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PRINCIPAL INVESTIGATOR: Dr. Dawn Elliott

CONTRACTING ORGANIZATION: University of Pennsylvania
Philadelphia, PA 19104

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Dawn Elliott; Robert Mauck
Nader Hebela

email: delliott@mail.med.upenn.edu

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3451 Walnut Street,
Philadelphia, PA 19104

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14. ABSTRACT

Disc injury through trauma, vibration loading, or mechanical overload, and the resulting disc degeneration in response to these insults over time are tremendous problems affecting the active and veteran military population. Current treatment options fail to restore disc structure and mechanical function. Our goal in this proposal is to develop methodologies for the engineering and implanting of a functional biologic disc replacement. Significant progress has been made in the last 12 months towards achieving this goal. We have successfully engineered a concentric annulus fibrosus, the functional properties of which improve with culture time. We have shown the dynamic culture further enhances functional matrix deposition. We have shown that a short period of exposure to transforming at a high dose is equal to or better than long term exposure for stem cells cultured in an engineered nucleus pulposus-like hyaluronan hydrogel. We have developed and validated a minimally invasive surgical technique for implantation of our engineered disc. We have successfully performed in implantation of acellular engineered discs. Finally, we have designed and implemented a novel internal fixation device to enhance retention of the engineered disc and stabilize the joint during healing.

15. SUBJECT TERMS
Intervertebral disc degeneration; low back pain; biological disc replacement; preclinical animal model

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Introduction:

The focus of this now completed project was on the restoration of the intervertebral disc through tissue engineering methodologies. Disc injury through trauma, vibration loading, or mechanical overload, and the resulting disc degeneration in response to these insults over time are tremendous problems affecting the active and veteran military population. Neither conservative treatments, such as stretching and exercise, nor surgical options, such as fusion and arthroplasty, restore disc structure or mechanical function. Total disc arthroplasty is relatively new to clinical practice, but suffers from the same problems as traditional implant materials – wear and the need for eventual replacement. An alternative to these methods involves implantation with a biologic tissue engineered replacement. Such a biologic tissue restoration method would not be subject to wear as occurs with prosthetic devices, and would restore flexibility and motion about that spinal segment. Because the function of the disc is mechanical, it is important to focus upon mechanics in the design of functional tissue engineered constructs (TECs) and to direct the biology (maintenance of phenotype and ECM deposition) towards these mechanical outcomes. Direct biologic restoration of the disc with a TEC that duplicates the mechanical properties of the native tissue and restores range of motion would be an ideal alternative. Our goal in this project was to develop methodologies for the engineering and implanting of a functional biologic disc replacement. **The objective of this proposal was to move to the translational space and towards clinical implementation by creating and implanting a tissue engineered disc-like angle ply structure (DAPS)**, and throughout this development, to remain mindful of the fundamental importance of the mechanical function.

During Year 3 of our funding on this project (which was extended to four years through a no cost extension), we made substantial progress, in particular with regards to development of our biomaterials and implantation models. Given the highly collaborative and interdisciplinary activities of this project, a single document detailing all progress is presented. The research team has met on a weekly or bi-weekly basis over this time course, and have established major advances in the formation of disc-like angle-ply structures for disc tissue engineering applications, as will be detailed below.

Body:

Our work stems from the translation of the single and bi-layer constructs with tensile properties approaching that of the native annulus fibrosus tissue into full 3D Disc-like Angle Ply Structures (DAPS), inclusive of a hyaluronic acid hydrogel seeded with adult stem cells, that can be used to replace the degenerate native disc. Years 1 and 2 were focused on material development and in vitro models. In Year 3, the bulk of our focus was on further refinement of the hydrogel NP region as well as establishment and validation of our disc replacement model in the rat caudal spine. Data on this progress are provided below.

<u>Aim 1</u>: Create a mesenchymal stem cell (MSC) seeded 3D structural TEC disc from concentric AF constructs surrounding an engineered nucleus pulposus (NP) composed of a hyaluronic acid (HA) hydrogel. Measure the disc structural mechanics in compression and torsion, and the isolated AF and NP substructures in compression following time in culture. Evaluate the molecular, histological, and biochemical

properties of these TEC discs as a function of time in culture and with variations in media conditions.

Optimization of the Tissue Engineered NP Region:

In addition to this work on the NP regions seeded with MSCs (reported on in our Year 1 and Year 2 Progress Reports), we also carried out a study to examine the growth of engineered NP constructs based on NP cells derived from native tissue (as an alternative to MSCs). Towards restoring the NP, a number of biomaterials have been explored for cell delivery. These materials must support the NP cell phenotype while promoting the elaboration of an NP-like extracellular matrix in the shortest possible time. Our previous work with chondrocytes and mesenchymal stem cells demonstrated that hydrogels based on hyaluronic acid (HA) are effective at promoting matrix production and the development of functional material properties. However, this material had not been evaluated in the context of NP cells.

Therefore, to test this material for NP regeneration, bovine NP cells were encapsulated in 1% w/vol HA hydrogels at either a low seeding density (20 x 10^6 cells/ml) or a high seeding density (60 x 10^6 cells/ml), and constructs were cultured over an 8 week period. Outcome assays for this study were similar to those noted above, with the addition of quantitative real time PCR for markers of the NP phenotype. These engineered NP cell-laden HA hydrogels showed sustained cell viability and functional matrix accumulation with increasing matrix content and mechanical properties with time in culture at both seeding densities (**Figs. 1-4**).

