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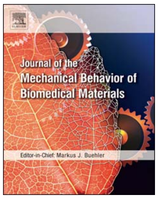

Effects of repeated biaxial loads on the creep properties of cardinal ligaments

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ABSTRACT

The cardinal ligament (CL) is one of the major pelvic ligaments providing structural support to the vagina/cervix/uterus complex. This ligament has been studied mainly with regards to its important function in the treatment of different diseases such as surgical repair for pelvic organ prolapse and radical hysterectomy for cervical cancer. However, the mechanical properties of the CL have not been fully determined, despite the important *in vivo* supportive role of this ligament within the pelvic floor. To advance our limited knowledge about the elastic and viscoelastic properties of the CL, we conducted three consecutive planar equi-biaxial tests on CL specimens isolated from swine. Specifically, the CL specimens were divided into three groups: specimens in group 1 ($n = 7$) were loaded equi-biaxially to 1 N, specimens in group 2 ($n = 8$) were loaded equi-biaxially to 2 N, and specimens in group 3 ($n = 7$) were loaded equi-biaxially to 3 N. In each group, the equi-biaxial loads of 1 N, 2 N, or 3 N were applied and kept constant for 1200 s three times. The two axial loading directions were selected to be the main *in-vivo* loading direction of the CL and the direction that is perpendicular to it. Using the digital image correlation (DIC) method, the in-plane Lagrangian strains in these two loading directions were measured throughout the tests. The results showed that CL was elastically anisotropic, as statistical differences were found between the mean strains along the two axial loading directions for specimens in group 1, 2, or 3 when the equi-biaxial load reached 1 N, 2 N, or 3 N, respectively. For specimens in group 1 and 2, no statistical differences were detected in the mean normalized strains (or, equivalently, the increase in strain over time) between the two axial loading directions for each creep test. For specimens in group 3, some differences were noted but, by the end of the 3rd creep test, there were no statistical differences in the mean normalized strains between the two axial loading directions. These findings indicated that the increase in strain over time by the end of the 3rd creep test were comparable along these directions. The greatest mean normalized strain (or, equivalently, the largest increase in strain over time) was measured at the end of the 1st creep test ($t = 1200$ s), regardless of the equi-biaxial load magnitude or loading direction. Mean normalized strains during the 2nd and 3rd creep tests ($t = 100$, 600, and 1200 s), along each loading direction, were not statistically different. Isochronal data collected at 1 N, 2 N, or 3 N equi-biaxial loads indicated that the CL may be a nonlinear viscoelastic material. Overall, this experimental study offers new knowledge of the mechanical properties of the CL that can guide the development of better treatment methods such as surgical reconstruction for pelvic organ prolapse and radical hysterectomy for cervical cancer.

1. Introduction

Pelvic floor disorders (PFDs), such as urinary incontinence, fecal incontinence, and pelvic organ prolapse (POP) are a growing component of women's health issues in the United States. It has been estimated that in 2010 over 28 million women had at least one PFD and this number is expected to increase to 44 million by 2050 (Wu et al., 2009). In particular, POP, the descent of a pelvic organ from its normal place towards the vaginal walls and into the vaginal cavity, is one of the most prevalent forms of PFDs. As of 2010, it is estimated that POP affects 3.3 million women in the United States, annually (Price et al., 2014). The onset of POP can be attributed to several factors, with the most common being age, labor, parity, menopause, and weight gain (MacLennan et al., 2000; Hendrix et al., 2002; Nygaard et al., 2008). For mild cases of POP, lifestyle changes such as a change in diet and exercise or muscle strengthening exercises such as Kegel exercises can help alleviate some of the symptoms. For more severe or extreme cases, the recommended course of treatment for the most common type of POP, the uterine

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prolapse, is typically a pelvic reconstructive surgery. The number of women who will undergo surgery to treat POP continues to dramatically increase, and it has been estimated that this number will increase from 166,000 in 2010 to approximately 250,000 in 2050 (Wu et al., 2011).

