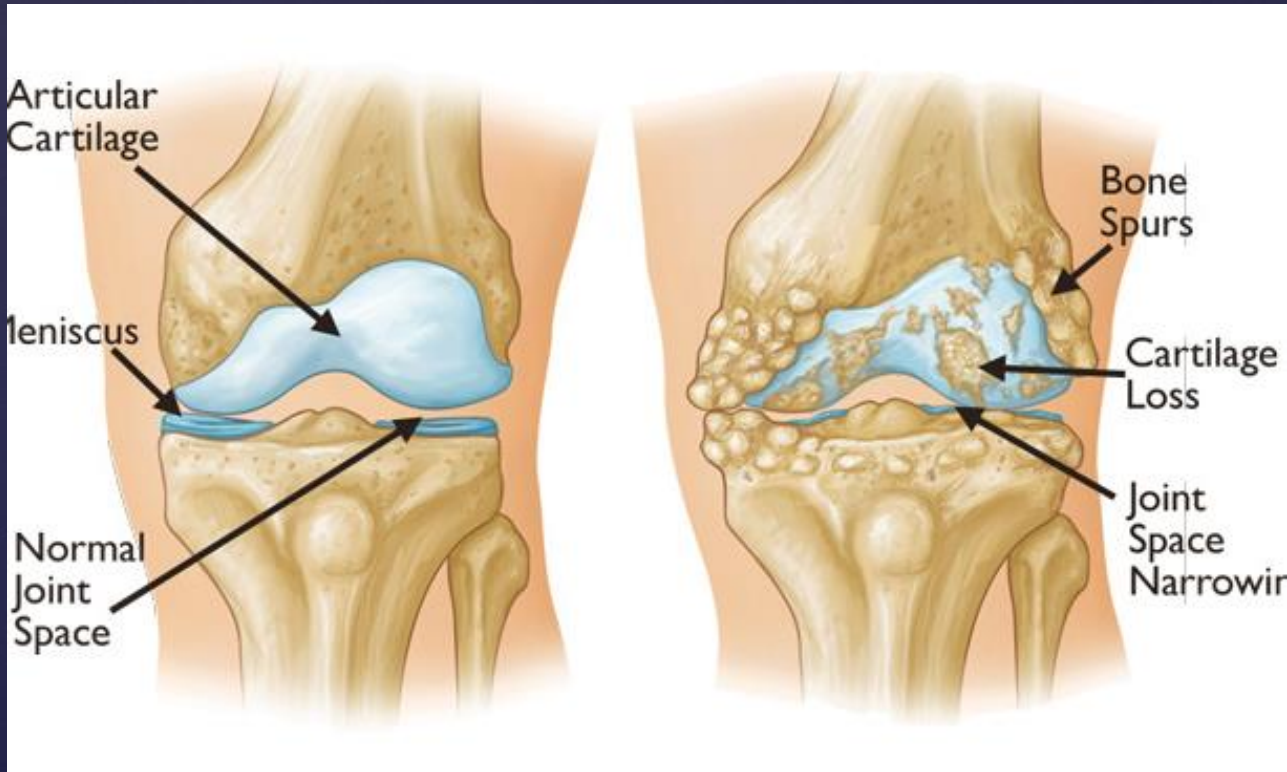
- Osteoarthritis is a painful condition that affects over 20 million adults in the US every year.
- In patients with Osteoarthritis, the articular cartilage of joints degenerates and ultimately is lost.



.

Osteoarthritis

- Destabilized Joint
- Cartilage loss
- Narrowed joint space
- Bone Spurs

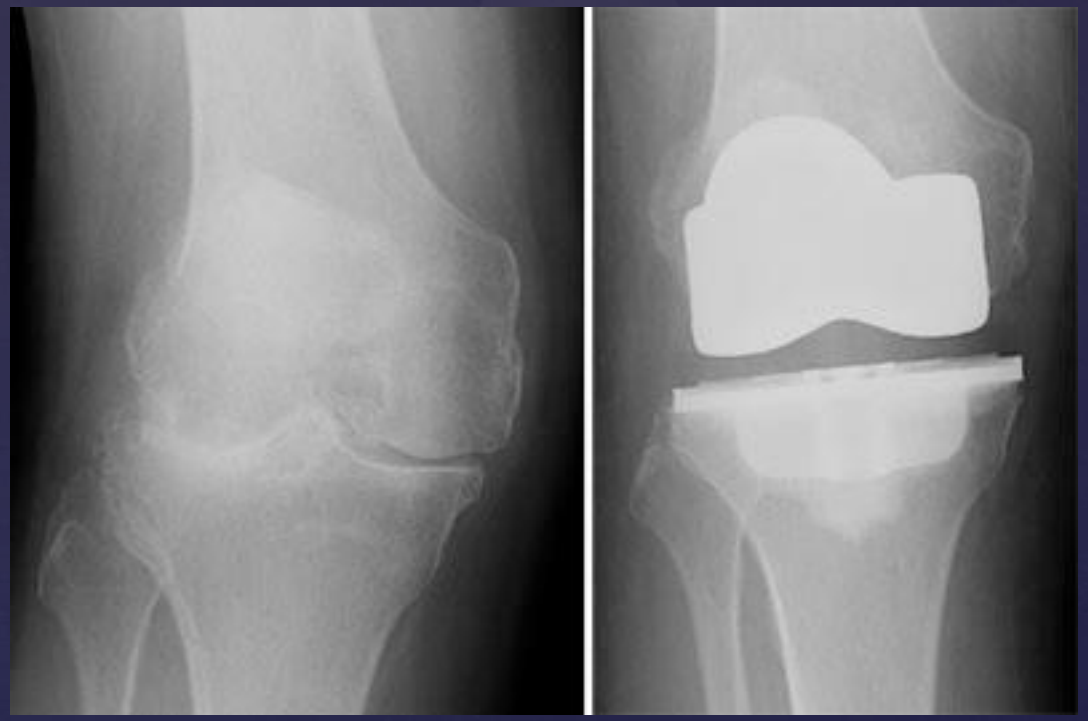


Osteoarthritis

- Precursors to Osteoarthritis can stem from traumatic events
  - Car accidents
  - Sporting injuries
  - Extreme Weight gain (Obesity)