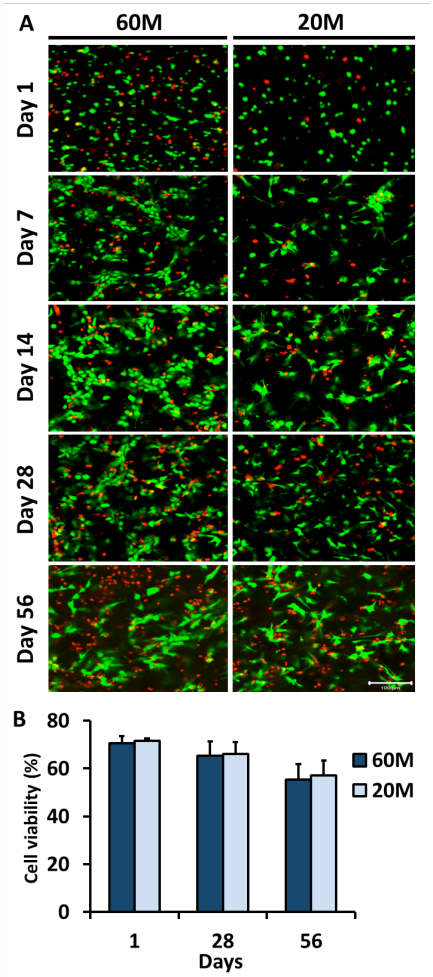

Figure 1: A) LIVE/DEAD staining of NPC-seeded HA constructs on days 1, 7, 14, 28 and 56 after encapsulation at either 60M or 20M seeding density (20X magnification, scale bar: 100 μm). B) Quantification of NPC viability in HA constructs on days 1, 28 and 56.

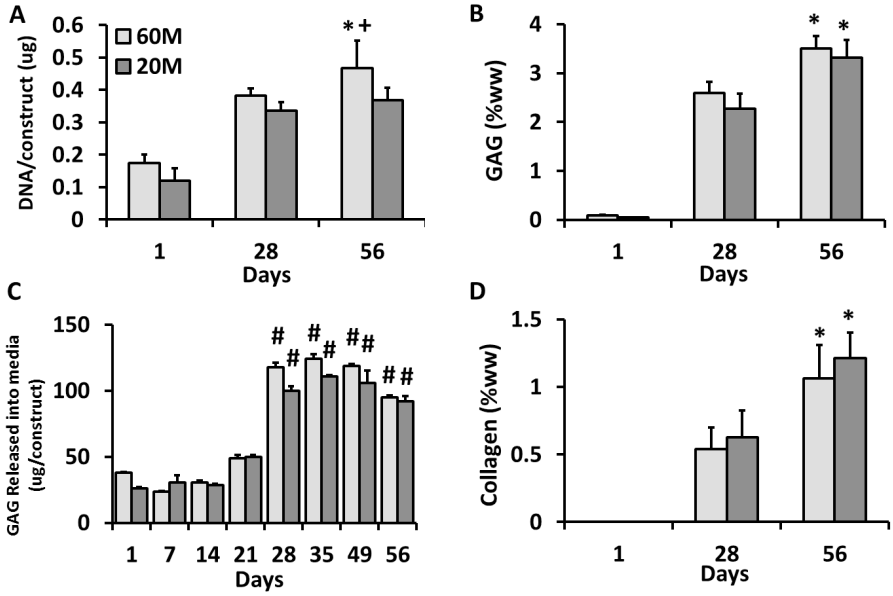

Figure 2: Biochemical composition of NP cell seeded HA constructs at seeding densities of 60M (grey) and 20M (dark grey) after 1, 28, 56 days of in vitro culture. A) DNA content per construct, n=5. B) GAG content per wet weight, n=5. C) GAG released to the media (per construct per day), n=3. D) Collagen content per wet weight, n=5. (* indicates $p < 0.05$ vs. day 28 within same group; + indicates $p < 0.05$ vs. 20M; # indicates $p < 0.05$ vs. day 21 within same group).

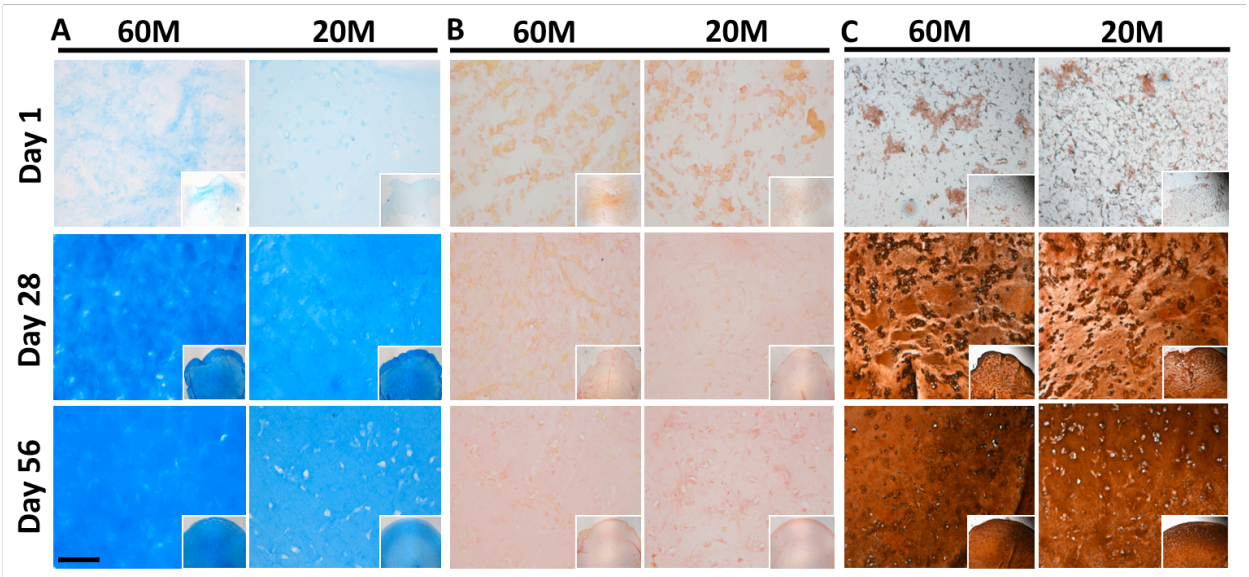

Figure 3: Matrix deposition in NPC-seeded HA constructs as a function of culture duration and seeding density. (A: Alcian blue staining of proteoglycans, B: Picrosirius red staining for collagens, C: immunostaining for collagen type II; 10× magnification (inset: 5×), scale bar: 100 µm).

