RESEARCH PROGRESS REPORT SUMMARY

Grant 02078: Development of a Regenerative Medicine Technique to Treat Cartilage Disorders in Dogs

Principal Investigator: Dr. William Brian Saunders, DVM, PhD

Research Institution: Texas A&M AgriLife Research

Grant Amount: $120,871.43

Start Date: 1/1/2014 **End Date:** 12/31/2016

Progress Report: FINAL

Report Due: 12/31/2016 **Report Received:** 3/20/2017

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Original Project Description:

Osteochondrosis is a common and debilitating disease affecting large, athletic dogs. Osteochondrosis is caused by abnormal endochondral ossification, the process by which growth plate cartilage adjacent to joint surfaces transitions from cartilage to bone. The result is excessively thickened cartilage that partially or completely separates from surrounding bone. Cartilage separation exposes the joint to underlying bone and creates a large loose body, termed a joint mouse, within the joint. Surgical or medical treatment results vary widely based on the affected joint, size of the osteochondrosisdefect, and intended purpose for each dog. Treatment options for osteochondrosishave remained essentially unchanged for decades. Tissue engineering represents a promising treatment alternative for dogs suffering from OC. Dr. Saunders believes the key to successful tissue engineering involves generation of regenerative osteochondral plugs, or ROPs. ROPs are tri-layered cylindrical plugs composed of hydrogels seeded with adult mesenchymal stem cells (MSCs). Each ROP layer is composed of materials that closely mimic specific zones of the joint and adjacent bone. ROP layers are bioactive, directing encapsulated MSCs to differentiate into specific tissues to more efficiently restore normal joint anatomy. Dr. Saunders will optimize the materials used to generate ROP layers and will determine if MSCs from tissue lining the joint (synovium) or inner cavity of bones (bone marrow) more effectively reconstruct native cartilage, transitional tissue, or bone. This work represents an important advance in canine regenerative medicine and is highly applicable to dogs with osteochondrosisor other common joint ailments such as osteoarthritis.

Publications:

Manuscripts in preparation.

Report to Grant Sponsor from Investigator:

Our project involves development of an innovative tissue engineering strategy for the treatment of osteochondrosis (OC) and other focal cartilage lesions in large and giant breed dogs. Osteochondrosis (OC) is a common and debilitating disease affecting large breed dogs that often results in reduced performance and poor quality of life. Current standard of care includes medical management, open or arthroscopic debridement of the OC lesion resulting in fibrocartilage ingrowth, and a procedure known as OATS (transfer of healthy cartilage from an un-affected joint to replace the OC lesion). Results of these treatment strategies vary depending on the affected joint, OC lesion size, and selected treatment. There is a need for improved treatment options for OC, and tissue engineering (a regenerative medicine strategy in which progenitor cells such as stem cells are combined with three-dimensional lattices to reconstruct or replace injured tissue) represents a promising treatment alternative for dogs affected by OC. Our project, which was originally funded by AKC-CHF Jan 1, 2014, aims to develop Regenerative Osteochondral Plugs (ROPs), which are cylindrical hydrogels composed of novel polymers and regenerative cells, created in a fashion to recreate healthy joint surface anatomy. Our team is uniquely suited to achieve our project goals, as we provide complimentary expertise in chemical engineering, stem cell biology, tissue engineering, and veterinary orthopedic surgery.

Our original central hypothesis of the grant proposal was that a material known as PDMSstar could be added to hydrogels to drive differentiation of canine stem cells into cartilage or bone, and that stem cells obtained from bone marrow or synovium (a tissue that lines joints) would respond differently to these hydrogels depending on the composition of the hydrogel.

We are testing our hypothesis through three aims:
1) Determine the effect of novel hydrogels on differentiation of canine stem cells toward cartilage, transitional tissue, or bone (this aim was partially met, blank PEG hydrogel scaffolds were examined extensively; PDMS-containing gels were not evaluated due to the fact that time and resources were allocated to successfully re-design the hydrogel scaffold for canine cells)
2) Determine the ability of tri-layered ROPs to induce zone-specific differentiation of canine stem cells (this aim was not met due to our need to develop a porous hydrogel fabrication method)
3) Evaluate biocompatibility of our novel hydrogels in rodents in preparation for use in client-owned dogs with OC. This important aim was fully met/completed.