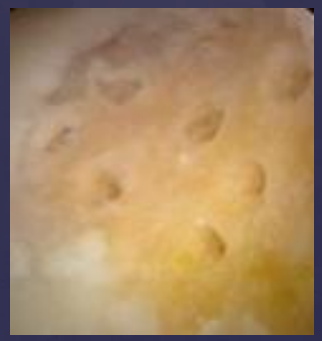
Current Treatment

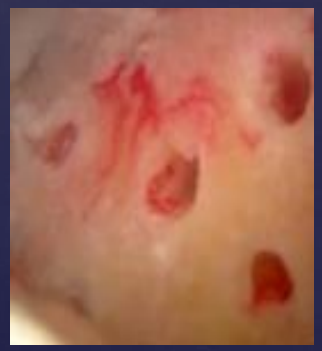
- Cartilage does not have robust healing capabilities so treatment is limited to…
  - weight management
  - physical therapy
  - anti-inflammatory prescriptions.
- But above treatment cannot repair damaged cartilage so….
- Joint replacement is used
- Other surgical procedures



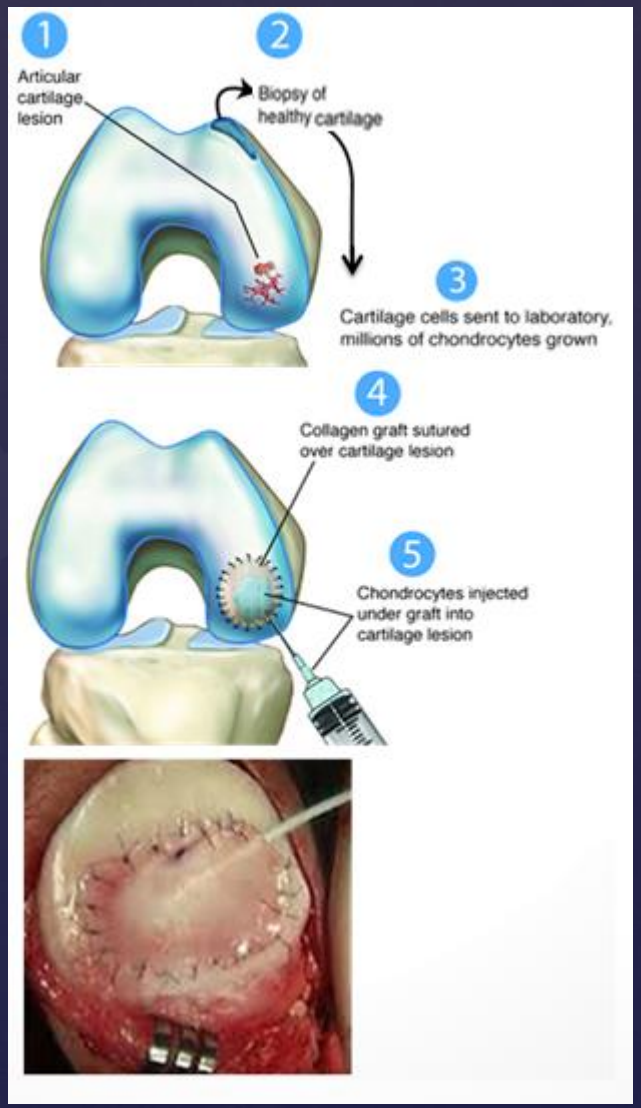
Surgical Treatment of Osteoarthritis

Microfracture





Autologus Chonodrocyte Implantation (ACI) "Carticel"