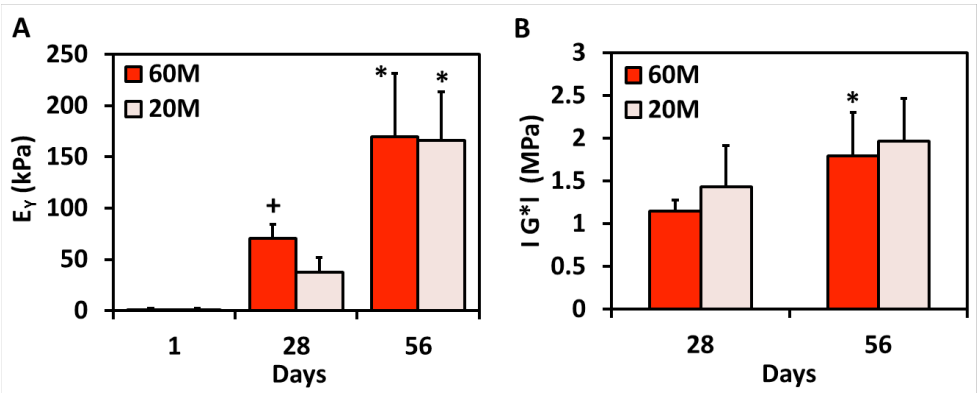

Figure 4: Equilibrium compressive modulus (A) and dynamic compressive modulus (B) of NPC-seeded HA constructs over 8 weeks of in vitro culture (*indicates $p < 0.05$ vs. at day 28; $^{+}$indicates $p < 0.05$ vs. 20M group, n=4~5/group/time point).

Furthermore, encapsulated cells showed NP-specific gene expression profiles that were significantly higher than monolayer expanded NP cells, suggesting a restoration of the NP phenotype (**Fig. 5**). Interestingly, these levels were higher at the lower seeding density compared to the higher seeding density.

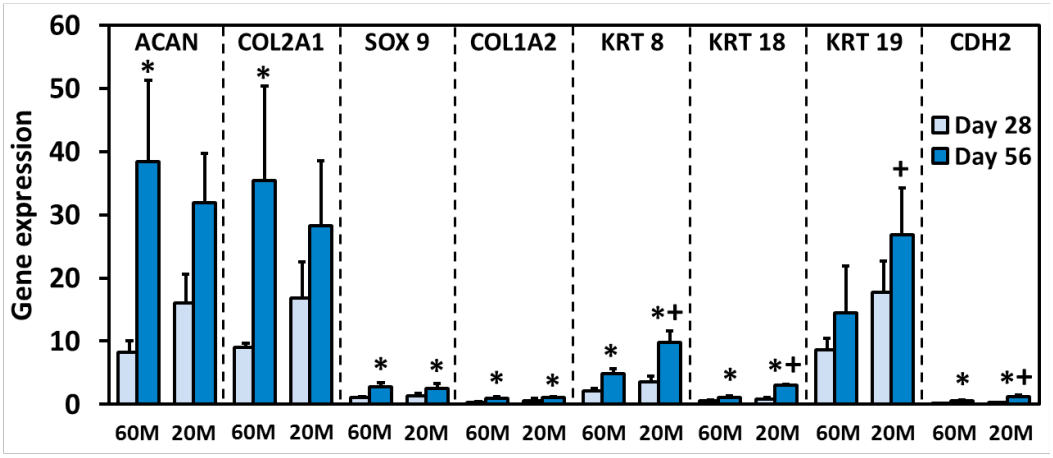

Figure 5: Expression of aggrecan (ACAN), collagen II (COL2A1), SOX 9, collagen I (COL1A2), cytokeratin (KRT) 8, 18, 19, and N-cadherin (CDH 2) after 28 and 56 day of culture for NPC-seeded HA constructs. Expression levels were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and expanded NP cells prior to encapsulation (* indicates $p < 0.05$ vs day 28; + indicates $p < 0.05$ vs 60M). (n=3/group/time point)

These findings support the use of HA-based hydrogels for NP tissue engineering and cellular therapies directed at restoration or replacement of the endogenous NP. This work was published as several abstracts and a full length manuscript in *Acta Biomaterialia* in 2015, as: **Phenotypic Stability, Matrix Elaboration, and Functional Maturation of Nucleus Pulposus Cells Encapsulated in Photocrosslinkable Hyaluronic Acid Hydrogels** by authors Dong Hwa Kim, John T. Martin, Dawn M. Elliott, Lachlan J. Smith, and Robert L. Mauck.

<u>Aim 2</u>: Implant the tissue engineered DAPS in a subcutaneous environment after varying period of in vitro pre-culture and evaluate maturation using the same assays and evaluation criteria as in Aim 1.
<u>Aim 3</u>: Implant the tissue engineered DAPS in situ using a rat tail disc replacement model. Evaluate DAPS maturation and in situ integration under static conditions and with resumption of mechanical loading. The engineered DAPS will be evaluated under two fixation conditions: an Immobilization group and Compression group, which is loaded axially with 0.5X body weight. Both of these fixation conditions will be applied for either short duration (8 weeks, followed by release and an additional 8 weeks normal loading) or held for the entire study duration (16 weeks). Structure, mechanics, and composition of the functional DAPS and its interface will be evaluated at 2, 8, and 16 weeks. Both fixation conditions will be compared against a sham control in which all surgery and fixation conditions are the same, but without disc removal or DAPS placement.

Given that Aims 2 and Aim 3 are both in vivo portions of the project, and so are related to one another, we will describe our accomplishments in these Aims together. Most of the work accomplished in Year 3 relates to the more challenging Aim 3, the implantation into the actual

disc space. This Aim represented the culmination of our project, and considerable effort was spent in Year 3 in realization this process. Note that deviations from the stated goals were necessary at times to further the experimental goals and based on the experimental findings, as will be described below.

