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TITLE: Electric Field Stimulation Enhances Healing of Post-Traumatic Osteoarthritic Cartilage

PRINCIPAL INVESTIGATOR: Chloë Bulinski, PhD

CONTRACTING ORGANIZATION:
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6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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New York, NY 10027

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Introduced early into the course of PTOA, electromagnetic energy may have the benefit of maintaining function in highly active individuals. Thus, electromagnetic fields are particularly suited to young active populations and for the military, in whom, PTOA is an unfitting condition and for whom joint replacement is an unsuitable salvage option. If successful, electromagnetic energy will maintain joint function and avoid surgery. The benefits to retain one's own joint are obvious. Electromagnetic energy devices are FDA approved for bone healing and have been use for 30 years in thousands of patients with an extremely low frequency of adverse events. So the risks of treatment are quite minimal. The clinical applications of electromagnetic field therapy would especially appealing to the young and middle aged patients with early PTOA who are symptomatic from pain and limited function. Benefits would be the reduction of pain and inflammation with concomitant improved function and also preservation of cartilage and bone with the potential avoidance, or at least delay, of joint replacement. Risks of this treatment would be so infrequent as to be anecdotal.

15. SUBJECT TERMS
Cartilage explants, fibrin glue, collagen, canine model system

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
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1. INTRODUCTION:

The causative role of trauma in the development of osteoarthritis (OA) has been well-described, and there are as yet no effective strategies for preventing the inflammation and apoptosis that develop post-trauma. Thus, injuries to joints caused by either sports or combat almost always develop into full-blown OA. Both basic and translational studies are needed to establish and therapeutically target the progression of traumatic injury into OA; a vital part of these studies involves use of animal models. To further our main objective of substantially improving the poor healing of OA defects in cartilage, we propose to develop strategies in which our model system is the dog, and we use electric fields (EFs) to direct movement of cartilage precursor cells to the site of OA damage, and to optimize differentiation at the site of injury. As precursor cells we will utilize either endogenous canine chondrocytes or the clinically relevant canine cartilage stem cells called Synovium-Derived Stem Cells (SDSCs). Moreover, we will develop and optimize matrix components that we will introduce into the site of injury in order to further enhance precursor cell motility and chondrogenic differentiation. We propose that our strategy will promote healing of articular cartilage defects in dogs. Further, we are confident that achieving healing in the canine system will both be useful for treating canines in the military and will also allow these strategies to be translatable to humans.

2. KEYWORDS: Cartilage explants, fibrin glue, collagen, canine model system

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.

Specific Aim 1: Apply EFs to canine cartilage explants to measure cell motility/ recruitment into an experimental wound.

Major Task 1: Perform in vitro studies of canine cartilage explants, measuring cell motility/ recruitment into an experimental wound.

Specific Aim 2: Apply EFs to 'wounded' canine cartilage explants in the presence of labeled synovium-derived stem cells (SDSCs).

Major Task 2: Perform in vitro studies of canine cartilage explants to which canine SDSCs have been added, measuring cell motility/ recruitment into an experimental wound.

Specific Aim 3: Perform *in vivo* studies investigating the efficacy of DC EFs for cartilage repair in a canine knee defect model.

Major Task 3: Make cartilage wounds in animals, surgically implant electrodes, and allow healing in the presence or absence of applied EFs.

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.

During Year 3, since experimental work for **Specific Aims 1 and 2** had been completed, including the milestones listed in the SOW for Months 0-24, we concentrated on 1) quantifying the motility and successful cartilage differentiation (with and without electric field administration) that we had induced with our experimental, injectable matrix components using in vitro cartilage explants, 2) presenting this work at orthopedic meetings and starting to prepare a full-length manuscript, and 3) performing the live animal work at the University of Missouri as described in Aim 3.

We began by analyzing and quantifying images of the specimens in which motility of cells was complete, a uniform distribution of cells and been achieved (+/- the application of the electric field), and cells in the lumen of the tissue explant (the so-called Chondrobagel) had been induced to differentiate (+/- the application of the electric field).

<u>In vitro work and quantification:</u> For these experiments, we used the Chondrobagel in vitro model of 3D cartilage wound-healing that we had previously devised for these studies, since this model is directly translatable to the in vivo experiments described in Aim 3. Briefly, our Chondrobagel model is a canine cartilage explant derived from the stifle joint, whose empty center is the exact dimensions of the in vivo 'wounds' we use in the canine trochlear groove in Aim 3.

We then explored electric field-induced migration (or galvanotaxis) of synovium-derived stem cells (SDSCs) in a collagen gel (lumenal patch) within the chondrobagel lumen (simulating an in vivo cartilage wound and generated, in turn, to simulate an in vivo cartilage injury). Synovial cells have been implicated in the limited repair response of cartilage in situ and can be differentiated to make cartilage in vitro, making them an attractive target cell source for us to use as simulated cartilage repair strategies.

