INTRODUCTION: Studies from our laboratory have shown that healing between tendon and bone occurs by formation of a fibrous, scar tissue interface rather than a normal ligament insertion site[1]. We have previously reported that inflammatory cells (neutrophils, macrophages) accumulate following tendon-to-bone repair prior to the development of the scar interface [2]. Since one of the principal functions of macrophages is cytokine production, we hypothesize that the rapid influx of macrophages and subsequent cytokine production (such as TGF-β) contribute to the early formation of the fibrous scar tissue interface, rather than a normal insertion site. It is also established that fetal wounds heal by regeneration of normal tissue morphology rather than by scar tissue, and that this response occurs in the absence of inflammation. Based on these findings, we hypothesize that attenuation of the rapid cytokine production by macrophage depletion may allow for regeneration of a normal insertion site rather than reactive scar tissue healing, in a form of “scar-less” healing. In this study we have examined healing of a tendon graft used for anterior cruciate ligament (A.C.L.) reconstruction in rats that were administered liposomal clodronate to deplete macrophages.

METHODS: Forty eight Sprague Dawley rats underwent A.C.L. reconstruction in the left knee using a flexor digitorum longus tendon graft. The animals were divided into two groups: controls (N=24) and liposomal clodronate-injected animals (LC) (N=24). Clodronate is a bisphosphonate that, when encapsulated within liposomes (liposomal clodronate), is actively phagocytosed by macrophages and induces apoptosis without stimulation [3]. Liposomes containing clodronate (a gift of Roche Diagnostics GmbH, Mannheim, Germany) were prepared as previously described [3]. In brief, a mixture of phosphatidylinoline (Lipoid GmbH, Ludwigshafen, Germany) and cholesterol (Sigma Chemical Co., St. Louis, MO) was resuspended in 0.6 M clodronate and sonicated to produce multilamellar liposomes. The liposomes were washed twice by ultracentrifugation to remove non-encapsulated clodronate and then resuspended in PBS for tail vein injection. All rats in group LC received 0.5ml/100gBW of clodronate two days prior to surgery, and then once per week (0.25ml/100gBW). An additional 0.5ml of LC was injected directly into the knee joint at surgery.

Twenty four rats were sacrificed at 2 and 4 weeks after surgery. After fixation in 10% buffered formalin for 3 days, the tissues were decalcified and then embedded in paraffin for routine histology (H & E, Alcian blue, and picrosirius red staining) and immunohistochemistry. The spleen and liver were also sectioned for immunohistochemical analysis. Immunohistochemistry was performed using mouse anti-rat ED1 (circulating macrophage) and anti-rat ED2 (resident macrophage) antibodies. Positively-stained cells were counted in ten high-power fields (50µm × 50µm at 200x) of the tendon-bone interface and grafted tendon in each limb. Collagen continuity between the grafted tendon and bone tunnel appeared significantly more organized in the LC group compared with controls at 4 weeks (ED1 and ED2, LC vs. control, p<0.0001 and p=0.006, respectively). Based on polarized light microscopy, collagen fiber continuity between the grafted tendon and bone tunnel appeared more robust and more organized in the LC group compared to controls.

RESULTS: Although macrophage depletion could increase the risk of post-operative infection, only one rat in the LC group developed a superficial infection in the operated knee. Liposomal clodronate effectively decreased the number of infiltrating macrophages at the healing tendon-bone junction after A.C.L. reconstruction in this model. Macrophage depletion resulted in decreased fibrous interface tissue and less newly formed bone at the tendon-bone interface. Although the width of fibrous interface tissue was decreased in the LC group, there was more organized collagen fiber continuity between the grafted tendon and bone. We hypothesize that the decreased amount of disorganized scar tissue is due, at least in part, to diminished TGF-β stimulation, resulting from less macrophage infiltration. This may allow expression of genes that will result in regeneration of normal insertion site morphology. Macrophage depletion in the intra-articular part of the graft may also reduce or diminish the deterioration in mechanical properties that occurs following graft transplantation. On-going studies will evaluate the biomechanical properties of the tendon-bone complex.

DISCUSSION: Liposomal-clodronate injection (intravenous and intraarticular) effectively diminished the number of infiltrating macrophages at the healing tendon-bone junction after A.C.L. reconstruction. On-going studies will evaluate the biomechanical properties of the tendon-bone complex.