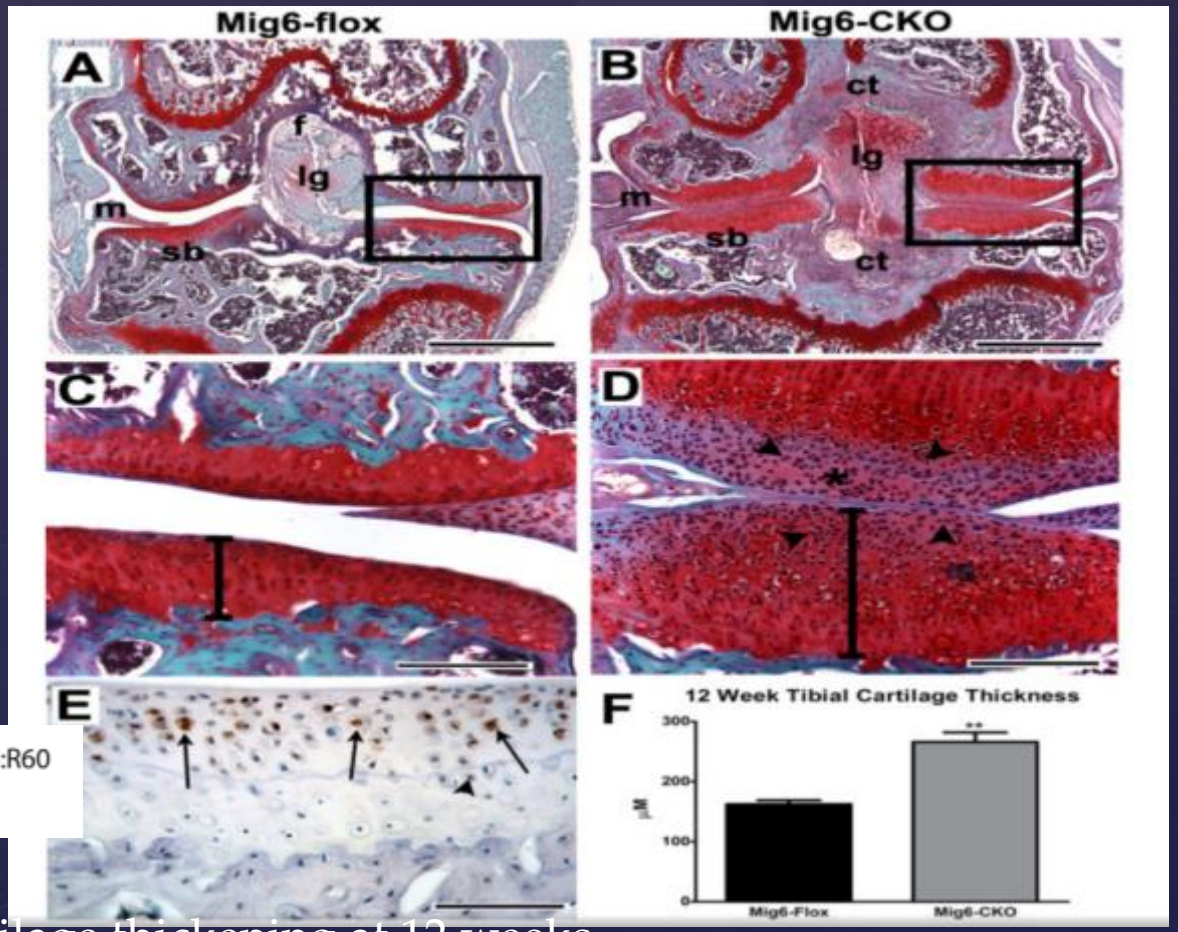


Problem to Address

- Our goal is to stimulate repair by the joints own cells
- We hope to do so by causing regeneration of the existing cartilage cells in the joint
- However, once osteoarthritis becomes severe and widespread, the cartilage damage becomes irreversible
- Over time, the cartilage will degrade so far that the joint will have bone to bone contact
- So we need to target an earlier stage injury, to repair, and thus prevent the disease from developing
- At an earlier stage, some cartilage will still be present in the joint, and this is useful for us.

EGFR Signaling

- Epidermal Growth Factor Receptor family play an important role in endogenous developmental processes.
- They are regulated by an inhibitor
- Research has shown that performing a Conditional knockout of this inhibitor promotes an over expression of the growth factor signaling
- The activated signals stimulate resident cells in the joint and cause cartilage to thicken