Development of an Engineered Disc Implantation Model in the Rat Caudal Spine:
Our first step in this work was pilot surgeries (with a few acellular DAPS) in Years 1 and 2 to establish the eventual implantation of the fully matured versions developed in later stages of the project. Specifically, we started by developing and validated the surgical technique for implantation of the tissue engineered disc into the rat caudal disc space. The surgical technique was designed to be minimally invasive in order to prevent vascular damage and promote rapid healing. Three surgical groups were evaluated: sham, discectomy and implantation of acellular disc constructs. Healing and integration were evaluated at time points up to one month using histology and microCT. At one week, histological evaluation showed the construct well placed in the disc space (**Fig. 6**), with lamellar architecture characteristic of the native tissue. At subsequent time points however, histology showed migration of the construct out of the disc space, and microCT (**Fig. 6**) suggested the disc space had subsequently collapsed.

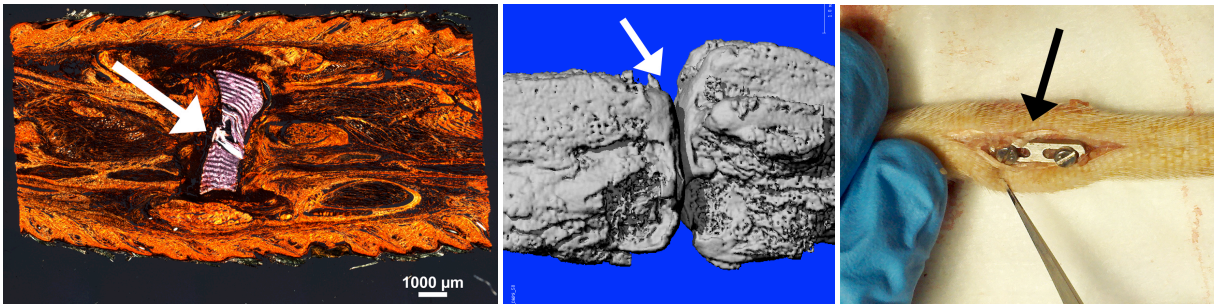

Figure 6: Left. Histology showing acellular disc implant (arrow) at 1 week. Middle. MicroCT showing collapsed disc space (arrow) at 4 weeks. Right. Original internal fixation device to (arrow) to stabilize joint and retain implant in disc space.

At the end of Year 2, and through Year 3, we worked to overcome this problem of disc collapse. To do so, we designed a series of internal and external fixation devices to stabilize the disc space in the immediate post-implantation time period. The goals of these designs were to enhance retention of the construct in the disc space and stabilize the joint during healing. Indeed, these early findings led to our next major time investment – improving implant stability and improving infiltration and anchorage of the implant via the ingrowth of endogenous cells in the disc environment. This work was published in 2014 in *Acta Biomaterialia* under the title **Translation of an Engineered Nanofibrous Disc-like Angle Ply Structure for Intervertebral Disc Replacement in a Small Animal Model** with authors John T. Martin, Andrew H. Milby, Joseph A. Chiaro, Dong Hwa Kim, Nader M. Hebela, Lachlan J. Smith, Dawn M. Elliott, and Robert L. Mauck, as well as at several conference abstracts.

In brief, this work built from our development of disc-like angle ply structures (DAPS) consisting of layered, oriented electrospun scaffold forming a concentric annulus fibrosus (AF) coupled with a hydrogel nucleus pulposus (NP). Based on early studies wherein DAPS were implanted into the rat caudal spine, approximately half of the implants were displaced, and for those that remained in place, there was limited cell infiltration into the dense scaffold. To address this, this study evaluated an external fixator to improve implant retention by stabilizing the rat caudal

spine and tested a new DAPS design wherein sacrificial layers were included to improve cell infiltration and anchorage by endogenous cells.

To carry out this study, poly(ε-caprolactone) (PCL) nanofibers were electrospun onto a rotating mandrel as aligned fibrous sheets (th=250μm). Strips were cut 30^{o} to the fiber direction and two strips with alternating $\pm 30^{o}$ alignment were wrapped concentrically to form the AF region of the DAPS (**Fig. 7**, top left and top right). DAPS with sacrificial layers (sDAPS) were fabricated with one layer (th=125μm or 250μm) of poly(ethylene oxide) (PEO, water soluble and rapidly degrading) or 75:25 poly(lactic-co-glycolic acid) (PLGA, slowly degrading) for every two layers of PCL (th=125 μm). Final dimensions were: OD=5mm, ID=1 mm, height=2 mm. To determine if cell infiltration is improved by including sacrificial layers, sDAPS were seeded with bovine AF cells and cultured for 28 days. First, sDAPS (with PEO) were washed to remove PEO, lyophilized and coated overnight in 20 μg/mL fibronectin. Then, bovine AF cells were seeded onto top and bottom surfaces ($2x10^{6}$ total cells), and sDAPS were cultured in serum-containing media. Infiltration was assessed via DAPI staining. An external fixator was designed to unload and stabilize the rat caudal spine (**Fig. 7**, bottom left and bottom right). Surgical wires were passed laterally through the mid-height of vertebrae adjacent to the C8/C9 disc, and the fixator was applied leaving access to the dorsal tail. A dorsal incision was made, the native disc was removed and AF-only DAPS or sDAPS were implanted into the disc space (DAPS: 14 days (n=4), 28 days (n=5), sDAPS: thin PEO, thick PEO, thin PLGA and thick PLGA, 14 days (n=3/group)). An additional discectomy control group (14 days (n=4), 28 days (n=4)) and external fixator plus discectomy control group (14 days (n=2), 28 days (n=4)) were included to verify disc height preservation with fixation. Rat tails were imaged longitudinally with a fluoroscope to quantify disc height index (DHI). Then, following euthanasia, tails were imaged via μCT to assess adjacent bone remodeling. Finally, histological sections were stained with H&E and imaged in bright field or polarized light, or stained with DAPI and imaged in fluorescent light.

