INTRODUCTION: Fibrillar type I collagen is the main constituent of soft connective tissues such as skin, ligament and tendon. Other constituents include other collagen types, elastin, proteoglycans (PGs) and glycosaminoglycans (GAGs). The proteoglycan decorin consists of a core protein covalently bonded to a single dermatan sulfate GAG. Decorin associates closely with fibrillar collagen, and it is believed to participate in regulation of fibrillogenesis of type I collagen. Decorin deficiency during development results in skin and tendon with both an altered fibrillar organization and inferior mechanical properties [1]. In vitro studies have shown that the presence of decorin during polymerization of type I collagen leads to altered fibrillar structure [2]. However, how this altered fibrillar structure translates to the mechanical behavior of these gels is unknown. Furthermore, it is unknown whether the entire decorin molecule is necessary to mediate this effect, or if the core protein or GAG alone can achieve the same results. Therefore, the purpose of this study was to determine how the presence of decorin, decorin core protein and dermatan sulfate during the polymerization of type I collagen gels influences the resulting mechanical properties.

METHODS: Gel Preparation. Type I collagen from rat tail tendon (BD biosciences) was polymerized into samples for tensile testing at a concentration of 2 mg/ml in silicone molds (Fig 1). Polyethylene mesh was placed in the ends of the sample for gripping and 300 μm black spheres were polymerized into the surface for tracking strain. A total of four groups of gels were tested (9 gels each): gels polymerized in the presence of intact decorin PG, decorin core protein, dermatan sulfate (DS) or bovine serum albumin (BSA), which was the control. Based on a dose-response experiment, the final concentration of decorin added was 50 μg/ml. This concentration was also used for the other groups. Decorin PG was purified from bovine tendons [3]. Decorin core protein was obtained by digesting decorin PG with Chondroitinase ABC (50 M U/ml) for 2 hours at 37°C. DS was derived from porcine intestinal mucosa (EMB Biochemicals).

Mechanical Testing. Gels were mounted in a tensile test setup consisting of a load cell, plastic clamps, a PBS test chamber and a movable linear stage [4]. Two video cameras (resolution 1024×1360 pixels) were placed directly above and to the side of the test chamber. Gels were placed in the PBS chamber. The gels were secured to the test clamps and then tested to failure at a strain rate of 10 mm/min. Force data was recorded at 5 Hz and image data was recorded at 2 Hz during testing.

Data Analysis. Three dimensional strain was computed from the image data. The strain along the long axis (ε2) was found by tracking the displacement of the black beads on the surface of the gel, while the two strains transverse to the test direction (ε1,ε3) were computed by tracking the change in the gel width and thickness. A Matlab program was written to threshold each image and then segment the boundaries of the gel and markers. Marker centroids, gel width and thickness were then extracted and the strain in the three principle directions was computed. The cross sectional area and tangent Poisson’s ratios (ν12, ν23) were computed for each time point, with the tangent Poisson’s ratio being defined as $\nu_{ij} = -\epsilon_{ij}/\epsilon_{ee}$. The engineering stress was found by dividing the force by the area of the gel in the reference configuration. The tangent modulus was computed from the slope of the stress-strain curves within the linear region and the tensile strength was computed as the maximum stress obtained prior to gel failure. The tangent modulus, tensile strength and maximum Poisson’s ratio were compared between groups using a one way ANOVA with a P value of ≤ 0.05.

RESULTS: The presence of decorin and the decorin core protein during polymerization of the type I collagen gels significantly increased the strength of the collagen gels as compared to the control, while the presence of DS alone was found to significantly decrease the strength of the gels as compared to control (Fig 2, left). The modulus and tensile strength of the gels polymerized in the presence of decorin and decorin core protein were increased by a factor of nearly two while, DS...