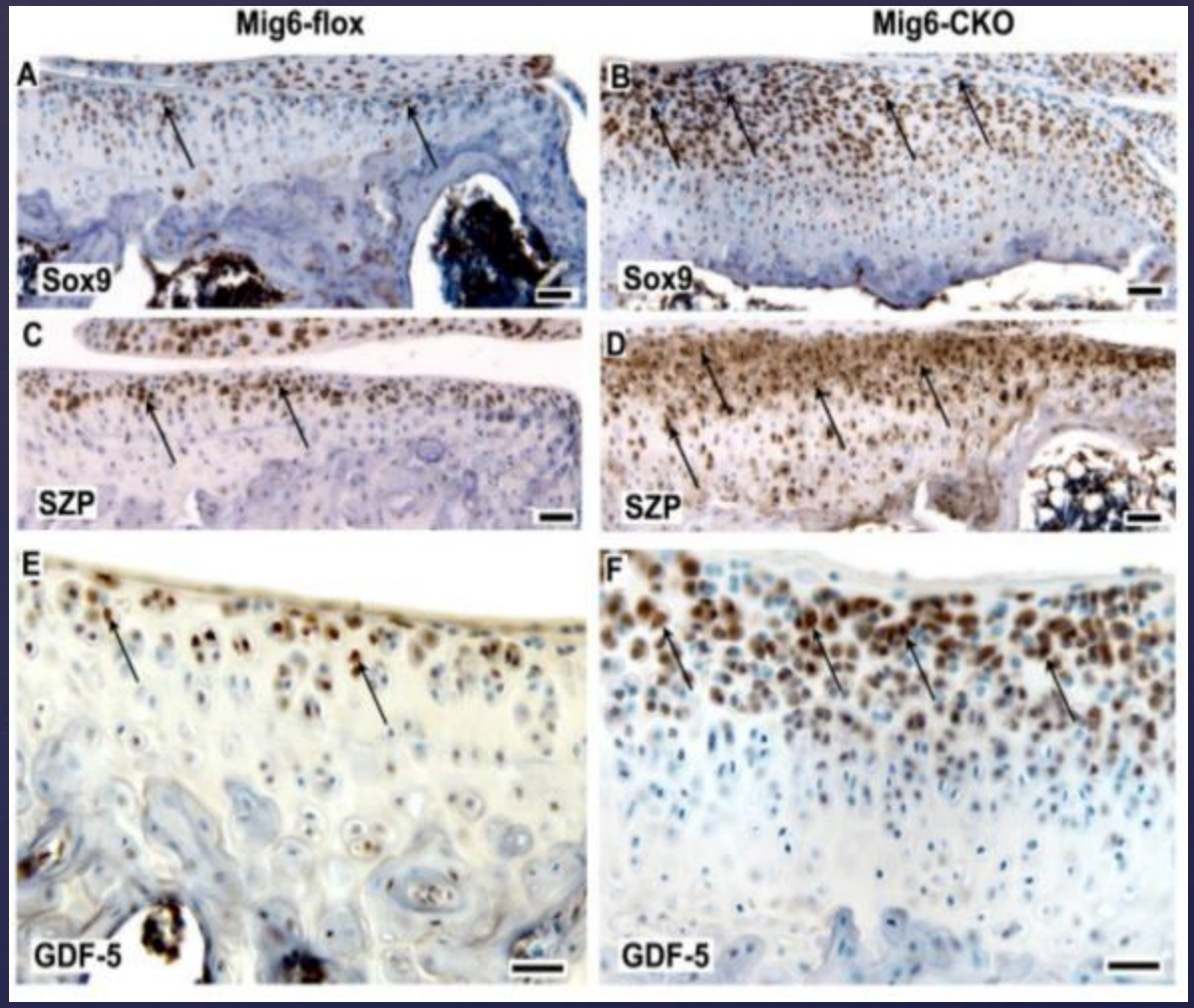


Shepard *et al. Arthritis Research & Therapy* 2013, **15**:R60
http://arthritis-research.com/content/15/3/R60

Research shows articular cartilage thickening at 12 weeks

Current Research

- A promising route of treatment
  - Resident progenitor cells in articular cartilage can respond to these growth factor signals by making new cartilage tissue.



Shepard *et al. Arthritis Research & Therapy* 2013, **15**:R60
http://arthritis-research.com/content/15/3/R60

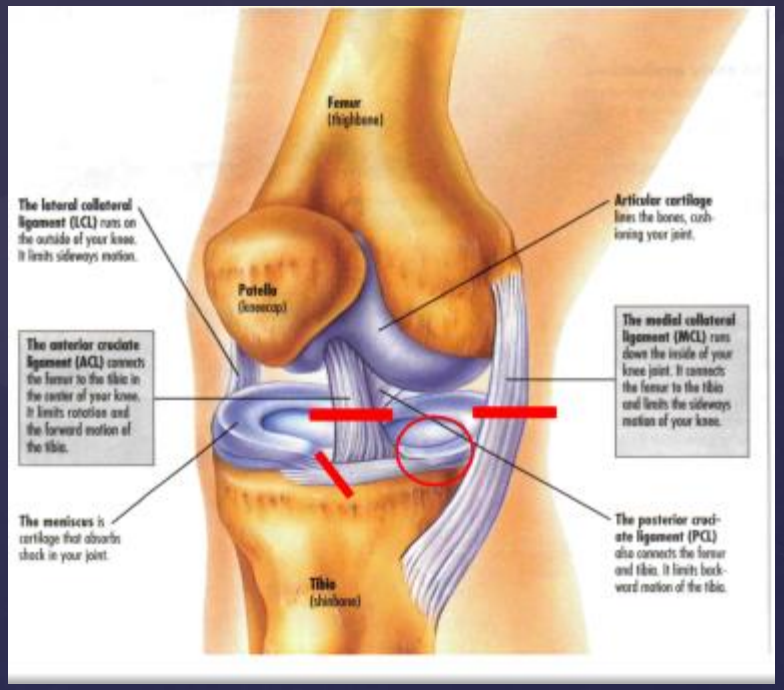
Project Goal

- The goal of this project is to obtain preclinical proof of concept for use of these growth factors to repair traumatic damage to the joint cartilage, which could prevent the development of osteoarthritis later on

- This will be achieved through a two step process:
  - Developing a repairable model of cartilage damage with osteoarthritic potential.
  - Using the model to test the role of EGFR to treat cartilage damage

Model development

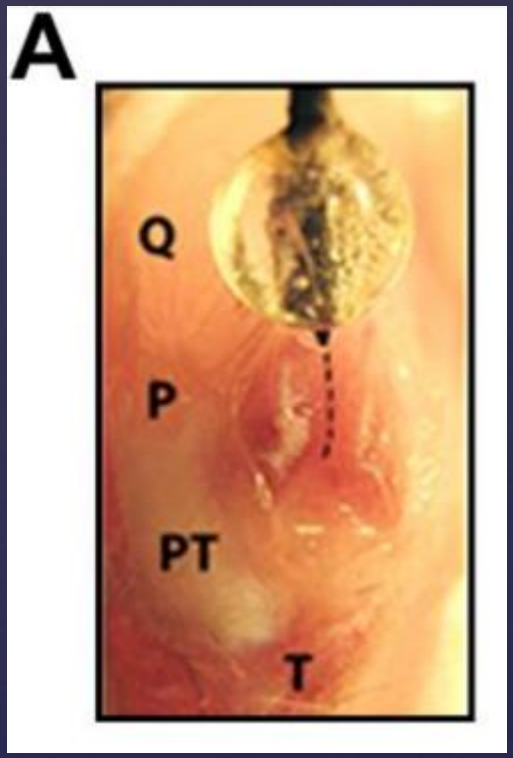
- In past models, osteoarthritis was induced by cutting the tendons surrounding the mouse knee joint and the instability of the joint will develop osteoarthritis.