Early in Year 3, we received the electric field chambers (see Appendix Figure 1, top) was that were configured to apply direct current electric fields to cylindrical cartilage tissue specimens in vitro and also in vivo. We were thus able to stimulate the migration and differentiation of endogenous/exogenous repair cells into a model defect (a Chondrobagel) and test whether in vitro effects of the electric field would predict in vivo success from the surgeries performed late in Year 3. We hypothesized that electric field orientation could be optimized to A) expedite and improve motility and differentiation/integration of tissue engineered cartilage grafts and ultimately this would induce a more favorable synovial joint environment for cartilage wound-healing in vivo. In Appendix Figure 1, bottom, we show the two field orientations we tested, namely electric field parallel or perpendicular to the explant surface. The chondrobagel models were placed in one of three conditions: no electric field (CTL) and electric field stimulation with coils oriented either perpendicular ($\perp$ PEMF) or parallel (|| PEMF) to the explant surface (Figure 1). PEMF (75 Hz, 1.5 mT) was applied for 8 hr/day, 7 days/week.

Constructs were cultured in chondrogenic medium supplemented with 10ng/ml TGFβ3, 100nM dexamethasone, and 50μg/ml ascorbic acid-2-phosophate. Specimens were evaluated for mechanical (not shown here) and biochemical properties at 0 and 6 wks. Electric fields differentially modulated the differentiation in the lumen of the explant (Appendix Figure 2). The %GAG/DW in the lumen increased relative to both control and perpendicular PEMF groups. In contrast, the %GAG/DW in the outer annulus was only affected by a perpendicular oriented PEMF.

In vivo work (to be completed during Extension Without Cost that is currently underway):
The original schematic for the in vivo experiments is shown in Appendix Figure 3. With the electric field devices and the in vitro results in hand, we designed the groups as depicted in the figure, and to administer a parallel electric field that would be the most likely to provide a significant enhancement of in vivo healing. Thus we obtained 9 dogs (4 groups as described in the proposal and shown in Appendix Figure 3, as well as one extra in case of surgical problem so this one was added to the + cells, + electric field group. We were unable to afford more dogs per group because of budget limitations and availability – at any price – of appropriate animals anywhere in the Midwest where our subcontracting investigator, Dr. James Cook, has his laboratory.) We also had to perform the labor-intensive and expensive task of grow up the necessary large quantity of canine SDSCs ($>10^7$ cells) and fit the surgeries into Dr. Cook's busy surgical schedule.

Thus, the surgeries were performed only 3 months before the end of Year 3. Each canine was 'wounded' by having 3 punches removed from trochlear groove cartilage, and matrix+/- SDSCs was placed in each of the cartilage wounds before the wounded area was closed up. All animals were fitted with electric field devices as shown in Appendix Figure 4. In order for the experiment to be done in a blind fashion, only the serial numbers on the devices indicated which were 'live' and which were sham devices that did not deliver an electric field.

All of the dogs received physical therapy and their gate was monitored to assess healing. The electric field applied was applied as described for in vitro work. At 3 months, all animals were sacrificed; we are now in the throes of analyzing the mechanical, biochemical and histological properties of the healed cartilage and comparing it to controls and to the surrounding tissue in each experimental animal. Our results will be forthcoming; we hope to test whether the electric field applied in parallel enhances migration of synovial repair cells, SDSCs, into the surgically administered wound, and repair of the cartilage in the wound.

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.

Work on the project provided a major amount of the Ph.D. training of the graduate student who has been working on the project, Robert Stefani, M.S. The work on this project will form a major part of his dissertation for his Ph.D. (See Appendix Figures for 2017 ORS Abstract on part of this work)

In addition, one undergraduate, Ms. Carina Sirochinsky, a Hunter College McCauley Scholar who started worked on the project in Summer 2016 as part of the Amgen Summer Program here at Columbia University, continued work through the Academic Year, 2016-2017 (during Year 3). She received training and mentored work with the PI, participated in the 2017 ORS Meeting, and she even presented a poster there (See Appendix Figures for 2017 ORS Abstract on part of this work); this is a tremendous addition to her professional development. Following her graduation, Ms. Sirochinsky was able to parlay this experience and training into a job at a small biotech startup in Brooklyn, NY, where she will work as she prepares for graduate school. Ms. Sirochinsky is planning to study for a Ph.D. in Material Science or Biomedical Engineering, and this emphasis stems partly from her focus is on biomaterials in the project.

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.

In addition to the ORS presentations by Sirochinsky and Stefani, made in order to share results with the orthopedic research community, the PI has given several talks to college students (Columbia Science Research Fellows, Biological Sciences Incoming Class of Ph.D. students) and the PI and Ms. Sironchinsky also presented this work at the ABRCMS (Annual Biomedical Research Conference for Minority Students). In this way, many young students new to science were introduced to the application of cell motility and differentiation studies to solving problems in human health.

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

In the final Extension Without Cost period of our project, we will quantify the extent cartilage repair of the dogs who were sacrificed at the end of Year 3. We will assay their level of differentiation via GAG, collagen, and measure their level of integration with the surrounding tissue optically and through mechanical assays. We will also use histology to determine whether the electric field application and the inclusion of SDSC repair cells affected the degree of wound-healing and integration with surrounding tissue.

