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

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CONTRACTING ORGANIZATION:
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14. ABSTRACT Post-traumatic osteoarthritis (PTOA) often follows joint fractures and dislocations, cartilage injuries, chronic ligament instability, among other traumatic affections of joints, and occupations or sports that subject joints to high levels of impact and torsional loading. One condition commonly associated with PTOA is rupture of the anterior cruciate ligament (ACL), which is particularly troubling because it occurs in young individuals and places them at high risk for PTOA. Reconstruction of the ACL, while providing stability, does not prevent the later onset of PTOA. Approximately 50% of individuals with an ACL injury develop PTOA 10-15 years after injury regardless of treatment of the ligament injury.					
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1. Introduction

Post-traumatic osteoarthritis (PTOA) often follows joint fractures and dislocations, cartilage injuries, chronic ligament instability, among other traumatic affections of joints, and occupations or sports that subject joints to high levels of impact and torsional loading. One condition commonly associated with PTOA is rupture of the anterior cruciate ligament (ACL), which is particularly troubling because it occurs in young individuals and places them at high risk for PTOA. Reconstruction of the ACL, while providing stability, does not prevent the later onset of PTOA. Approximately 50% of individuals with an ACL injury develop PTOA 10-15 years after injury regardless of treatment of the ligament injury.

PTOA and the trauma that leads to it have a disproportionate burden upon members of the Armed Forces, their families, and the Veteran population. A report surveying the Army Physical Evaluation Board recently described that OA and back pain were the two most common “unfitting conditions” leading to separation or retirement from active duty with OA accounting for 31.4% of all unfitting conditions. In 2009, OA was more prevalent than PTSD, TBI and extremity amputation. Recommendations for research priorities in combat casualty care include PTOA in its top 7 debilitating conditions. “PTOA is the most common chronic, debilitating condition of combat-injured warriors” (Department of the Army, Medical Research and Materiel Command, 2011).

The research proposes a technique to treat PTOA. It examines the effects of exposure to electromagnetic energy on cartilage and bone structure as well as inflammation in the hopes of aborting the destructive processes in PTOA.

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 used 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 appeal 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.

2. Key Words

Chondrogenesis, Cell migration, osteoarthritis, traumatic injury

3. Accomplishments

Specific Aim 1 Apply electric fields (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. (Months 1-12)

Milestone(s): 1. Deliverables will include canine knee joints from which cartilage explants for the culture studies at Columbia, and synovium from which synovium-derived stem cells (SDSCs) can be isolated for *in vitro* and *in vivo* studies at Columbia and University of Missouri, respectively. 2. A suitable design for an implantable direct current (DC) electrode system with cathodal electrodes and battery-controller (anode) that can be implanted into the knees of dogs will be described and the electrode systems fabricated by Dr. Cadossi. 3. Data on the specific EF parameters (field strength, duty cycle, duration) that demonstrate recruitment of endogenous chondrocytes to the experimental wound (as noted by cell counts in the wound) and the properties of the repair tissue that develop. These data will be used in part to determine the EF conditions to be studied for the *in vivo* studies. (Month 18)

Local IRB/IACUC Approval (for Tasks in Aim 3) (Month 18)

Milestone Achieved: HRPO/ACURO Approval (for Tasks in Aim 3) (Month 24)

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.

Milestone(s): 1. Generate data on the specific EF parameters (field strength, duty cycle, duration) that demonstrate recruitment of labeled SDSCs to the experimental wound (along with endogenous chondrocytes as noted by unlabeled cells in the wound) and the properties of the repair tissue that develop. These data will be used in part to determine the EF conditions to be studied for the *in vivo* studies. 2. Final design for an implantable DC electrode system with cathodal electrodes and battery-controller (anode) that can be implanted into the knees of dogs will be described and the electrode systems fabricated by Dr. Cadossi. (Month 24)

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.