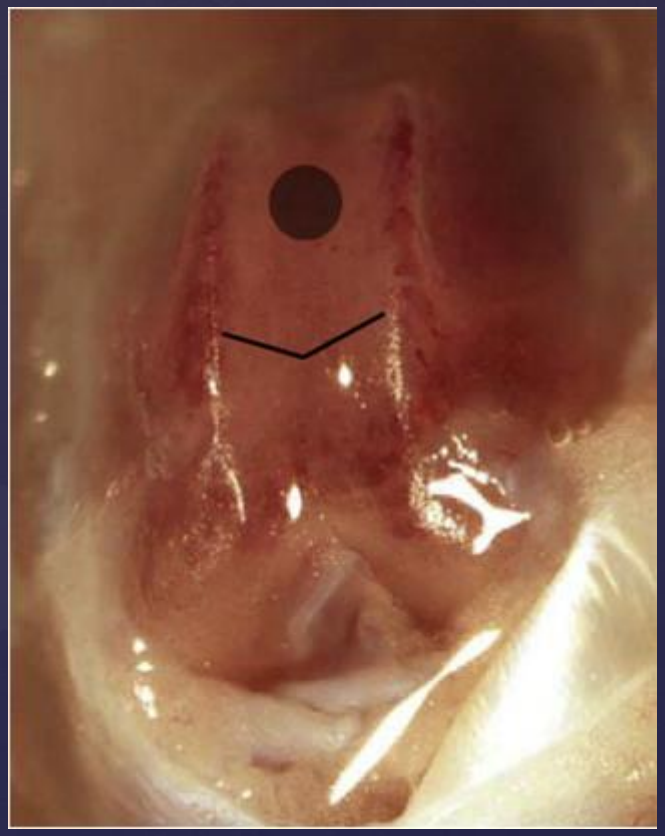


- However this model is non repairable and would not be appropriate for studies with cartilage regeneration by growth factor stimulation.
- Thus a repairable model must be developed.

Experiment

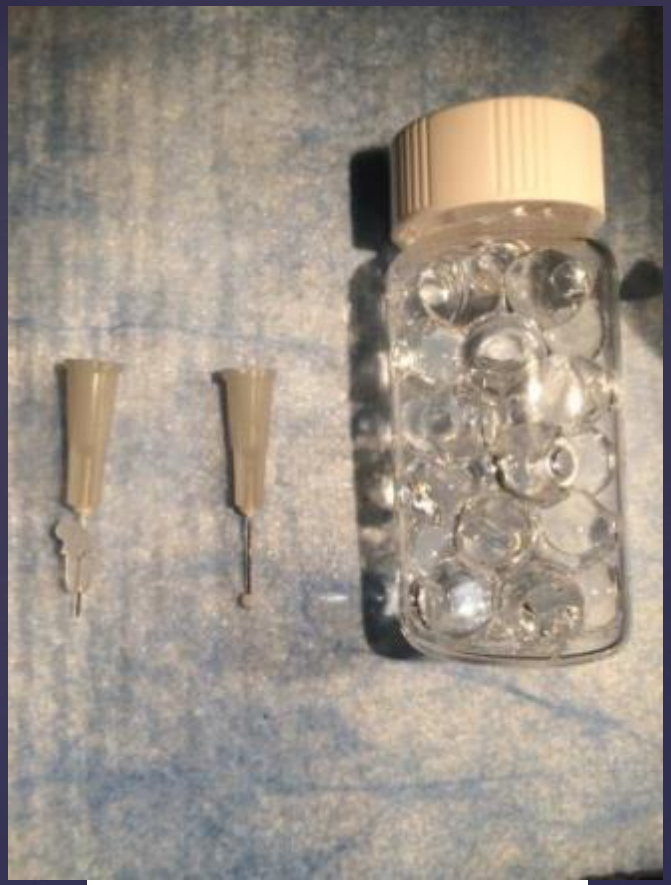
- Microsurgery will be used to create a defect (hole or groove) in the articular cartilage of the knee joints of mice.