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).

The work in which we identified appropriate matrix and cell conditions as well as appropriate electric field parameters to substantially 'heal' in vitro wounds is important and impactful. Given the success of our in vitro strategies, we feel that we have a good chance of success in vivo, as well. If the success of our 3D in vitro wound-healing system is mimicked in the analysis of our in vivo work, our study will have had a transformative impact on the field.

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.

In other fields such as cell biology, investigators have had difficulty coming up with 3D models that can be used to study cell motility, cell differentiation, wound-healing, etc. Our system satisfies most of the criteria demanded for these types of investigations and in addition, our model system is amenable to real-time observation of the migrating cells. Thus, our system has been noted by cell and developmental biologists for its ingenuity, health impact and convenience.

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

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

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

Nothing to Report

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

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

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

Nothing to Report

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

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

Nothing to Report

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 are completing our project so the delays we had in getting the dogs, scheduling the surgeries, getting the electrical devices, and growing up the huge quantity of cells have all been resolved by the CDMRP's granting us an Extension Without Cost. We feel we now have ample time to complete and write up the remaining work.

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

Nothing to Report

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

Significant changes in use or care of human subjects

Not Applicable

Significant changes in use or care of vertebrate animals

No Changes

Significant changes in use of biohazards and/or select agents

Not Applicable

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

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

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

Nothing to Report

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

1. Sirochinsky et al (see Appendix): "**Effect of Genipin on Engineered Tissue Integration in an Injectable Cell-Seeded, Collagen and Fibrin Glue Matrix" ORS 2017 Abstracts. Accepted for Presentation at Annual Meeting in San Diego in March, 2017**

2. Stefani et al. (see Appendix): **"Electric Field Modulation of Synovial Fibroblast Migration for Cartilage Repair" ORS 2017 Abstracts. Accepted for Presentation at Annual Meeting in San Diego in March, 2017**

- **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.*

1. Sirochinsky, C., Pino, N., and Bulinski, J.C. "**Effect of Genipin on Engineered Tissue Integration in an Injectable Cell-Seeded, Collagen and Fibrin Glue Matrix", presented at ABRCMS in Tampa Fl, November, 2016**

- **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.

Nothing to Report

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

Nothing to Report

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

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: J. Chloë Bulinski
Project Role: Principal Investigator
Nearest person month worked: All of Year 3 (and up to the present)
Contribution to Project: Dr. Bulinski has trained all students, designed all experiments and participated in the set-up, analysis and troubleshooting of experiments.

Name: Clark T. Hung
Project Role: Co- Investigator
Nearest person month worked: All of Year 3 (and up to the present)
Contribution to Project: Dr. Hung has trained all students, designed all chambers and apparati, and participated in set-up, analysis and troubleshooting of experiments.

Name: Roy Aaron
Project Role: Partnering P-I
Nearest person month worked: Year 3 - present
Contribution to Project: Dr. Aaron has participated in design and analysis of electric field application experiments.

Name: James L. Cook
Project Role: Partnering P-I
Nearest person month worked: Year 3 - present
Contribution to Project: Dr. Cook has participated in design and functional application of 3D matrix to support cell motility. He has also supplied canine tissue used in Quarter 3 and up to the present

Name: Rob Stefani
Project Role: BME Graduate Student
Nearest person month worked: 1 months of Quarter 4 (and he will continue until at least June 1, 2017)
Contribution to Project: Mr. Stefani generated the figures for the Appendix Results and one of the abstracts therein; he has also performed the Z-stack imaging and analysis of cell densities.

Name: Carina Sirochinsky
Project Role: Hunter College McCauley Honors Undergraduate Student
Nearest person month worked: All months of Quarter 4 (and she will continue part-time, until at least May 1, 2017)
Contribution to Project: Ms. Sirochinsky generated the data and figures in one of the abstracts Results and she also performed the SDSC labeling studies and the analysis of matrix components.

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.

Nothing to Report

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

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

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

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

Ruggero Cadossi, President and CEO, IGEA Medical
Carpi, Italy

Dr. Cadossi and his team participated in the project in Year 3 by helping design the in vivo experiments and by providing – gratis – the electrical devices used on each of the dogs studied. Dr. Cadossi also met with us twice and contributed advice in several phone/SKYPE conversations to enhance the collaborative work.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

None

Total Spent Project to Date as per Columbia University's Financial System: $578,486

Total Spent in Year3 as per Columbia University's Financial System: $240,418

Current Unspent Balance as of 9/30/17 $63,770

QUAD CHARTS: See Quad Chart for More information

9. APPENDICES:

OR130124 - Electric Field Stimulation Enhances Healing of Post-Traumatic Osteoarthritic Cartilage

PI: Bulinski, Jeannette Chloë, Ph.D. **Org:** Columbia University in the City of New York **Award Amount:** $500K

