INTRODUCTION

Pelvic floor disorders such as urinary incontinence, fecal incontinence, and pelvic organ prolapse represent a major public health concern in the United States affecting one third of adult women [1]. These disorders are determined by structural and mechanical alterations of the pelvic organs, their supporting muscles and connective tissues that occur mainly during pregnancy, vaginal delivery, and aging [1].

The cardinal ligaments play an important role in supporting the uterus-vagina complex. Knowledge about the mechanical behavior of these ligaments is essential for the development of improved clinical interventions for pelvic floor disorders. For this reason, in this study we will examine the structure of the swine cardinal ligament using scanning electron microscopy (SEM) and perform uniaxial tests on these ligaments to quantify their elastic and failure properties.

EXPERIMENTAL METHODS

Dissection. The cardinal ligaments were obtained from two full term sows which gave birth immediately before they were sacrificed. First, the skin, adipose tissue, and muscle in the lower abdomen was cut to reveal the pubic symphysis. Next, a hack saw was used to split the pubic symphysis and a rib spreader was inserted to open the pelvis so that the vagina, rectum, and bladder were clearly visible. Using a scalpel the vagina, rectum, and bladder were extracted from the abdominal cavity as a unit. Then, the bladder and rectum were carefully dissected away from the vagina and discarded. The vagina was laid flat on a dissection table and any excess adipose tissue was removed from the cardinal ligaments. Finally, the cardinal ligaments were separated from the vagina. For SEM and uniaxial tension testing, the cardinal ligaments were cut into rectangular strips approximately 7 mm wide by 115 mm long. These strips were oriented so that the physiological loading direction coincided with the long axis of the sample. The samples were individually wrapped in plastic and stored frozen (-20°C).

SEM Examination. Prior to SEM examination, one ligament was allowed to thaw for one hour and three samples were cut down to a length of approximately 30 mm. The specimens were placed in 2% glutaraldehyde-0.01 M sodium cacodylate buffer, fixed in osmium tetraoxide and then critical point dried. To visualize the cross-section, each sample was frozen in liquid nitrogen and cut with a sharp razor blade; then a gold coating was applied by vacuum evaporation. Images of the samples were collected using an environmental scanning electron microscope (Quanta 600 FEG, FEI).

Uniaxial Testing. Ten samples were removed from the freezer and allowed to come to room temperature for one hour. They were kept continuously hydrated by spraying them with PBS throughout the sample preparation. Images of each sample were collected using a microscope (Stemi 2000C, Zeiss) to determine the width of each specimen at five locations. A caliper instrumented with a force gauge was used to measure the thickness of each specimen at five locations under a 50 g compressive load (Mitutoyo 573-291-20). Assuming a rectangular cross-section, the area for each specimen was calculated using the average width and thickness. Black ink was sprayed on the surface of the ligament to produce marks with suitable contrast for strain calculation.

The specimens were tested using a tensile testing machine.
FIGURE 1: SCANNING ELECTRON MICROGRAPHS OF THE CARDINAL LIGAMENT ILLUSTRATING (A) THE BUNDLED GROUPS OF COLLAGEN FIBERS AND (B) AN INDIVIDUAL BUNDLE WITHIN THE LIGAMENT.

(ElectroPuls E1000, Instron). Each sample was pre-loaded to 0.1 N. It was then stretched at 0.75 mm/sec until failure occurred. The motion of the ink marks was tracked using a digital image correlation method (MATLAB v. 7.10, MathWorks) and the axial strain was computed. The nominal axial stress was calculated by dividing the load by the measured cross-sectional area.

RESULTS

Scanning electron micrographs of the ligament cross-section revealed multiple bundles of collagen fibers [Fig. 1(a)]. When examining the collagen network within one bundle one finds collagen fibrils approximately 60 nm in diameter that are organized into fibers primarily pointing into the page [Fig. 1(b)]. However, the fibers at the bottom center of the image are clearly aligned in the vertical direction [Fig. 1(b)]. This arrangement of the collagen network suggests that the cardinal ligament may need to resist loads in multiple directions.

A representative stress-strain curve for the cardinal ligament is shown in Fig. 2. The ligaments exhibited the strain-stiffening behavior generally observed in soft collagenous tissues. In the failure region of the stress-strain curve, multiple peaks can be observed when a tear initiates in one location and is then halted. While the tear is halted the load increases, until the tear begins to propagate again. Eventually the tear propagates through the width of the tissue and the stress drops to zero.

To quantify the mechanical properties of the cardinal ligament, the average values of the elastic modulus of the linear region and the ultimate stress were determined. A line was fit to the points in the linear region using the linear least squares fitting routine polyfit in MATLAB. The slope found from the linear regression is taken as the modulus of the linear region. Across all 10 samples the average elastic modulus of the linear region was found to be 0.40 ± 0.19 MPa, while the average ultimate stress was 1.49 ± 0.66 MPa.

DISCUSSION

In this study, a procedure for obtaining the swine cardinal ligaments was presented, the cross-sectional structure of these ligaments was clearly visualized by SEM and a series of tensile tests up to failure were completed. While these preliminary uniaxial tensile tests provide knowledge of the mechanical properties of cardinal ligaments, they only represent the first step in assessing their complex mechanical response. It is important to note that the sows used in this study were full-term when sacrificed. Moreover, in vivo the cardinal ligament is physiologically loaded in a manner that changes during the course of gestation [1]. Therefore, additional studies that account for the stage of gestation need to be conducted in order to accurately characterize the mechanical behavior of these tissues.

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REFERENCES