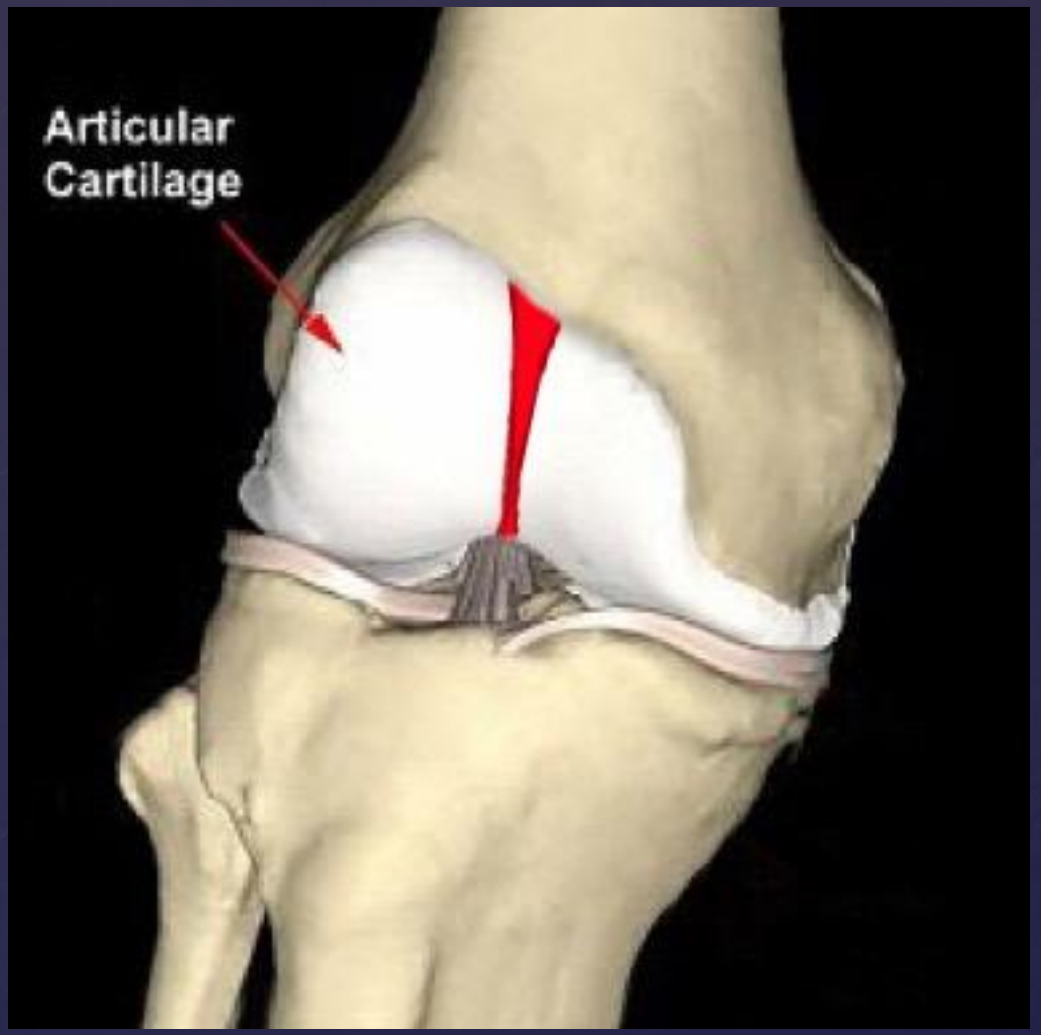




J. Fitzgerald et al: Articular cartilage regeneratiob in MRL/MpJ mice

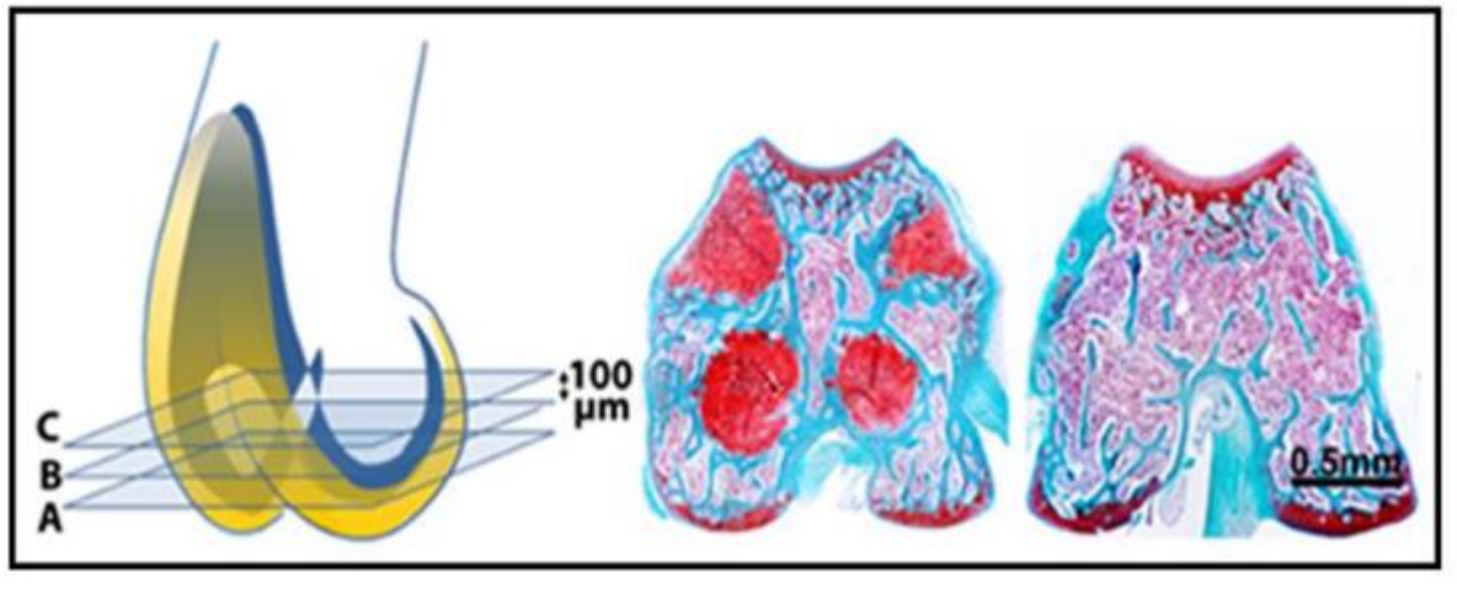
N.M. Eltawil et al: A novel in vivo murine model of cartilage regeneration