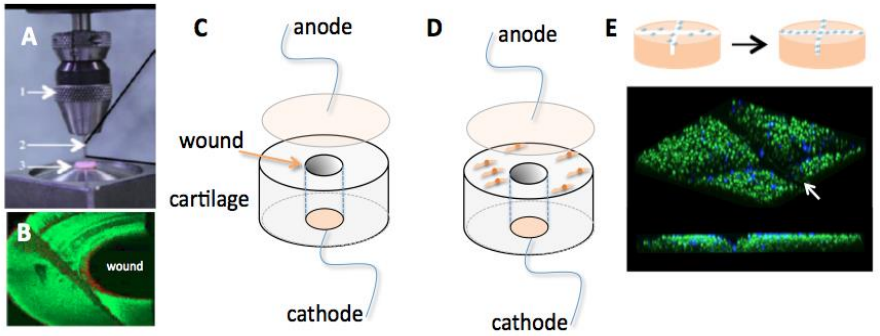
Study Aims

- Test EF capacity to 'heal' wounds in canine cartilage explants.
- Test EF capacity to 'heal' cylindrical wound in cartilage explants as above, but with added synovium-derived stem cells (SDSCs).
- Test EF capacity to heal *in vivo* osteochondral defects in canine knees.
- Test EF capacity to heal *in vivo* canine cartilage defects as above but with added SDSCs that may home to wounds to promote healing.

Approach

In vitro studies: We will prepare cylindrical explants of canine knee cartilage, simulating a focal defect in canine cartilage. We will fill the centers with a gel of Type I collagen to simulate a fibrous scar. We will measure how well the applied EFs induce migration of chondrocytes and/or labeled SDSCs (added to ½ the explants) into the wound area of the explant.

In vivo studies: We will generate focal defects in the trochlear groove of canine knee cartilage and use applied EF to promote movement of endogenous chondrocytes and added canine SDSCs into the lesion. We will measure recovery of gait, artheroscopic imaging, and endpoint histology.



A) Apparatus we will use to A, Create cylindrical defects in cartilage explants with minimal cell death at wound edge (B, live cells: green and dead cells: red). Schematic of cartilage explant wound model subject to DC electric field (EF) ± SDSCs (C, D). E) Confocal stack image of cell migration (blue label) into cruciform wound on living cartilage explant (green chondrocytes). Not shown: Application of EFs to cylindrical defects made in canine knee cartilage (focal defect model), to promote migration of endogenous chondrocytes or injected SDSCs with the capacity to heal the cartilage.

Time and Cost

Activities / FY	14	15	16	17
Specific Aim 1 – apply EFs to canine cartilage explants to measure cell motility recruitment into an experimental wound.				
Specific Aim 2 – apply EFs to 'wounded" canine cartilage explants in the presence of SDSCs				
Specific Aim 3 – Perform *in vivo* studies investigating the efficacy of DC EFs for cartilage repair in a canine knee defect model				
Estimated Budget		$110,594	$169,802	$240,418

Updated: 18 Oct 2017

Goals/Milestones

FY14 Goal – Measurements of the efficacy of EFs to activate cell motility/recruitment into an experimental wound within canine cartilage explants (obtained from euthanized animals).

FY15 Goal – Measurements of the efficacy of EFs to activate motility/recruitment of Synovium-Derived Stem Cells (SDSCs) into experimental wounds within canine cartilage explants.

FY16 Goal – Testing the recovery of mechanical properties, biochemistry, and histology of .canine knee joints which we treated with EF, SDSCs, both, or neither, to evaluate the efficacy of healing of in vivo cylindrical wounds (i.e., 'focal defect' lesions).

Comments/Challenges/Issues/Concerns

- N/A at this time

Total Budget Expenditure to Date

Actual Expenditure: $578,846

1

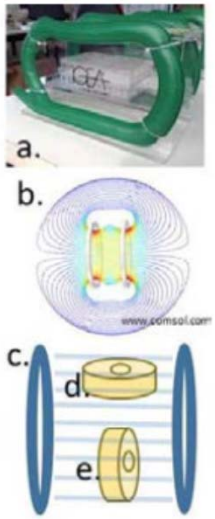
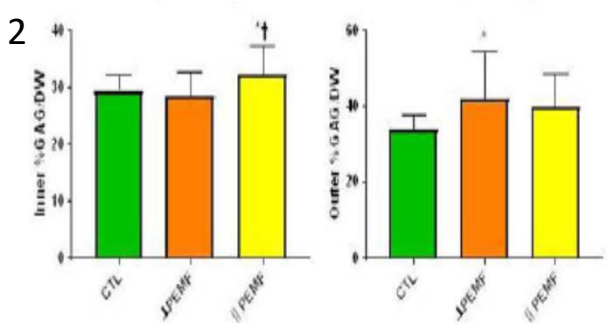


Figure 1: a. In vitro electric field (EF) chamber to be used also in vivo; b. Schematic of EF generated by dual coils; c. Coils with EF; d. Construct orientation perpendicular to EF (orange bars in Figure 2); e. Construct orientation in parallel to EF (yellow bars in Figure 2).

