Testing Micronized Amnion as a Therapeutic for OA in a Rat Model using Contrast Based Micro-CT Imaging

Tanushree Thote, B.S.1, Sanjay Sridaran2, Angela S.P. Lin3, Nick J. Willett, Ph.D.2, Robert E. Guldberg2.
1Georgia Inst. of Technology, Atlanta, GA, USA, 2Georgia Institute of Technology, Atlanta, GA, USA, 3Georgia Tech, Atlanta, GA, USA.

Disclosures:
T. Thote: None. S. Sridaran: None. A.S. Lin: None. N.J. Willett: None. R.E. Guldberg: 3C; Robert Guldberg serves on the Medical Advisory Board of MiMedx Group, Inc.. 4; Robert Guldberg owns stock options in MiMedx Group Inc.. 6; Robert Guldberg received a monetary gift and materials for experiments from MiMedx Group.

Introduction: Osteoarthritis is the leading cause of disability in the US. There are currently no clinically viable disease modifying osteoarthritis drugs (DMOADs) and hence, there is a need to develop and test new OA therapies. One potential intervention for OA is extracellular matrix (ECM) derived from amniotic membrane which possesses anti-inflammatory and anti-angiogenic properties coupled with low immunogenicity(1). An injectable micronized dehydrated human amnion/chorion membrane (dHACM) formulation has been developed that allows for intra-articular delivery (MiMedx Group, Inc.). Equilibrium partitioning of an ionic contrast agent based CT imaging (EPIC-µCT) has been previously used to examine compositional and morphological changes in rat models(2,3) of joint degeneration. In this study, EPIC-µCT was used as a tool to analyze the effects of micronized amnion as a therapeutic in a rat OA model. A previous study has demonstrated the therapeutic benefit of a single intra-articular injection of micronized dHACM in the rat medial meniscal tear (MMT) model, where OA progression was attenuated at 3 weeks as indicated by histology and EPIC-µCT data(4).

The objective of this study was to analyze the longer term effects of micronized dHACM (at 6 weeks) via a single injection 24 hours post-surgery or a delayed injection 3 weeks post-surgery, in the rat MMT model using EPIC-µCT. Our hypothesis was that a delayed amnion injection (3 weeks post-surgery) would be more effective in reducing joint degeneration compared to a single injection (24 hours post-surgery) at a 6 week time point due to limited duration of particle retention in the synovium

Methods: Animal model: Weight matched male Lewis rats (275-300g) underwent MMT surgery on the left leg with the uninjured right leg serving as a contralateral control. The rats were divided into three experimental groups - (1) Only MMT (no treatment) (2) MMT + micronized dHACM injection at 24 hours post-surgery and (3) MMT + micronized dHACM injection at 3 weeks post-surgery (n = 6-7/group). All surgeries were performed at Georgia Tech and approved by the Institutional Animal Care Use Committee. All animals were sacrificed at 6 weeks. µ-CT Imaging: Joints were dissected and scanned following equilibration in Hexabrix contrast agent. For analysis, images were resectioned sagittally and coronally, contoured and analyzed at suitable thresholding levels. µ-CT Image Analysis: Three volumes of interest (VOIs) were evaluated: (1) Only osteophytes (OP) (2) Medial 1/3 region of medial tibial plateau cartilage (3) Only focal cartilage lesions on the medial plateau(3). To evaluate osteophytes multiple quantitative measures were established - Total osteophyte volume, mineralized osteophyte volume and average 3D osteophyte thickness based on expanding spheres (direct distance transformation). To quantitatively assess focal defects, lesion volume was calculated (lesion was defined as a defect > 50% of cartilage thickness). Lesion volume was only calculated for samples that showed occurrence of lesions. Representative images show delineation of the medial 1/3 tibial plateau from the medial tibial plateau and illustration of lesion sites (Fig 1 A, B).

Statistics: Data are shown as means ± SEM.
Differences were evaluated using ANOVA, with individual comparisons made by Tukey's post analysis (p<0.05)

**Results: Cartilage:** Cartilage thickness was significantly lower in the contralateral controls compared to the MMT & MMT+Amnion@24hr group (Fig 2A). Cartilage attenuation, an indicator of proteoglycan content, was significantly lower in the contralateral control and MMT+Amnion@3wk group compared to the MMT & MMT+Amnion@24hr groups (Fig 2B). Lesion volume was significantly lower in the MMT+Amnion@3wk group and contralateral controls compared to MMT and MMT+Amnion@24hr group (Fig 2C). Contralateral controls showed no lesions on the articular surface.

**Osteophytes:** Representative coronal grayscale CT images show the outline of an osteophyte and depiction of mineralized and total osteophyte in an MMT sample (Fig 3). Quantitative EPIC-µCT showed significantly lower total osteophyte volume, mineralized osteophyte volume and average 3D osteophyte thickness in the MMT+Amnion@3wk and contralateral control groups compared to MMT and MMT+Amnion@24hr groups (Fig 3).
Subchondral bone: Subchondral bone volume and thickness for the medial 1/3 tibial plateau was significantly lower in the contralateral controls and MMT+Amnion@3wk group compared to MMT and MMT+Amnion@24hr group (Fig 4).

Discussion: This is the first study demonstrating a rescue effect of micronized dHACM on OA progression. Our previous results demonstrated a therapeutic benefit at 3 weeks with a single intra-articular injection. This study showed that a single intra-articular injection was not able to attenuate OA progression over a longer 6 week time course, but a delayed injection of micronized dHACM appeared to have a chondroprotective effect in the rat MMT model. Delayed intra-articular injection of micronized dHACM reduced incidence of lesions, cartilage attenuation, osteophyte development and subchondral bone sclerosis. The increase in cartilage volume has been demonstrated in previous studies and is associated with swelling of the cartilage extracellular matrix in response to injury in the MMT model[3]. EPIC-µCT was suitable as an analytical tool to examine differences in the articular cartilage surface. Traditionally, articular cartilage is analyzed via 2D histopathological scoring which requires large sample sizes and is semi-quantitative. EPIC-µCT is fast, non-destructive and provides quantitative data making it a desirable tool for relatively high-throughput screening of DMOADs as demonstrated by this study. Ongoing work includes histology to examine composition of cartilage, retention of micronized dHACM particles in the synovium as well as EPIC-µCT analysis of MMT rats treated with multiple dHACM injections (at 24 hours and 3 weeks).
Significance: There is a need to explore and assess novel OA therapeutics in an efficient and quantitative manner. Micronized dHACM presents an ECM based material that has potential to treat OA. The rescue effect of a delayed dHACM injection is clinically relevant because OA is already established and progressing when patients present with symptoms and seek treatment. Additionally, EPIC-µCT can be utilized as a tool to test effects of new therapeutics in small animal models.

Acknowledgments: The contribution of MiMedx Group, Inc. Kennesaw, GA in providing EpiFix® Injectable is gratefully acknowledged.


ORS 2014 Annual Meeting
Poster No: 0195