Our initial funding was for a 24-month period. We requested and were granted an additional 12-month extension on the project. We successfully isolated stem cells (hereto referred to as

MSCs) from bone marrow, synovium (the membrane that lines joint cavities), and fat (adipose tissue) from five donor dogs. Each donor's cells were extensively evaluated in the lab for ease of isolation, growth rate of cells, and the ability of isolated MSCs from these tissues to differentiate (transform) into cells that resemble bone, cartilage, and fat. We have also assessed the ability of these MSCs to interact with the immune system in an in-vitro co-culture assay in which our MSCs are cultured with mouse immunologic cells known as macrophages. A comprehensive immunologic screen of canine MSCs had not been performed prior to initiation of this work.

Many more MSCs can be rapidly isolated from synovium and fat tissue as compared to bone marrow, suggesting that these tissues could be used to provide a more rapid source of MSCs as compared to bone marrow. When evaluated for flow cytometry markers (essentially a cellular fingerprint), MSCs from all three tissues have similar flow cytometry profiles, although bone marrow MSCs stain less consistently for a marker known as CD105. However, when evaluated for their ability to differentiate into more specialized cells using long-standing laboratory assays, synovium and bone marrow MSCs exhibit superior differentiation into bone and cartilage, whereas fat-derived MSCs are incapable of differentiating into bone in short term assays. In contrast, fat-derived MSCs are superior to synovium and marrow MSCs in their ability to form cells that resemble fat. Lastly, cells from all three tissues appear to have some ability to interact with the immune system, as culturing increasing numbers of canine MSCs with mouse macrophages that are activated to produce an inflammatory protein known as TNF-alpha results in a decreased production of TNF-alpha by the mouse cells. Collectively, we have comprehensively characterized canine MSCs from three clinically relevant tissues using assays updated and optimized for the canine species. Our results suggest that while fat and synovium can be used to rapidly obtain MSCs as compared to bone marrow, bone marrow and synovium MSCs may be superior to fat-derived MSCs in their ability to form bone and cartilage like tissue. We believe the comprehensive characterization we have provided will serve as a landmark, foundational type study in the field of canine stem cell biology and regenerative medicine. A manuscript reporting these results is currently under review in a well-respected scientific journal.

During the course of Aim 1 studies, we discovered that canine cells cultured within hydrogels fabricated through "traditional" techniques experienced substantial stress and cell death, regardless of the source of MSC (all three tissues). While these results may have seemed discouraging at the time, we were prepared for challenges and in our "Potential Pitfalls and Proposed Alternatives" section of the grant application, we proposed to use an alternate fabrication technique for our hydrogel scaffolds known as SIP/Salt fabrication. This technique results in a scaffold with slightly different structural and material properties. The main advantage of this system is that it introduces a series of interconnected pores to the scaffold. The size and number of pores can be precisely controlled during fabrication. The pores allow room for cells to attach, spread, move, and we believe will also allow surrounding host tissue

to grow into the scaffold, allowing for an improved bonding, or interlock of the scaffold with the surrounding patient tissue.

We performed comprehensive in vitro analysis of the new porous hydrogels and results were quite impressive. The scaffold out performed any three-dimensional synthetic scaffold I had worked with to-date. In short, the new fabrication method, combined with our characterized MSCs, represents a groundbreaking discovery that may be clinically applicable for veterinary patients (and perhaps some day human patients as well) and we are grateful to the AKC-CHF and partner breed clubs for the support. Due to the time necessary to re-design the scaffold, we did not complete Aim 2 aims regarding the effect of the polymer PDMS on osteogenic differentiation of canine MSCs. This work will be the focus of an additional grant proposal to the AKC-CHF.

Lastly, we evaluated biocompatibility of traditional and novel 3D hydrogels in rodents. This was necessary prior to utilizing our new scaffold in client owned dogs with bone and cartilage injuries. Biocompatibility was assessed by implanting cell-free hydrogel scaffolds into the subcutaneous space (below the skin) or intralarticular space (in the joint) of 12 week old rats. These experiments were performed with approval of our institutional IACUC and with a strong focus on ethical use of animals, pre and post surgical pain control, and regular monitoring of the rats. At 21 days the rats were humanely euthanized and either the subcutaneous or joint tissues evaluated using histology (examining the tissues with microscope slides). The results were extremely promising. The SIPS/SCPL (porous) hydrogels were biocompatibility in both spaces. Results suggest that the SIPS/SCPL fabrication method does not increase the body's response to the scaffold. These experiments were necessary prior to placing a new implant into a client-owned dog.