Figure 2: The parallel EF enhanced differentiation (%GAG/DW) within the inner matrix-filled core region of each Chondrobagel. *p<0.05 compared to no PEMF control (CTL) group.

2



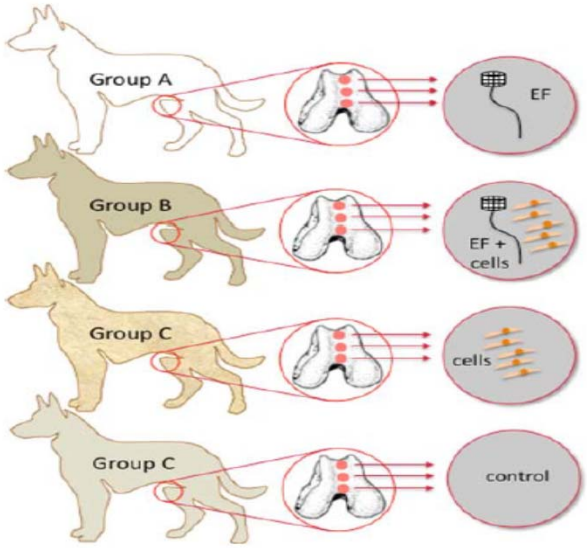


Figure 3: Nine animals were used in the study; they were divided into groups A-D as outlined in the original proposal (for convenience, the schematic, Figure 5 from the original grant proposal is reproduced here). Results will be measured during the Extension Without Cost that we have been awarded (as animals were sacrificed on the last day of Year 3 and we have not yet had sufficient time to analyze results)

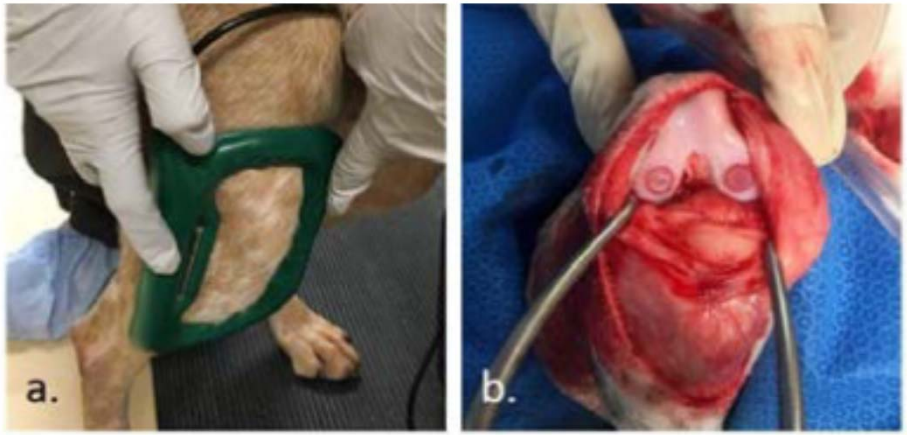


Figure 4: a. Coil placement on canine stifle joint following b. In vivo implantation of grafts on canine femoral condyle.

Electric Field Modulation of Synovial Fibroblast Migration for Cartilage Repair

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Disclosures: RMS, BLR, AMS, RJN, JHL, GAA, JCB (None), CTH (7- JOR)

INTRODUCTION: As articular cartilage is not vascularized, it exhibits a poor healing capacity, creating a condition where localized regions of damage will lead to full blown osteoarthritis if untreated. Applied electric fields (EFs) can be applied *in vitro* to foster development of functional tissue grafts in culture as well as *in vivo* for promoting tissue repair. The application of EFs is already used clinically to promote wound healing of various tissues, including skin, bone and cartilage [7]. In the context of cartilage repair, the system described herein allows EF gradients to be applied to cells cultured in in 3D, whereas such studies have been more typically performed in 2D [3]. The current study explores EF-induced migration (or galvanotaxis) of synovial fibroblasts in collagen gel (study 1) or on cartilage explants in which cylindrical wounds have been made to simulate cartilage injury (study 2). Synovial fibroblasts have been implicated in the limited repair response of cartilage *in situ* [5] and can be differentiated to make cartilage *in vitro* [6], making them an attractive target cell source for cartilage repair strategies.