Results from this study showed that disc height was preserved by external fixation, even after total discectomy. At days 14 and 28, the fixator+discectomy and fixator+DAPS DHI were not different from immediate post-operative values (**Fig. 7**). μCT confirmed that fixation prevented disc space collapse, with minimal superficial bone remodeling at the wire insertion sites (**Fig. 8**). In addition, all DAPS implanted with fixation remained in the disc space and maintained their lamellar architecture (**Fig. 8**). sDAPS maintained interlayer gaps, enabling cell infiltration into the lamellar structure both in vitro and in vivo. In in vitro studies, AF cells penetrated the full height of both thick and thin layer PEO sDAPS after one week, and proliferated, filling the lamellar structure over 28 days (**Fig. 9**). PCL-only DAPS were poorly infiltrated, with central areas completely devoid of cells. When implanted in vivo, sDAPS remained in place and maintained their structure over time. Matrix deposition between layers was evident for both thin and thick layer PEO sDAPS, and appeared more rapidly than in PLGA sDAPS, though PLGA sDAPS better maintained their structure during implantation (**Fig. 9**). While some matrix deposition was apparent in thin PLGA sDAPS, thick PLGA sDAPS were not infiltrated by cells, as the implant was encapsulated by fibrous tissue before PLGA degraded.

This study advanced the development of an engineered disc for total disc replacement. We established a viable in vivo model by stabilizing the rat caudal spine and improved DAPS by including routes for cell migration. These findings demonstrate that application of an external fixator preserves disc height and markedly improves retention of the DAPS. A previous study had shown that a disc comprised of a collagen AF and an alginate NP was retained in the rat tail without fixation. This may be due to differences in the surgical approach or the physical properties of the construct, however, with the external fixation developed herein, one can

implant constructs with a range of physical properties and protect implants for a defined period after surgery. PCL-only DAPS were poorly infiltrated by cells, a common drawback of electrospun scaffold. Rapidly degrading layers of PEO allowed colonization of the DAPS by cells that secreted extracellular matrix, while slowly degrading layers of PLGA marginally improved infiltration. This suggests that early migration of endogenous cells will be important for integration. These data established the rat caudal spine (stabilized by external fixation) as a test bed to assess long term implant biocompatibility and, in addition, that sacrificial layers in the DAPS AF region improve implant patency and subsequent cell infiltration.

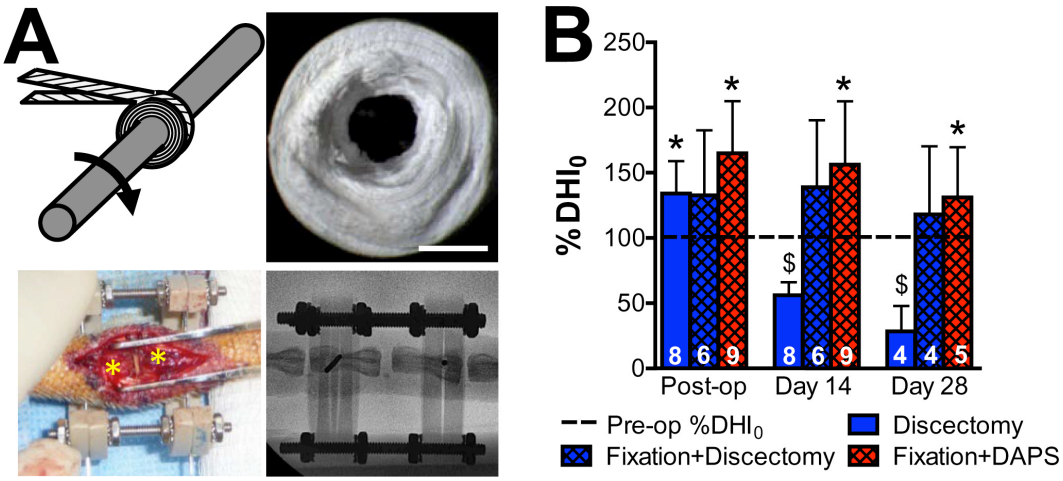

Figure 7: (A, top left and top right) DAPS were fabricated by wrapping strips of electrospun PCL with alternating alignment into concentric layers, scale=2mm. (A, bottom left) An external fixator was used to stabilize adjacent caudal vertebrae (asterisk). (A, bottom right) The fixator was radiolucent by design to enable disc height quantification. (B) Disc height index (DHI). Sample number is indicated on each column, *, p<0.05 from pre-op and $, p<0.05 from all other groups.

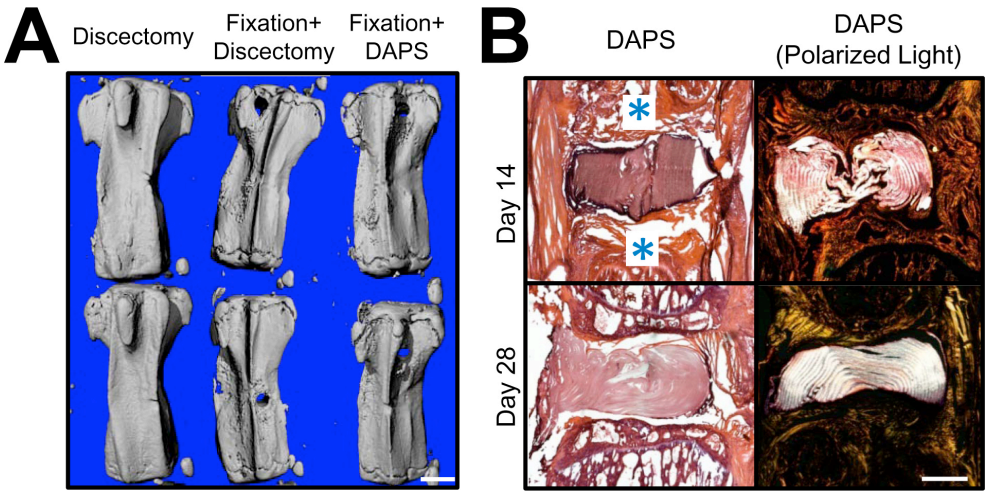

Figure 8: (A) µCT reconstructions after 28 days in vivo, scale=2mm. (B) H&E-stained DAPS in brightfield and polarized light after 14 or 28 days in vivo, scale=1mm. Vertebrae are marked with an asterisk.

