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Research Paper

Histo-mechanical properties of the swine cardinal and uterosacral ligaments

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ABSTRACT

The focus of this study was to determine the structural and mechanical properties of two major ligaments that support the uterus, cervix, and vagina: the cardinal ligament (CL) and the uterosacral ligament (USL). The adult swine was selected as animal model. Histological analysis was performed on longitudinal and cross sections of CL and USL specimens using Masson's trichrome and Verhoeff–van Giesson staining methods. Scanning electron microscopy was employed to visualize the through-thickness organization of the collagen fibers. Quasi-static uniaxial tests were conducted on specimens that were harvested from the CL/USL complex of a single swine. Dense connective tissue with a high content of elastin and collagen fibers was observed in the USL. Loose connective tissue with a considerable amount of smooth muscle cells and ground substance was detected in both the CL and USL. Collagen fibers, smooth muscle cells, blood vessels, and nerve fibers were arranged primarily in the plane of the ligaments. The USL was significantly stronger than the CL with higher ultimate stress and tangent modulus of the linear region of the stress–strain curve. Knowledge about the mechanical properties of the CL and USL will aid in the design of novel mesh materials, stretching routines, and surgical procedures for pelvic floor disorders.

1. Introduction

Pelvic floor disorders (PFDs) such as urinary incontinence, fecal incontinence, and pelvic organ prolapse affect millions of women every year. These disorders, which are mainly caused by pregnancy, vaginal delivery, and aging (MacLennan et al., 2000; Kepenekci et al., 2011), have devastating consequences not only on the quality of life of women but also the healthcare system (Kenton and Mueller, 2006). The annual direct cost of prolapse surgeries alone exceeds 1 billion dollars in the United States (Subak et al., 2001). The burden placed by PFDs on women and the healthcare system will become even more significant with the projected increase in the aging population. Indeed, it has been estimated that the number of American adult women who suffer for PFDs will rise from 28.1 million in 2010 to 43.8 million in 2050 (Wu et al., 2009).

PFDs occur due to structural and mechanical alterations of pelvic organs, muscles, ligaments, and fasciae. Recent studies

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have suggested that "problems of bladder, bowel, prolapse, and some types of pelvic pain, mainly originate from the vaginal ligaments, not from the organs themselves (Petros, 2010)." The vaginal ligaments are mainly composed of collagen fibers interlaced with elastin, smooth muscle cells, nerve fibers, fibroblasts, and vascular structures. During pregnancy and childbirth, these ligaments are likely to lose their strength and increase their laxity due to the release of relaxin, a placental hormone that reduces the production of collagen and increases collagen breakdown (Sherwood, 2004). The mechanical properties of the entire vagina/supportive ligaments complex have been shown to be restored after parturition (Lowder et al., 2007). However, in many cases, the structure of the ligaments is permanently altered due to childbirth trauma and, consequently, their mechanical function is likely to be compromised. With menopause and aging, elastin and collagen degradation may also lead to laxity of the vaginal ligaments (Ewies et al., 2003; Goepel, 2008; Chen et al., 2002). These morphological changes in the ligaments are, most probably, linked to a reduction in estrogen (Mokrzycki et al., 1997).

The two major suspensory ligaments of the uterus, cervix, and vagina are the uterosacral ligament (USL) and the cardinal ligament (CL) located in a posterior direction over the levator plate of the pelvic diaphragm. The USL provides support to the cervix and the upper vagina and is connected to the sacrum (DeLancey, 1994; Buller et al., 2001; Amundsen et al., 2003). Pelvic pain in pregnancy, nocturia, urgency, and abnormal bladder emptying are believed to be caused by the laxity of the USL (Petros, 2010). The CL is linked to the USL at the cervix and extends to the upper fascia of the pelvis. Prolapse of the vagina and uterus has been associated with the laxity of both USL and CL (Miklos et al., 2002; Petros, 2010).