METHODS: Chamber Design: The galvanotaxis chamber (Figure 1A) was configured to apply direct current (DC) electric fields to cylindrical cartilage tissue specimens to simulate the migration of endogenous/exogenous repair cells into a model defect (Figure 1B). A gasket between the top and bottom portion provided a tight seal and accommodated irregularly shaped specimens. The cathode (-) and anode (+) were positioned above and below the sample chamber, respectively. Study 1: Synovial fibroblasts were seeded at a density of 50,000 cells/cm^2 on the surface of a 2 mg/ml type I collagen gel. After an overnight pre-culture to allow adhesion, gels were placed in the chamber and exposed to 3.33mA current for either 90 or 180 min to achieve an applied field strength E= 1 V/cm. Study 2: A section of native synovium was labeled with DiI (yellow) and placed in direct physical contact with native cartilage for EF stimulation. 10 mm diameter cylindrical explants (thickness of 1 mm), with 1mm diameter concentric cores removed, representing cartilage with a lesion were studied. Explants were cultured in serum-free chondrogenic defined media [8]. Imaging: For study 1, samples were stained with DAPI for cell localization. For study 2, the synovium was removed from the explant for subsequent fixation in 4% PFA and imaging, leaving migrated cells behind. Specimens were subsequently stained with DAPI (blue) for co-localization of endogenous cartilage cells with the migrated synovial cells on the cartilage explant. A confocal microscope was used to visualize cell accumulation at the cartilage surface and defect site. Chamber Characterization and Validation: Electrical properties of the specimens were computed assuming an applied current of 3.33 mA, media resistivity of 0.59 Ωm, cartilage resistivity of 1.56 Ωm, and sample geometry. Current density was 17 mA/cm^2 and voltage drop (E) was 1V/cm in the collagen gels in study 1. Current density was 422 mA/cm^2 and voltage drop (E) was 25 V/cm at the defect site for study 2. The overall chamber resistance was estimated theoretically and agreed with measurements. Finite element analysis of the configuration in study was also performed using FEBio, (Figure 1C and 1D) [1]. The cartilage ring was modeled as a triphasic material: porous solid (cartilage matrix), interstitial solvent (PBS/media), and two monovalent counter-ions (sodium and chloride) [2]. The current density profile at equilibrium showed high ion flow at the defect site and relatively low flow in the cartilage explant, as expected. Statistics: Comparisons were analyzed using ANOVA with Tukey's post-hoc test ($p<0.05$).

RESULTS: In study 1, a proportion of synovial fibroblasts migrated to a maximum depth of ~250 µm after 90 min and ~650 µm after 180 min, for a an average speed toward the cathode (-) in the range of 167 µm/hr to 217 µm/hr. For comparison, no-EF control cells migrated ~50 µm/hr (Figure 2). For the given region of interest, ~11% of cells migrated after 90 min and ~23% in the 180 min sample. In study 2, a small number of synovial cells migrated to the cartilage surface regardless of treatment conditions. Control specimens (no-EF) showed minimal synovial cell recruitment to the cartilage surface (Figure 3A) and no visible migration into the defect site (Figure 3B). On the other hand, specimens exposed to a single three hour EF treatment showed a high degree of synovial cell recruitment (Figure 3C). Additionally, synovial cells migrated to a depth of up to 200 µm into the defect after 180 min, for an average speed of ~66.7 µm/hr (Figure 3D).

DISCUSSION: In the current study, a specialized galvanotaxis chamber was designed and tested to evaluate 3D cell migration to applied EF strengths at levels similar to those reported to promote galvanotaxis in 2D. The system was modeled and validated using finite element analysis and basic circuit computations. This study demonstrated enhanced cathodal migration of synovial repair cells in both a collagen gel scaffold and a cartilage explant defect repair model. Synovial fibroblasts have been shown to have a speed of up to 10 µm/hr in 2D galvanotaxis systems (E=6V/cm) [4], whereas speeds of an order of magnitude greater were observed currently in 3D collagen gels (1 V/cm) and cartilage defects (25 V/cm). Cell sorting or passaging techniques may help to optimize the cell population for galvanotaxis and subsequent tissue repair [4]. While the present system is unable to perform real-time cell tracking, it does permit for maintenance of aseptic conditions, multiple treatments over time, and subsequent cell, tissue and media analyses. We anticipate that insights gained from the current studies may foster development of therapeutic strategies to promote endogeneous cartilage repair by recruitment of resident or exogenously-delivered cells, such as synovial fibroblasts, via EF-induced homing.

SIGNIFICANCE: This chamber will allow subsequent studies to determine the optimal EF strength, electrode placement, and parameters of electrostimulation for cartilage repair, such as EFs to recruit or guide repair cells to the cartilage wound and then to stimulate their biosynthesis of tissue.

REFERENCES: [1] Maas+ *J. Biomech. Eng.* 2012. [2] Ateshian+ *J. Biomech. Eng.* 2013. [3] Chao+ *Connect. Tissue Res.* 2007. [4] Tan+ *OAC* 2015. [5] Hunziker+ J Bone Joint Surg. 1996. [6] Sampat+ Tissue Eng 2011. [7] Balakatounis+ Eplasty 2008. [8] Ng+ Annals Biomed Eng. 2011.

ACKNOWLEDGEMENTS: This work was supported by CDMRP W81XWH-14-1-0591, NIH AR061988 and Columbia SURF-Amgen.