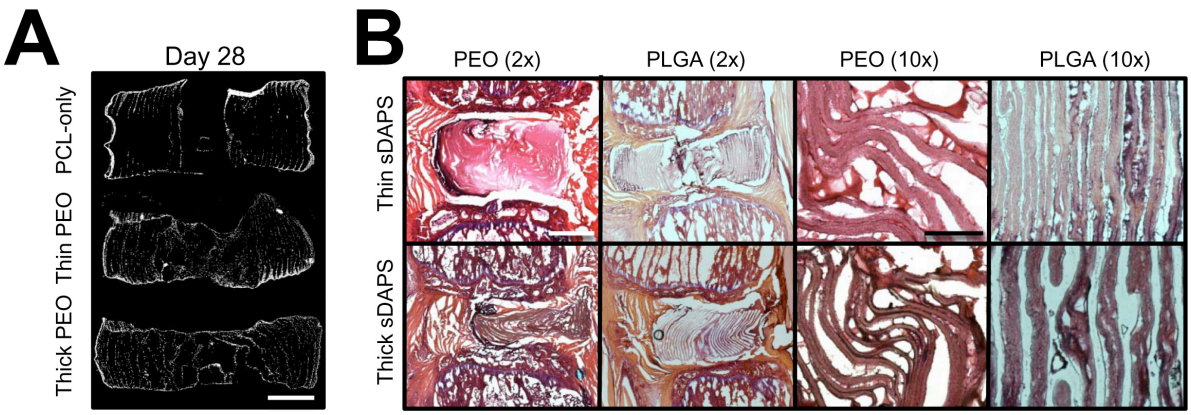

Figure 9: (A) DAPI-stained DAPS after in vitro culture for 28 days, scale=1mm. (B) Low and high magnification H&E-stained sDAPS after 14 days in vivo, scale: 2x=1mm, 10x=500μm.

Development of Radio-opaque Implants to Monitor Position Non-Invasively:

Given that implant migration was a potential problem in this rat tail model, we also developed methods to non-invasively image and monitor implant position as a function of fixation. To do so, we developed a radiopaque nanofibrous scaffold by electrospinning a polymer/heavy metal salt solution, and used this material as a diagnostic tool for model development. In this study, we characterized the radiopacity, structure and mechanical behavior of the scaffold, and showed its utility in an *in vivo* model of intervertebral disc replacement. This work was published in abstract form at the 2014 Orthopaedic Research Society Meeting and a full length manuscript titled **A Radiopaque Electrospun Scaffold for Engineering Fibrous Musculoskeletal Tissues: Characterization and *In Vivo* Application** with authors John T. Martin, Andrew H. Milby, Subash Poudel, Kensuke Ikuta, Dong Hwa Kim, Christian G. Pfeifer, Harvey E. Smith, Dawn M. Elliott, and Robert L. Mauck is now in review.

To carry out this study, radiopaque scaffolds were generated from a 14.3% w/v slurry of poly(ε-caprolactone) (PCL) mixed with zirconium(IV) oxide (zirconia), a radiodense powder with a characteristic dimension <100 nm. Slurries were electrospun while continuously mixing, and collected onto a rotating mandrel to create an aligned nanofibrous sheet (th.=200μm). Four scaffold-types with varying radiodensity were fabricated: 100% PCL, 90% PCL/10% zirconia, 75% PCL/25% zirconia, 50% PCL/50% zirconia. Each scaffold was assayed for structural continuity, radiodensity and mechanical strength. To assess nanostructure, samples were imaged by SEM (n=1/group). To measure radiodensity, 8 mm diameter samples were punched from each scaffold and scanned by μCT (vivaCT 75, SCANCO) at 20μm resolution (n=5/group). The linear attenuation coefficient of each sample was calculated from volumetric reconstructions. Scaffold strips (5mm x 40mm) were tested in uniaxial tension in the fiber direction (n=5/group). The mechanical testing protocol consisted of a 0.05 N preload, followed by extension to failure at a rate of 0.1% strain/s. Tensile modulus was calculated as described previously.

Disc-like angle ply structures fabricated from radiopaque scaffold (rDAPS) were then implanted into the rat caudal spine. Strips were cut from aligned radiopaque scaffold 30 degrees to the fiber direction and two strips with alternating ±30 degrees alignment were wrapped concentrically to form the annulus fibrosus region of the rDAPS. To implant rDAPS, surgical wires were passed laterally through the mid-height of vertebrae adjacent to the C8/C9 disc, and an external fixator was applied (**Fig. 10**). A dorsal incision was made, the native disc was removed and rDAPS were inserted into the disc space. Rats were returned to cage activity and

euthanized at 28 days. Two implant types were used in this study: a radiodense implant, “50/50 rDAPS” with 2 layers of 50% PCL/50% zirconia (n=2); a radiolucent implant,”75/25 rDAPS” with 1 layer of 75% PCL/25% zirconia, 1 layer 100% PCL and one layer of degradable 75:25 poly(lactic-co-glycolic acid) (PLGA, to provide a route for cell migration once degraded) (n=3). To monitor changes in implant position and structure *in vivo*, rat tails were imaged longitudinally with a fluoroscope. Then, following euthanasia, tails were imaged via μCT to assess implant structure and histological sections were stained with picrosirius red (for collagen).

