Mechanical Properties in vivo conjugated (HVJ)-liposomes containing antisense or sense decorin antisense-treated (n=24) and sense treated (n=24) groups, Sendai virus ligament contains large and small diameter fibrils. Previous studies have suggested a correlation between collagen fibril sizes in ligaments and tissue mechanical properties. Therefore, production of larger collagen fibrils could improve the mechanical properties of ligament scar. Decorin is a proteoglycan which is suggested to be an inhibitor of collagen fibril assembly in vitro and in vivo. We hypothesized that the inhibition of decorin expression in an early ligament scar in vivo may potentially improve collagen fibril assembly and thus healing quality. Here we recently established a model of efficient antisense gene therapy in healing rabbit medial collateral ligament (MCL) of the knee. Here, we investigated the effect of this decorin antisense gene therapy in ligament healing with a multidisciplinary assessment, using the model.

Methods: Seventy-two mature New Zealand White rabbits had a 4-mm gap injury in the right medial collateral ligament (MCL) of the knee. For antisense-treated (n=24) and sense treated (n=24) groups, Sendai virus conjugated (HVJ)-liposomes containing antisense or sense decorin oligonucleotides (ODN) were injected into the ligament scars after 2 wks of healing. As the negative control group (n=24), ligament scars were poked with a needle without injection of any solution. All rabbits were sacrificed 4 wk post-injection (6 wks after injury). For molecular analysis (n=6 from each group), RT-PCR was performed on RNA extracted from whole scar tissue. Decorin mRNA levels were normalized to GAPDH values and were compared among the treatment groups. For protein analysis (n=5 from each group), guanidine-chloride extracted scar samples were analysed by western blotting using a specific antibody to the C-terminus of decorin core protein. The TEM (n=6 from each group), ultra-thin sections from the center area of scars were examined and a total of 28756 collagen fibrils were measured and histogram was generated. For mechanical analysis (n=7 from each group), the femur-MCL-limb complex was subject to failure testing. For statistical analysis, ANOVA (single factor) and Student’s t-tests were used with a significance at p<0.05.

Results: Results showed first that mRNA levels of decorin were significantly lower in antisense treated scars than those in sense treated and negative control scars (p<0.005). TEM study showed the presence of some correlation between large diameter collagen fibrils (> 150 nm) in antisense treated scars, while the fibrils in all sense treated and negative control scars were of uniform smaller size (<100 nm) (Fig. 2). Furthermore, antisense treated 6 wk scars were stronger than both sense treated and negative control scars at high load stress. Antisense treated scars had an average failure stress of 15 MPa, while sense treated and negative controls failed at 8 MPa (p=0.023) (Fig. 4). There was no significant difference in scar cross sectional area among the groups.

Discussion: In vivo results in this rabbit MCL scar model suggest that suppression of decorin was associated with the development of large collagen fibrils in antisense treated scars within 4 wks of the treatment. This supports the potential involvement of decorin in the regulation of de novo collagen fibrillogenesis in early ligament healing. Notably, with the alteration of collagen profile, antisense decorin treatment also had a significant effect on the mechanical strength of early MCL scars. These results suggest that enhanced de novo collagen fibrillogenesis could improve the high load mechanical properties of healing ligaments and also supports the hypothesis of some correlation between tensile strength and the presence of large diameter collagen fibrils in soft tissues. However, it is not yet clear whether

Relevance to Musculoskeletal Condition: Gene therapy is a promising treatment for potential manipulation of tissue healing. Here we demonstrated the improved quality of early ligament scar in an animal model by suppressing decorin gene expression, using an in vivo antisense gene therapy.