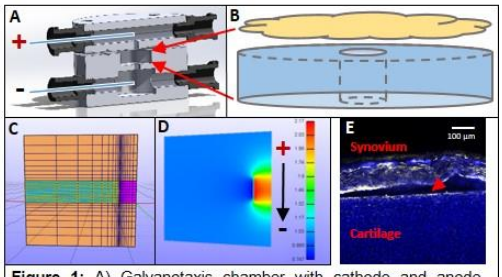

Figure 1: A) Galvanotaxis chamber with cathode and anode positioned above and below the sample chamber. B) Synovium, labeled with DiI (yellow) prior to EF-stimulation and Cartilage labeled with DAPI (blue) post-stimulation. C) FEBio model of chamber represented as two bathing solutions (orange) above and below the cartilage explant (yellow), with a biopsy punch defect in the center (purple). To simplify computations, the symmetric sample was modeled as a wedge. D) Total current density (µA/mm^2) profile at equilibrium. E) Confocal image of specimen *in situ*.

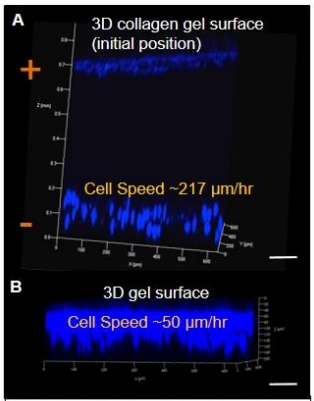

Figure 2: Confocal Z-stack images from Study 1 at T=180 min, A) E=1V/cm, B) E=0V/cm; scale bars= 100 µm

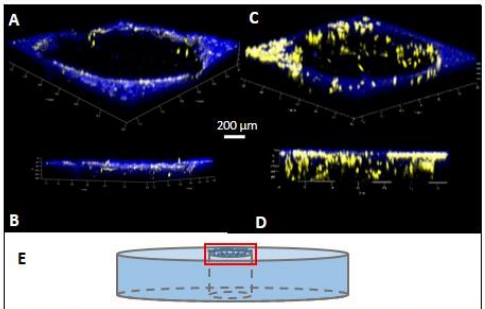

Figure 3: Representative top (A) and side (B) view of control explant (E=0V/cm) showing minimal synovial cell recruitment and migration onto the cartilage and into the defect site. Representative top (C) and side (D) view of explant exposed to DC EF (E=25 V/cm) for three hours, showing high degree of synovial cell recruitment and migration up to 200 µm into the defect. E) Synovium explants were removed for imaging, leaving migrated cells behind in the specified region of interest.

Effect of Genipin on Engineered Tissue Integration in an Injectable Cell-Seeded, Collagen and Fibrin Glue Matrix

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Disclosures: None

INTRODUCTION: Successful engineering of the repair of osteoarthritis lesions would allow for integration between the construct and the native tissue into which it is implanted. To address the issue of tissue integration, we propose an injectable, cell-seeded matrix that contains collagen, fibrin glue, and aprotinin, structurally stabilized by genipin. Genipin, a biocompatible cross-linker, has previously been used to fix collagen-based scaffolds prior to cell-seeding or in vivo implantation [1]. Our approach is novel, as we incorporate genipin with synovium-derived stem cells (SDSCs) in the liquid matrix that subsequently polymerizes into a solid scaffold in our *in vitro* model construct. We hypothesized that the genipin would promote crosslinking between the collagen in the scaffold with that in the native tissue. The incorporation of genipin with living cells warranted a monitoring of the effect of different genipin concentrations of the viability of the SDSCs, as well as testing the effects of addition of chondroitin/hyaluronic acid into the matrix to better model the *in vivo* 3D chondrogenic environment. Upon determining the biological threshold concentration of genipin, we tested the effects of genipin on construct longevity and integration of the construct matrix with the native cartilage tissue.

METHODS: Materials: Unless otherwise noted, all materials and tissue culture solutions were obtained from Sigma Chemical or Life Technologies. Harvest of Cells and Explants: Articular cartilage was harvested from the knee joints of freshly slaughtered 3-4 month old bovine calves, digested with collagenase IV and plated and expanded as described [2]. Biopsy punches (8 mm dia) were used to create cylinders of femoral cartilage. These cylinders were sliced into disks and a biopsy punch was used to remove a 6 mm dia section in the center of the disk (see Fig. 1), creating an explant modeling osteoarthritic lesions. Construct Creation: SDSCs were trypsinized from culture at 90% confluence and labeled with DiI solution (Molecular Probes). The following components were combined to create a liquid matrix with final concentrations as follows: Collagen type I (1 mg/mL bovine Nutragen Collagen, Advanced Biomatrix), fibrinogen (10 mg/mL), thrombin (100 un/mL), aprotinin (3000 un/mL), SDSCs (5×10^6 cells/mL), and genipin at various concentrations (0, 22, 44, 110, 220, 2200µM). The explant lumens were then filled with the liquid matrix (~60 µL of matrix per explant), which was allowed to polymerize at 25°C for 30 minutes (Figure 1). Construct Culture: The constructs were cultured in CM supplemented with 10 ng/mL TGF-β3 for the first 14 days and then in unsupplemented CM for the remainder of the study. Histology: Samples were fixed in acid formalin on day 1, day 14, day 28, day 42, and day 56 of the study to assess the cell distribution, integration with surrounding cartilage tissue, and the GAG (Alcian blue staining) and collagen (Safranin-O staining) distribution in the constructs at each time point. Imaging: Constructs were stained with DAPI nuclear stain prior to imaging. Images were taken with channels for DiI (ex/em 549/565) and DAPI (ex/em 358/461). Live/Dead assays were also carried out to monitor cell death over the course of the study. Integration monitoring: Integration between the artificial and native tissue was monitored by tracking the gap size between the matrix and explant over time as well as tracking the migration of chondrocytes out of the explant and into the lumen region.