Milestone(s): 1. Deliverables will include *in vivo* gait analyses of dogs implanted with DC electrode system ± injected SDSCs or control defects. 2. At sacrifice, CT images, arthroscopy, and clinical examination will provide results about the efficacy of EF application. 3. Tissues sent to Columbia for processing and analyses (mechanical and biochemical properties, and histology) will provide results about the efficacy of EF application. (Month 36)

In the past quarter we made major inroads in the experiments targeting Specific Aims 1 and 2 by identifying and overcoming four (4) challenges in the project. Our goal was to refine and perfect our 3D wound-healing system for in situ use in canine cartilage. The system we developed in previous project periods allows us to induce motility of either endogenous cartilage cells or exogenous Synovium-Derived Stem Cells into bovine or canine cartilage explants.

Thus, in this quarter, we addressed four previous obstacles, and here we describe steps taken to start to solve each one:

First, in previous progress reports we describe difficulty documenting and, especially, quantifying the motility of cells into the engineered matrix (collagen and fibrin) at the center of the bagel-shaped cartilage explant (which we call a 'chondrobagel'). Since explants are too thick to image throughout their depth even in a confocal microscope, we have had to perform paraffin-embedding and histology, and often the central matrix becomes dislodged during the embedding process. Obviously, we cannot assay the density of cells within the matrix if the matrix is gone. Thus we have switched to canine explants (average thickness 0.8 mm, as described previously) and also bovine explants cut to 0.8- 1 mm thickness. This has permitted us to use DAPI-fluorescence of nuclei to quantify the motility without having to embed, as confocal imaging is possible throughout the depth of these explants (see Appendix Results).

Second, we have had difficulty determining the appropriate electric field strength to use in order to optimize motility without damaging cells or surrounding tissue. Doing a wide number of runs necessitated having lots of chambers. Thus we have streamlined the process of 3D-printing a number of chambers in which to run different electric field strengths. Thus, we have tried 5 different voltages (not shown) in order to optimize migration and minimize heating. Of course, to overcome this obstacle we also needed to be able to quantify the cell migration. Thus, as shown in Appendix Results, we have now found that an electric field strength of 1 V/cm, i.e., one-sixth as great as the electric field strength we used previously, achieves very rapid migration, little to no cell death, and a final cell density that is conducive to chondrogenesis (see below).

Third, our overall goal in healing cartilage injury instead of allowing it to progress into osteoarthritis is to achieve differentiation once directed migration has allowed sufficient cell numbers to arrive into the lumen of the 'chondrobagel' explant. Recently, we achieved a cell density in the lumen of the explants that is 80% of the density of the authentic chondrocytes in the explant (i.e., cell density in lumen compared with walls of the chondrobagel, not shown). Accordingly, we have removed these sterile explants to chondrogenic medium, where they will remain for the 6-week differentiation period, after which we will assess their level of differentiation via GAG, collagen and eventually, mechanical assays. In the latter assay, we will compare the 'chondrobagels' with reconstituted lumen to explant cylinders of the same dimensions, but without the punched out and reconstituted lumen. These experiments are underway (see plans for the upcoming project period for more details) but they seem promising.

Fourth, the student who has been working diligently on the project this year, Amy Silverstein, is required to serve as a teaching assistant this year in order to graduate in 2017. The teaching assistant position will cover her salary which we have budgeted for. Thus, we have recruited another student from the Bulinski and Hung groups, Rob Stefani, to work full-time on the project (and to be paid a stipend from it). Rob is uniquely qualified as he worked before graduate school with our collaborator, Roy Aaron, and Rob thus has almost 5 years of experience investigating electric field effects on cells. In fact, it is Rob, working together with Amy Silverstein, who has produced the data described in our Appendix Results.

In the next quarter of our project, we will optimize the migration including a) maximizing the homogeneity of cell density throughout the explant lumen (one can see from the figure in Appendix Results) that cells may be moving in clumps such that there are areas of 120% the density of native cartilage, and some areas with practically 0% of the native density), b) finding conditions such as time of electric field application whereby either bovine or canine cells have achieved the optimal cell density in the lumen of the explants (i.e., compared with walls of the chondrobagel, not shown), and c) optimizing cell health in the cells that have migrated in electric field. We have not yet assayed for any cell death, and beyond that, we hope to assure the health of the cells (perhaps by shortening the electric field exposure) in order to obtain the best chondrogenesis (see below).

