

Development of Human iPSC-Derived Macrophages as Preventative Therapy for Post Traumatic Osteoarthritis in a Rat Model of ACL Tear

Melissa Chavez, BS^{1,2}, Patricia Del Rio, MS^{1,2}, Thomas Spaeter, PhD^{1,2}, Giselle Kaneda, BS^{1,2}, Jacob Wechsler, BS^{1,2}, Julia Sheyn, BPharm^{1,2}, Dave Huang, MS^{4,5}, Victoria Yu, BS^{1,2}, Oksana Shelest, MS², Melodie Metzger, PhD^{4,5}, Alexander Moser, PhD^{2,6}, Wafa Tawackoli, PhD^{1,2,3,5,6}, Dmitriy Sheyn, PhD^{1,2,3,5,6}

¹Orthopaedic Stem Cell Research Laboratory, ²Board of Governors Regenerative Medicine Institute, ³Department of Surgery, ⁴Orthopedics Biomechanics Laboratory, ⁵Department of Orthopedics, ⁶Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA

Disclosures: M Chavez (N), P Del Rio (N), T Später (N), G Kaneda (4), J Wechsler (N), J Sheyn (N), D Huang (N), V Yu (N), O Shelest (N), M Metzger (5), A Moser (N), W Tawackoli (N) and D Sheyn (N)

Introduction: Post-traumatic osteoarthritis (PTOA) is a degenerative cartilage disease that occurs in a relatively young patients following injury like ACL tear, causes pain, disability, and economic burden. The iPSC-derived macrophages have the potential to prevent the inflammatory insult to the joint and PTOA induction. Non-surgical model of PTOA using a noninvasive ACL tear mimics the human condition as close as possible. Macrophages are a critical breakthrough in PTOA therapy since there is an imbalance in the immune microenvironment. Immunogenicity poses challenges for development of human stem cell therapies in animal models. In this study we are developing the models and setting the stage for the iMAC treatment of PTOA (Fig. 1a).

Methods: To study the potential iPSC-derived treatments on PTOA, immunocompromised Nude and wild type Sprague Dawley (SD) rats were used to study immune reactivity to human iPSC-derived cells. Since the joint is considered immune privileged site, we hypothesized that SD rats would not have a greater immune reaction compared to Nude rats when human iPSC-derived cells were injected into the injured knee. SD and Nude rats went noninvasive ACL injury (Fig. 1b) and were injected with human cells 2 weeks later (Fig. 1c). Serum was collected at the day of injury, injection day, day 0, 7 10 and 14 post injection to analyze IgM levels. At 2 weeks the joint tissues were digested to identify T cells or processed for histology. Human iPSCs were differentiated to macrophages using an optimized protocol, characterized via flow cytometry, and confirmed functionality through phagocytic assay. To further develop the PTOA model, SD rats underwent non-invasive ACL tear and were followed for 16 weeks and were sacrificed after 18, 20 or 25 weeks. Knee joints were processed for histology and evaluated using a OARSI score.

Results: The human cell were detected in IHC (Fig. 1d) and no visible inflammation was detected in histology of both rat types (Fig. 1e). We observed no significant difference in IgM levels and CD8⁺ cells between Nude and SD rats, demonstrating no immune reaction in immunocompetent rats after human cell injection to the joint (Fig. 1f). We have successfully differentiated iPSCs to macrophages showing their expression of macrophages surface markers (Fig. 2a,b) and detected phagocytosis capacity using the zymosan (Fig. 2b). To further characterize the PTOA model, SD rats underwent non-invasive ACL injury (Fig. 1b) and were followed for 16 weeks. Gait analysis of rats with knee injury demonstrated an increase in left and right stride length, a decrease in sway width and a significant increase in left paw angle (Fig. 3a). ELISA for collagen fragments in the rat serum detected significant elevation on week 18 (Fig. 1b) indicating cartilage degradation. Rats were sacrificed after 18, 20 or 25 weeks, the knee joints were processed for histology and used for OARSI score, showing development of knee OA as early as week 18 post injury (Fig. 1c).

Discussion: Our results confirmed the feasibility of non-invasive ACL tear to model PTOA in rats and a feasibility of human stem cell therapy using iPSC-derived macrophages. Our data also shows that the joint is an immune privileged site due, thus there is lack of immune response and no rejection of the human cells. We also show the feasibility of iMAC differentiation and function, which can lead to preventive therapy development for PTOA in the future.