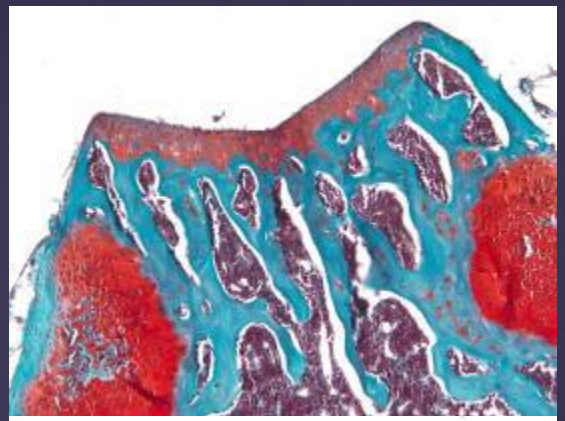
Experiments done on control C57/Bl 6 12 week old mice

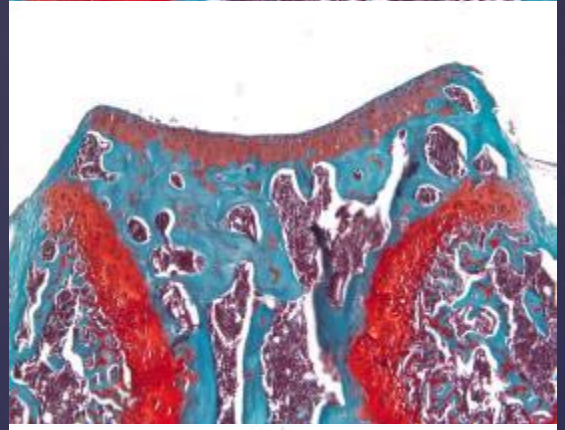
- The critical defect will injure the cartilage. Over time, the joint of the mouse will develop osteoarthritis
- Histology will confirm that the defect does not heal on its own, and that osteoarthritis is induced



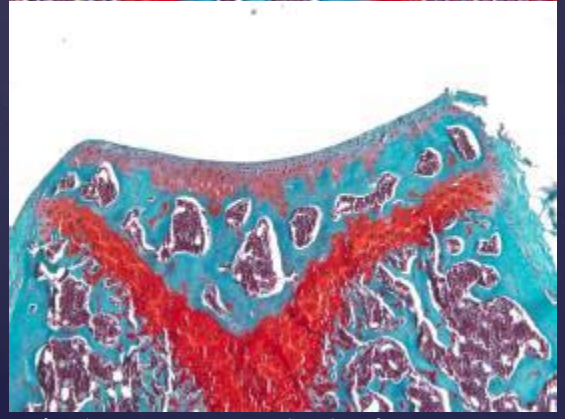
N.M. Eltawil et al: A novel in vivo murine model of cartilage regeneration