We have already started to test whether the newly migrated cells can undergo differentiation. In the next Quarter, we will optimize the chondrogenic differentiation removing sterile explants to chondrogenic medium and feeding them chondrogenic medium for the 6-week differentiation period. We will then assay their level of differentiation via GAG, collagen and eventually, mechanical assays. In the latter assay, we will compare them to explant cylinders of the same dimensions, but without the punched out and reconstituted lumen.

We will add these data to a manuscript that we have already started to draft. We feel strongly that development of our system and strategy is important as a model for 3D wound healing studies in the musculoskeletal field.

4. Changes/Problems

One can never anticipate all problems that may arise. Here, we may have significant cell death and thus the cells may differentiate poorly (or not at all, due to the presence of necrotic cells).

Another difficulty might be obtaining a uniform density of cells throughout the explant; we are trying to make sure that incident cells are migrating singly rather than as clumps.

Finally, results with our pilot studies with bovine cells may not be similar to results with canine cells. For example, canine cells have proven difficult to culture and differentiate as the components that cause them to proliferate and undergo chondrogenesis are markedly different from those that act on juvenile bovine cells.

5. Products

Nothing to report.

6. Participants & Other Collaborating Organizations

Name: J. Chloë Bulinski

Project Role: Principal Investigator

Nearest person month worked: All of Quarter 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 Quarter 3 (and up to the present)

Contribution to Project: Dr. Hung has trained all students, designed all chambers and apparatus, and participated in set-up, analysis and troubleshooting of experiments.

Name: Roy Aaron

Project Role: Partnering P-I

Nearest person month worked: January, 2015 - 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

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: January, 2015 - 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: Amy Silverstein

Project Role: Graduate Student

Nearest person month worked: 3 months of Quarter 4 (and she has continued up until the present)

Contribution to Project: Ms. Silverstein has performed work in preparing the explants and culturing them, as well as preparing the matrix to fill them and running them in the electric field.

Name: Rob Stefani

Project Role: BME Graduate Student

Nearest person month worked: 1 months of Quarter 4 (and he will continue up until at least June 1, 2015)

Contribution to Project: Mr. Stefani generated the figures for the Appendix Results and he has also performed the Z-stack imaging and analysis of cell densities.

7. Special Reporting Requirements:

Projected expenditure to date: \$167,235.00

Actual funds expended to date: \$\$137,033.22

See Quad Chart for more information.

8. Appendices

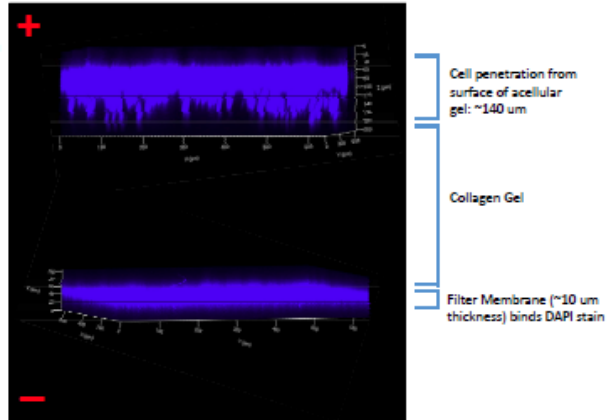
See next pages

Appendix Results

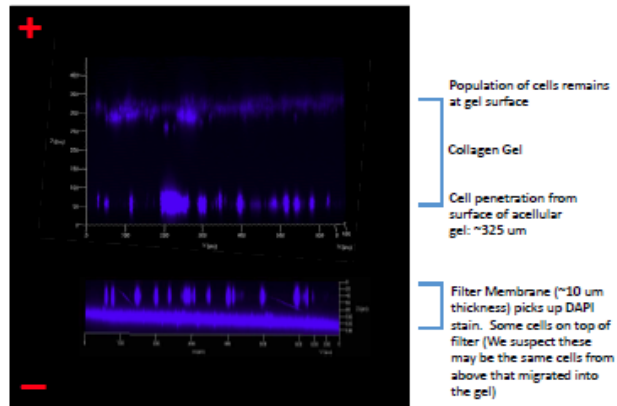
3-hr application of electric field yields 80% of the cell density of native cartilage