Significance/Clinical Relevance: Non-invasive ACL tear demonstrated development in PTOA human stem cell therapy development have demonstrated promising results for studying PTOA in rat model. Future steps of this study will include introducing differentiated iPSC-derived macrophages into an ACL injured rat model to modify the inflammatory environment and hinder OA onset, progression, and associated pain. This potentially of the shelf therapy can be further developed to prevent the onset of PTOA.

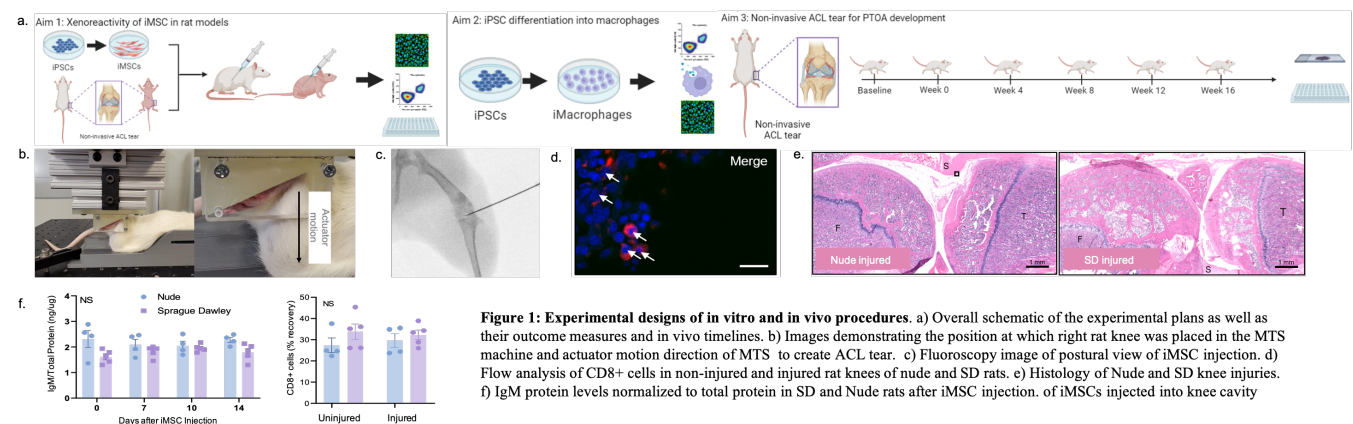


Figure 1: Experimental designs of in vitro and in vivo procedures. a) Overall schematic of the experimental plans as well as their outcome measures and in vivo timelines. b) Images demonstrating the position at which right rat knee was placed in the MTS machine and actuator motion direction of MTS to create ACL tear. c) Fluoroscopy image of postural view of iMSC injection. d) Flow analysis of CD8⁺ cells in non-injured and injured rat knees of nude and SD rats. e) Histology of Nude and SD knee injuries. f) IgM protein levels normalized to total protein in SD and Nude rats after iMSC injection. g) iMSCs injected into knee cavity

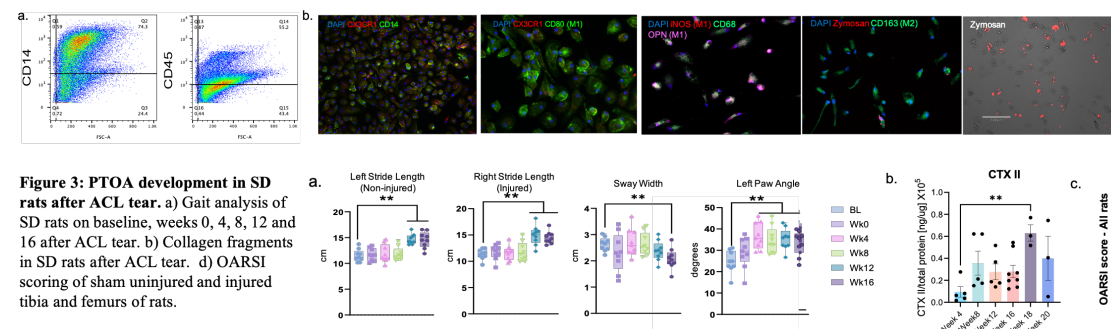


Figure 2: iMacrophage differentiation and characterization. a) Flow cytometry of iMacrophages demonstrating CD14 and CD45 biomarkers. b) Immunofluorescent images of iMac differentiation, polarization and phagocytic activity with Zymosan

Figure 3: PTOA development in SD rats after ACL tear. a) Gait analysis of SD rats on baseline, weeks 0, 4, 8, 12 and 16 after ACL tear. b) Collagen fragments in SD rats after ACL tear. d) OARSI scoring of sham uninjured and injured tibia and femurs of rats.