Results from this study showed that we could create a trackable radio-opaque construct. SEM revealed that all formulations of radiopaque scaffold had continuous and aligned fibers (**Fig. 11**). Zirconia was embedded within fibers at lower concentrations, but at the highest concentration it was evident that zirconia aggregated into large pellets exterior to the fibers (**Fig. 11**). In addition, as zirconia increased fiber diameter decreased. Scaffold modulus increased with ziroconia content, except at the highest concentration (**Fig. 11**). Scaffold radiation attenuation increased with zirconia content plateauing at 25% zirconia (**Fig. 11**); PCL alone had no signal and thus 3D reconstruction was not possible. Both formulations of rDAPS were visualized intra- and post-operatively. 50/50 rDAPS had a distinct signal (**Fig. 12**), more intense than that of the native bone, while the 75/25 rDAPS cast a radiolucent shadow similar to bone (**Fig. 12**). rDAPS remained in the disc space over 28 days with no change in position or shape. These results were confirmed by μCT; both types of rDAPS had a signal distinguishable from bone allowing for the reconstruction of each separately. Reconstructions of 50/50 rDAPS demonstrated that the radiopaque implants occupied the disc space and did not cause an adverse reaction (**Fig. 13**). 3D reconstructions of 75/25 rDAPS demonstrated that lamellar structure was intact after 28 days (**Fig. 12**). Images of histological sections confirmed that new collagen was deposited between layers, though the PLGA had not completely degraded (**Fig. 13**).

In this study, we developed and characterized a radiopaque electrospun scaffold composed of PCL, a standard tissue engineering polymer, and zirconia, a heavy metal salt. We demonstrated that scaffold radiopacity and mechanics can be tuned by altering the concentration of zirconia. In addition, we fabricated radiopaque implants and validated a rat caudal spine model of disc replacement. The radiation attenuation of each scaffold increased with zirconia content and plateaued at 25% zirconia. Radiopaque implants made with high and low concentrations of zirconia were visualized in the rat caudal spine. In addition, the radiopaque scaffold was compatible with the formation of new collagen by endogenous cells.

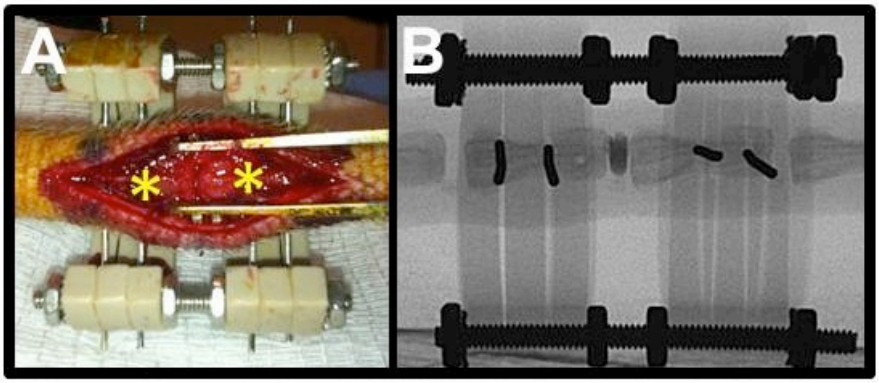

Figure 10: A) rDAPS were implanted between vertebrae (asterisk) of the rat caudal spine to demonstrate the utility of radiopaque scaffold. B) The vertebrae were stabilized with a radiolucent external fixator.

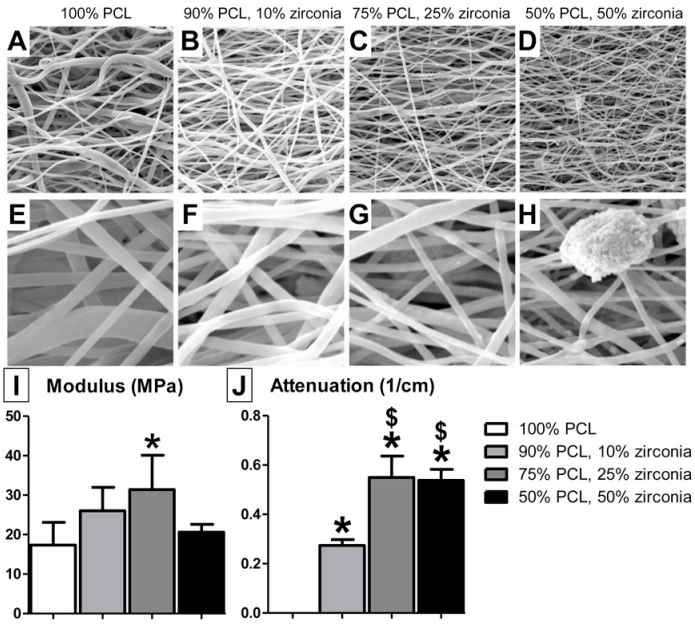

Figure 11: Characterization of radiopaque scaffold. SEM images at A-D) 2,000x magnification and E-H) 10,000x magnification, I) tensile modulus and J) linear attenuation.

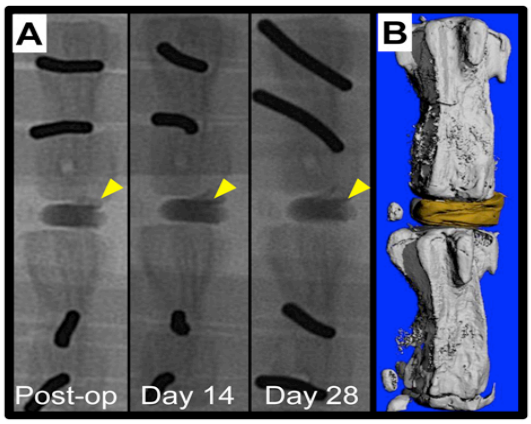

Figure 12: *In vivo* implantation of high radiodensity rDAPS. A) Fluoroscopy over 28 days. B) µCT reconstruction after 28 days.