Time of Electric Field Application

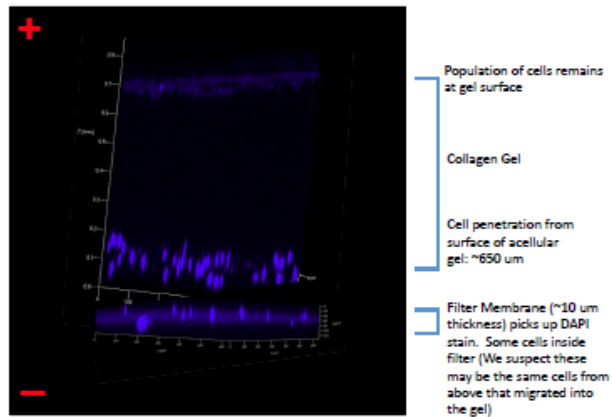
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T=90 min

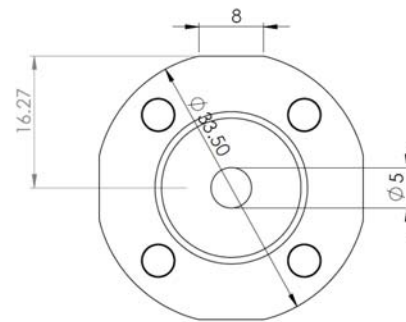
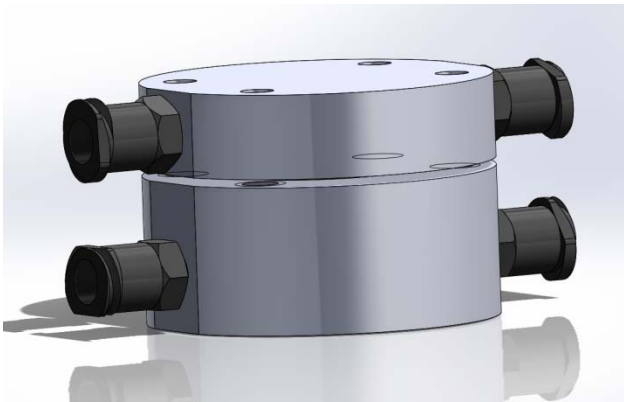


T=180 min



Appendix Results-Methods

- Electric Field 3D chamber as shown below
- Acellular 2mg/ml Type I collagen gel applied to the lumen of 'Chondrobagel' with 10 mm O.D., 6 mm I.D.
- 2×10^5 Passage 2 SDSCs seeded on top of lumen at T= -1 Da
- Cells allowed to adhere overnight
- Following day, gels were place in chamber on top of filter
- 3.33 mA applied for 0, 1.5, or 3 hr (calculated electric field strength E: 1 V/cm, validated with measurements of chamber resistance)
- Chondrobagels were fixed with formaldehyde, stained with DAPI and subjected to confocal imaging



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

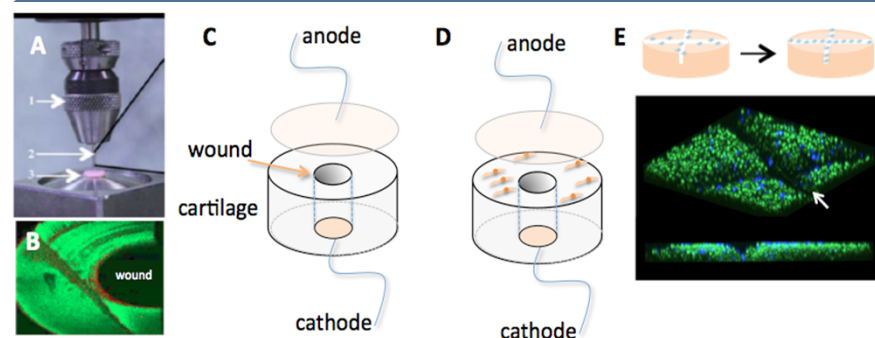
Study Aims

- 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, arthroscopic 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 <i>in vivo</i> studies investigating the efficacy of DC EFs for cartilage repair in a canine knee defect model					
Estimated Budget		\$110,594	\$169,802	\$220,687	

Updated: 14 July 15

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

Budget Expenditure to Date

Projected Expenditure: \$167,235

Actual Expenditure: \$114,874.22