RESULTS: Genipin concentrations from 0 µM up to 221 µM were tolerated by SDSCs in the matrix, yielding a uniform distribution of cells and matrix filling the lumen of the construct (Fig. 2). Higher genipin concentrations led to a change in cell morphology from fibroblastic to spherical, potentially indicating cell death; Live/Dead assays confirmed the cell viability in the samples. Samples with genipin concentrations above the tolerated level, however, showed the best construct longevity. In addition, the otherwise toxic genipin concentration had no effect on the viability of cells migrating out of the explant tissue (Figure 3). Addition of chondroitin sulfate (C6S) and HA in physiological concentrations seemed to have no apparent effect on cell viability or longevity of the construct.

DISCUSSION: We have successfully combined collagen and "Fibrin Glue" into a matrix in which SDSCs thrive and fill in the engineered 'wound' in the cartilage explant. Others [3] have reported in vivo incorporation of a similar matrix expected to recruit host cells into an induced cartilage defect. However, testing out similar approaches, with and without stem cells incorporated into the matrix in the lumen of the cartilage explant, we determined that the SDSCs tended to move on, and thus deform, the collagen fibers, and to degrade the fibrin, possibly via secreted metalloproteases, even in the presence of aprotinin to inhibit these enzymes. This resulted in inhomogeneous matrix in the explant lumen and poor integration with the surrounding explant cartilage tissue. Our results here demonstrate that addition of genipin to cross-link the matrix could produce a more stable, better integrated matrix. We determined conditions whereby the addition of genipin during matrix formation was compatible with SDSC viability, and the protocol we have developed appears amenable to extrapolation to an *in vivo* cartilage defect repair model.

SIGNIFICANCE: Creating an engineered tissue that successfully integrates with native cartilage in osteoarthritic joints is a major challenge that has yet to be overcome in the field of cartilage engineering. Incorporating genipin into an injectable matrix may provide a solution to this problem and allow for the development of more effective, long-term and even preventative treatments for osteoarthritis. Our results here, in developing a protocol in which stem cells, matrix components are mixed and allowed to polymerize in the lumen of a cartilage defect model are readily translatable into an in vivo setting or potentially into a human therapy.

ACKNOWLEDGEMENTS: CS was supported by the Amgen Summer Program at Columbia University; the project was supported by CDMRP Award # number W81XWH-14-1-0591 to JCB.

REFERENCES: [1] Lima+ J. Biomed. Mater. Res. 2008. [2] Tan+ Clin Orthop Relat Res 2011. [3] Shetty+ Orthopedics 2013.

FIGURES:

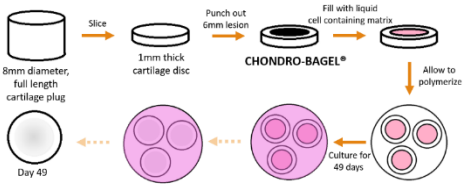

Figure 1: Construct creation and culture schematic. Target end product at day 56 is fully integrated and developed artificial cartilage in the center of cartilage explant.

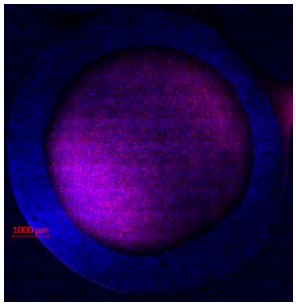

Figure 2: Genipin cross-linking of matrix: 220µM genipin was added to matrix mixture and SDSCs (1.7×10^5 cells/ construct). Construct was imaged after 14 da. Note SDSCs (in red with DiI membrane stain) fill the lumen, All nuclei are in blue with DAPI label.

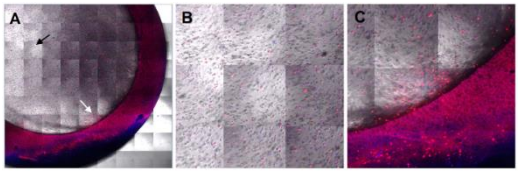

Figure 3: Genipin cross-linking at supra-toxic concentration (2.2 mM): (A) SDSCs seeded in matrix shown in red (DiI membrane stain) with SDSC and chondrocyte nuclei in blue (DAPI nuclear stain). Red fluorescence (ex/em ~549/560) in explant tissue arises from genipin crosslinking. Black/white arrows: area enlarged in 3B/3C, respectively. (B) SDSCs in the center of matrix with round morphology and are DiI stained. (C) Cells in matrix near explant tissue not DiI stained (visible with DIC) near the edges of the explant tissue presumably migrated out of the explant and into the lumenal matrix.