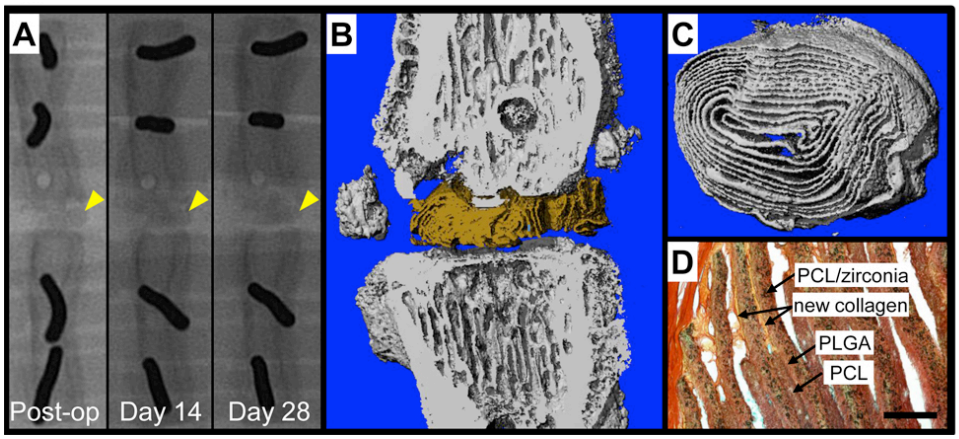

Figure 13: *In vivo* implantation of low radiodensity rDAPS. A) Fluoroscopy over 28 days. B) μCT reconstruction after 28 days. C) Reconstruction of scaffold only after 28 days. D) Picrosirius red-stained section at day 28.

Collectively, the advances to our in vivo model and capacity for monitoring implant position set the stage for our final experiments in the no cost extension year (as detailed in the final report).

Key Research Accomplishments:

According to the progress we have made on the Aims outlined above, we have achieved the following Key Research Accomplishments in Year 3.

- We have shown that NP cells in HA perform well, and promote the NP cell phenotype. (Aim 1)
- We fully developed and validated a rat tail model of disc implantation (Aim 3), including the development of an external fixation system for protecting the implant during early stages of in vivo growth and maturation. (Aims 2&3)
- We successfully performed implantation of acellular engineered discs, and modified scaffolds to promote endogenous tissue infiltration. (Aims 2&3)

Reportable Outcomes:
Based on funding from the DOD, our team published three peer-reviewed manuscripts through Year 3 and have another 3 manuscripts that are either in review or about to be submitted based on work done during the no-cost extension year. In addition, we published 6 abstracts at national and international meetings on this work. Funding from the DOD supported one doctoral student in mechanical engineering at the University of Pennsylvania as well as a postdoctoral fellow during Year 3.

Conclusions:
With support from the DOD, our made marked progress in Year 3. We built on this success to realize the Aims of our initial proposal during the no cost extension year that followed. In Year 3 in particular, we validated an in vivo rat tail model for the implantation of our DAPS construct, and designed novel fixation devices for protecting the DAPS post implantation. After successfully developing and validating our preclinical animal model, we evaluated acellular implants, setting the stage for evaluation of cellular implants during the no-cost extension year.

References/Publications:

Full Length Manuscripts:

1. Martin JT, Milby AH, Chiaro JA, Kim DH, **Hebela** NM, Smith LJ, **Elliott** DM, **Mauck** RL (2014). Translation of an engineered nanofibrous disc-like angle ply structure for intervertebral disc replacement in a small animal model. *Acta Biomaterialia*; 10(6):2473-2481
2. Kim DH, Martin JT, **Elliott** DM, Smith LJ, **Mauck** RL (2015). Phenotypic stability, matrix elaboration and functional maturation of nucleus pulposus cells encapsulated in photocrosslinkable hyaluronic acid hydrogels. *Acta Biomater* 15(12):21-9.

3. Martin JT, Collins CM, Ikuta K, **Mauck** RL, **Elliott** DM, Zhang Y, Vaccaro AR, Albert TJ, Anderson DG, Collins CM, Smith HE (2015). Population average T2 MRI Maps reveal quantitative regional transformations in the degenerating rabbit intervertebral disc that vary by lumbar level. *J Orthop Res*; 33(1):140-148
4. Martin JT, Milby AH, Pfeifer CG, Smith LJ, **Elliott** DM Smith HE, **Mauck** RL (2015). Short and long-term outcomes with an acellular disc-like angle ply structure for total disc replacement in a small animal model. (in preparation)
5. Martin JT, Milby AH, Poudel S, Pfeifer CG, Smith HE, **Elliott** DM, **Mauck** RL (2015). A radiopaque electrospun scaffold for engineering fibrous tissues: characterization and *in vivo* application. (in review)

Abstracts and Conference Proceedings:

1. Martin JT, Milby AH, Chiaro JA, **Hebela** NM, **Elliott** DM, **Mauck** RL (2013). Translation of a nanofibrous disc-like angle ply structure for intervertebral disc replacement in a small animal model. *Philadelphia Spine Research Society, Second International Spine Research Symposium*, Philadelphia, PA (Podium Presentation)
2. Martin JT, Milby AH, Chiaro JA, Kim DH, Smith LJ, Smith HE, **Elliott** DM, **Mauck** RL (2014). Engineered nanofibrous disc-like angle ply structures with sacrificial layers for intervertebral disc replacement in a small animal model. *60th Annual Meeting of the Orthopaedic Research Society*, New Orleans, LA (Poster Presentation)
3. Martin JT, Poudel S, Milby AH, Pfeifer C, Smith HE, **Elliott** DM, **Mauck** RL (2014). A radiopaque electrospun scaffold for engineering fibrous tissues: characterization and in vivo application. 6*0th Annual Meeting of the Orthopaedic Research Society*, New Orleans, LA (Podium Presentation)
4. Kim DH, Smith LJ, **Elliott** DM, **Mauck** RL (2014). Maturation and Material Dependent Response of AF and NP Cells to Mechanical Perturbation. 6*0th Annual Meeting of the Orthopaedic Research Society*, New Orleans, LA (Poster Presentation)
5. Milby AH, Martin JT, Smith LJ, Chiaro JA, Smith HE, **Elliott** DM, **Mauck** RL (2014). Nanofibrous disc-like angle structures for intervertebral disc tissue engineering in a small animal model. *7th Annual Lumbar Spine Research Society Meeting*, Chicago, IL (Podium Presentation)
6. Martin JT, **Mauck** RL, Zhang Y, **Elliott** DM, Smith HE (2014). Population average T_2 MRI maps reveal quantitative transformations of the degenerating disc in a rabbit puncture model. *7th World Congress of Biomechanics*, Boston, MA (Poster Presentation)

Appendices: N/A