The importance of investigating the biomechanical properties of the USL and CL for the treatment of PFDs has been recognized only in the past few years (Weber et al., 2004). Force and displacement data have been collected on the ligaments by employing different techniques (Reay Jones et al., 2003; Cosson et al., 2003; Moalli et al., 2005), including *in-vivo* measurement methods (Luo et al., 2014). These data are, however, highly dependent on the dimensions of the tested specimens. Consequently, the biomechanical properties computed from them cannot be generalized to specimens of different dimensions. Stress and strain data have been collected to characterize the elasticity and viscoelasticity of USLs via uniaxial tests (Vardy et al., 2005; Martins et al., 2013; Rivaux et al., 2013) and of USLs and CLs via biaxial tests (Becker and De Vita, 2014). These data describe the mechanical behavior of USL and CL, independently of their size. In the experimental study by Vardy et al. (2005), quasi-static tensile tests and incremental stress relaxation tests of USLs from monkeys were performed, demonstrating their nonlinear elasticity and viscoelasticity. The study by Vardy et al. (2005) is notable for being the first attempt to determine the mechanical behavior of USLs. Tensile properties such as ultimate tensile strength and stiffness of female cadaveric USLs were computed for the first time by Martins et al. (2013). In the study by Martins et al. (2013), stress and strain data were reported, although strain data were computed from the clamp displacement (and not using more accurate video strain measurement methods). Mooney–Rivlin constitutive parameters were employed by Rivaux et al. (2013) to quantify the nonlinear elasticity of female cadaveric USLs that were uniaxially tested. Biaxial elastic and viscoelastic material properties were computed very recently by our group for both the swine USL and CL using novel constitutive parameters (Becker and De Vita, 2014).

In this study, we determine both the histological and mechanical properties of the USL and CL using the swine as an animal model. Toward this end, we perform scanning electron microscopy (SEM) and histological studies on specimens isolated from one entire USL/CL complex. We conduct tensile tests on specimens located in different anatomical regions within another entire USL/CL complex. From accurate stress and strain data measurements, tensile properties such as elastic moduli of the toe and linear regions of the stress–strain curve, ultimate tensile strength (UTS), and strain at UTS are computed. We then evaluate a possible relation between the composition and structure of these ligaments and their tensile properties.

2. Material and methods

2.1. Harvesting technique

Two full term sows (weights = 261 kg and 234 kg) were acquired from a different study in accordance with an approved Virginia Tech IACUC protocol. The sows were euthanized immediately after giving birth (each sow delivered 13 piglets) and their lower abdomen and hindquarter were isolated and firmly secured to a dissection table. In order to identify the vaginal canal, cervix, and uterus, a plastic rod was inserted in the introitus of the vagina (Fig. 1(a)). By using a scalpel, a midline vertical incision was made until the peritoneal cavity was entered. The pubic symphysis was then separated using a hack saw. A rib spreader was utilized to separate the pubic symphysis for access to the vagina and support structures. Using the plastic rod and a scalpel, the vagina, cervix, uterus, and support structures together with the rectum and bladder were extracted from the abdominal cavity as a single complex. This procedure minimized damage to the CL and USL needed in this study (Fig. 1(b)). The bladder and its connective tissues were carefully removed from the vagina–cervix–uterus complex and discarded.

The USL connected the proximal vagina, from the interdigitating pads (cervix in the swine) to the sacrum. Taking care to retain as much of the USL as possible, a scalpel was used to remove it from its attachments to the vagina and sacrum. The CL fanned out laterally from the lateral vagina, through the broad ligament, including the uterine artery and vein, to the pelvic side wall. The CL was also cautiously dissected to preserve its full course (Fig. 1(b) and (c)).

The vagina and the attached USL and CL were then laid flat on a dissection table, and any excess of adipose or muscular tissue was removed from these ligaments. Finally, the USL and CL were separated from the vaginal wall and kept hydrated with phosphate buffered saline (PBS) solution. After dissection, the ligaments were wrapped in plastic and stored at $-20\,^{\circ}$C. Before each mechanical test, SEM or histological analysis as described hereafter, the ligaments were removed from the freezer and allowed to thaw at room temperature for 30 min.