INTRODUCTION: Previous animal studies found that tendon-to-bone healing occurs by formation of a reactive scar tissue, resulting in a mechanically inferior interface rather than a regeneration of normal insertion site morphology. We hypothesize that the rapid accumulation of macrophages and other inflammatory cells at the healing tendon-bone interface results in reactive scar formation rather than regeneration of a normal insertion site. Ineffective healing may be due to an insufficient number of undifferentiated cells at the healing tendon-bone interface. Thus, the application of mesenchymal stem cells at the tendon bone junction could improve healing. There is little information available in the literature on cell therapy applications in tendon-to-bone healing. Two prior rabbit studies found that bone marrow stromal cells could improve fibrocartilage formation at the healing tendon-bone interface (1, 2). No previous studies have evaluated human mesenchymal stem cells (hMSCs) in tendon-to-bone healing. We hypothesized that hMSCs applied to the healing tendon-bone junction would improve insertion site healing.

METHODS: Twenty four athymic rats underwent bilateral ACL reconstruction using a flexor digitorum longus tendon graft. After passing the graft into the bone tunnels, 1.10^6 hMSCs (Center for Gene Therapy, Tulane University Health Sciences Center) suspended in 0.1 ml of a fibrin glue carrier vehicle (Baxter, Illinois.) were injected in the femoral and tibial tunnels. The contralateral limb (controls) received the fibrin glue carrier vehicle alone. Four animals were sacrificed at 2, 4 and 6 weeks after surgery. The tissues were fixed in 10% neutral buffered formalin, decalcified, embedded in paraffin, and cut into five-micron thick sections perpendicular to the bone tunnels in the tibia and the femur. Alternate sections were stained with hematoxylin and eosin, safranin-O, or used for immunohistochemical analysis with monoclonal anti-collagen II antibody. Twelve animals were sacrificed at 6 weeks after surgery and used for biomechanical testing. After careful dissection leaving only the ACL graft across the knee, specimens were loaded on a material testing machine (MTS Systems Corp., Minnesota) with a displacement rate of 0.17mm/sec until failure. The load-to-failure was recorded, the stiffness was measured from the load-deformation curve, and the site of failure was observed macroscopically. Statistical analysis was performed by Kruskal-Wallis nonparametric test with significance set at p<0.05.

RESULTS: There were differences in the morphology of the healing attachment site between the groups. A large number of cells were present at the interface in the hMSC-treated limbs. Chondrocyte like cells were observed at 2, 4 and 6 weeks postoperatively (Fig. 1). These cells were surrounded by proteoglycan-rich matrix, as demonstrated by safranin-O staining. Furthermore, we found progressive structural organization of the newly formed fibrocartilage-like tissue. At 4 weeks postoperatively there was a large number of osteoblastic-like cells lining the edge of the bone tunnel in a very organized manner (Fig. 2). Large zones of newly-formed bone were found at the interface (Fig. 3). Robust collagen fibers crossing from bone to tendon were observed in the two groups (Fig. 4). There was collagen II staining in areas with newly-formed cartilage-like tissue. Four animals (2 in the histology group and 2 in the biomechanics group) exhibited complete degeneration of the graft at 6 weeks postoperatively. There was no evidence of infection in these animals. Biomechanical testing showed high failure loads and stiffness compared with previous studies using the same model, but no significant differences were observed between the two groups (Fig. 5).

DISCUSSION: This preliminary study established the feasibility of using an athymic rat model to evaluate hMSC’s in tendon-to-bone healing. Further studies with this model will allow definition of the cellular and molecular events in tendon-to-bone healing. The fibrin glue carrier was effective for delivery of the hMSCs. Human MSCs applied to the tendon-bone interface are likely to differentiate into chondroblastic or osteoblastic lineage cells. The activities of these cells seem to enhance bone ingrowth. The fibrin glue carrier itself may play a role in collagen fiber formation at the healing interface, as the biomechanical properties were significantly improved in both groups when compared to the results of previous studies in our laboratory using the same animal model. Immunohistochemical analysis will help to better characterize cell differentiation and provide information about the ability of these cells to regenerate a fibrocartilage insertion site. Further studies will be necessary to determine the optimal hMSC concentration for this application. The cause of the apparent graft degeneration seen in several animals is unclear, but may relate to excessive production of matrix-degrading enzymes during remodeling. Mesenchymal stem cells may be used for therapeutic gene delivery to improve tendon-bone healing in this model.