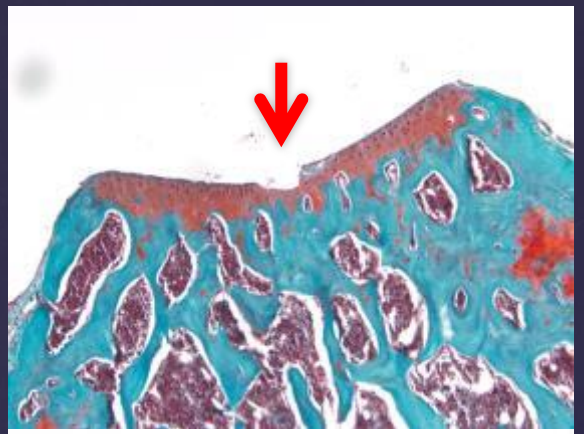
Results



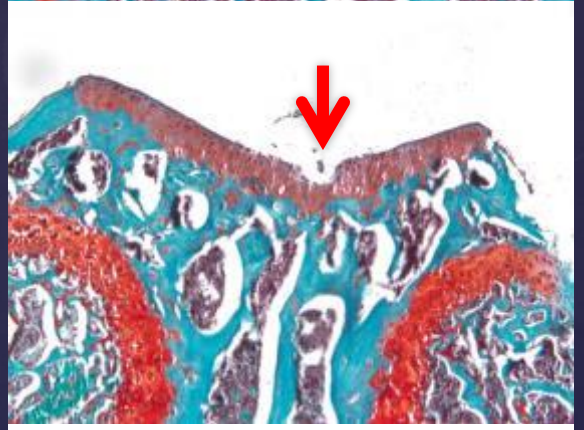


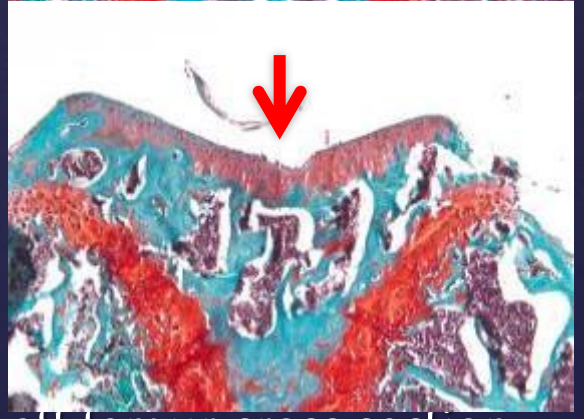
CT



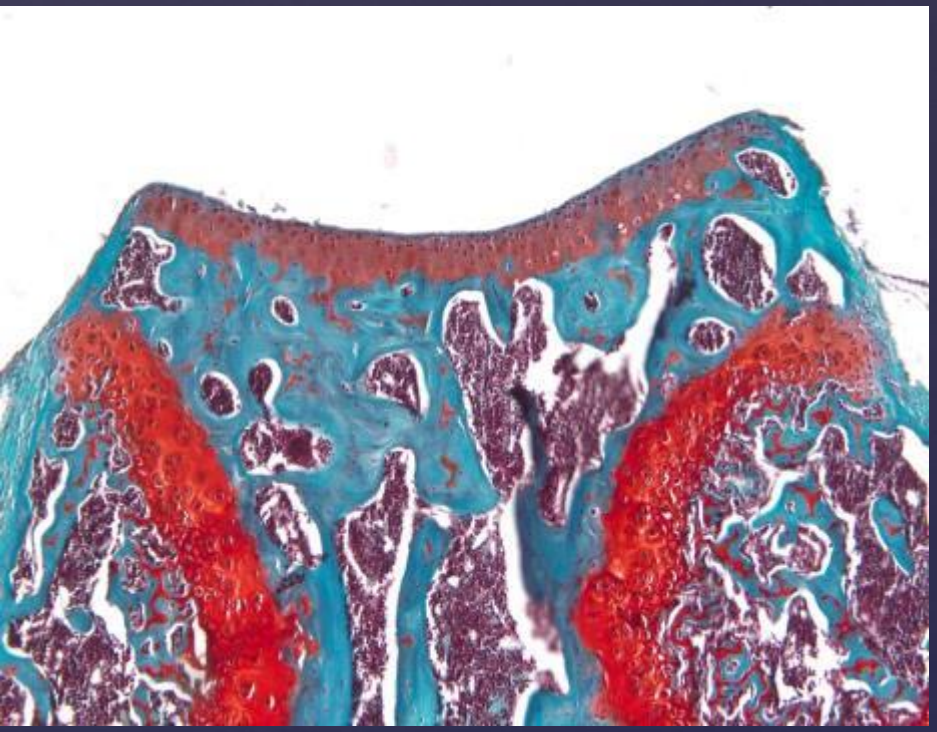


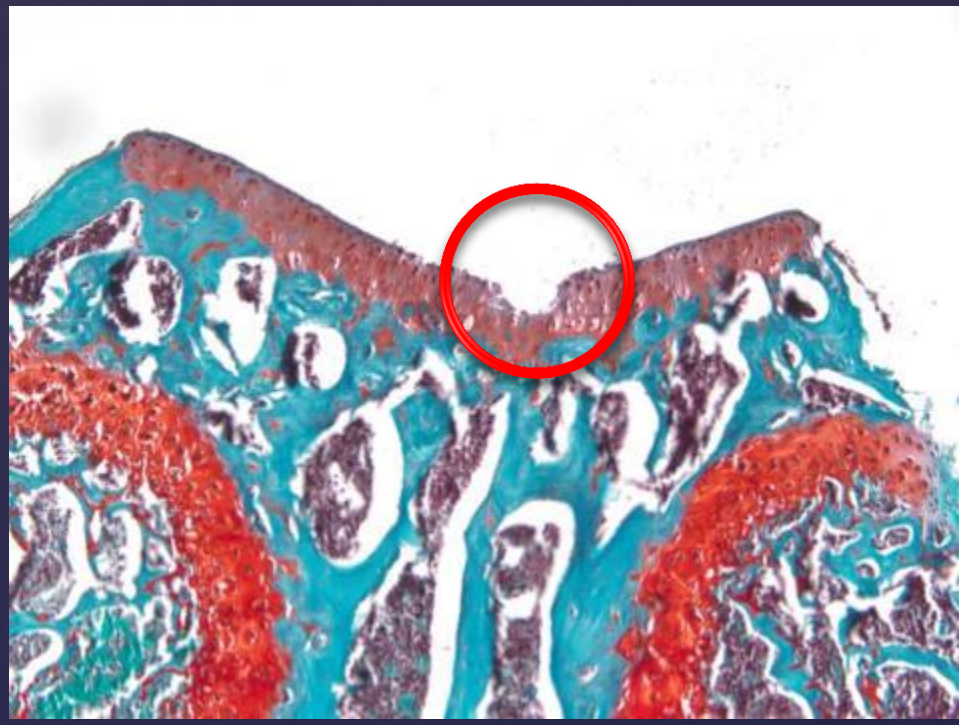
Defect Surgery





- C57/Bl6 Critical defect surgery +1 day post surgery. Left femur cross section
- C57/Bl6 Right femur cross section. No surgery control





Results

Next step future work

- Compare healing responses between normal mice and mice in which the growth factor signaling had been genetically activated.
  - Examine Progenitor cells to validate that growth factor signaling activation plays a part in the regeneration of cartilage.
- Examine the ability of nanoparticles as a delivery method for the growth factor to repair joint cartilage damage

Conclusions

- If successful, this will provide preclinical evidence for growth factor signaling in stimulating progenitor cells to regenerate cartilage.
- Together these studies will support development of a clinically relevant approach to prevent osteoarthritis by stimulating cartilage repair by the body's own cells.

Acknowledgements

- Dr. Caroline Dealy
- Dr. Melanie Fisher
- Mayuko Sonokawa
- Lane Wilson
- J.B. Shepard
- UCONN Stamps Scholarship
- Dr Dealy's DOD Grant "New Strategies for Targeted Intervention in Post-Traumatic Osteoarthritis"

- Tyler Ackley
- Ayesha Ejaz
- Luiz Reza