Traditionally, native tissue repairs have been adopted to treat POP but mesh augmented repairs have become more common over the past years. However, many women experienced adverse side effects to mesh augmented procedures, such as pain, mesh erosion, dyspareunia, and recurrence of POP. A comprehensive study by Maher et al., who collected data on surgical management of POP of approximately 6000 women, found that 14% of patients who received a transvaginal mesh experienced some form of POP recurrence (Maher et al., 2011). The study also found that 18% of patients who received a transvaginal mesh experienced mesh erosion and 11% of patients underwent reoperation. Surgical meshes used for PFDs were developed in the 1950s initially to treat abdominal hernia repairs and, due to their success, in the 1970s gynecologists started using these abdominal meshes for repair of POP (Ellington and Richter, 2013). However, women experienced many of the complications outlined above and these were, most likely, triggered by the mismatch in properties between the native tissue and the synthetic mesh.

Damage to pelvic supportive ligaments, such as the uterosacral ligament (USL) and the cardinal ligament (CL), contributes significantly to the development of PFDs (DeLancey, 1992; Wei and de Lancey, 2004; Nygaard et al., 2008). The USL and CL are visceral ligaments that connect the upper vagina/cervix to the sacrum and pelvic sidewalls, respectively, and provide support to the vagina, cervix, and uterus (Ramanah et al., 2012) (Fig. 1). These ligaments are often characterized together and are commonly referred to as the USL/CL complex (Dwyer and Fatton, 2008; Ramanah et al., 2008; Chen et al., 2013). They are, however, quite different and, for this reason, they deserve to be studied also independently. The CL is parallel to the body axis and is vertically oriented when a woman is an upright position, while the USL is dorsally directed toward the sacrum. Using an MRI based 3D technique, the CL was found to be much longer and more curved than the USL (Chen et al., 2013).

One of the first studies to investigate the existence of the CL was conducted by Mackenrodt who described the CL as a transverse cervical ligament that is the chief supporting structure of the uterus (Mackenrodt, 1895). By the 1960s, Range and Woodburne conducted an anatomical analysis of the CL, finding that it is mostly made of blood vessels, nerves, lymphatic vessels, and loose connective tissue with collagen and smooth muscle fibers (Range and Woodburne, 1964). Through a more recent structural characterization of the CL, Samaan et al. suggested the CL may be a suitable attachment point for a synthetic mesh in surgical repair of POP (Samaan et al., 2014). The CL has also been found to play a pivotal role in the treatment of cervical cancer via radical hysterectomy. Historically, the CL and its surrounding connective tissue were removed in radical hysterectomy, following a procedure that was established by Latzko and Shiffmann (1919) and Okabayashi (1921). However, studies conducted by Yabuki et al. in the 1990s and 2000s determined that preservation of the CL is crucial in order to prevent neurogenic bladder and excessive bleeding, given the proximity of the CL to the neural pathway responsible for the control of bladder function (Yabuki et al., 1991, 1996, 2005).

Investigating the effect of repeated constant loading on the time-dependent mechanical behavior of the CL and other supportive ligaments is essential since these ligaments are constantly under tension and experience large changes in length and curvature *in vivo* (Luo et al., 2014). Recently, Chen et al. used geometrical data collected via an MRI based 3D technique and developed a four-cable mechanical model in order to quantify the geometrical and mechanical characteristics of CL and USL in living healthy women (Chen et al., 2013). After reconstructing the pelvic anatomy of 20 healthy women, the authors deduced that the CL is parallel to the body axis and, as a woman stands upright, the CL becomes vertically oriented. Due to its alignment, the CL experiences greater tension than the USL and its curvature allows the apical support to have a large range of motion. In everyday life, the CL undergoes changes in tension as a woman sits and stands upright. These changes are exacerbated with fluctuations in weight and during pregnancy when the growing fetus exerts additional tension on the pelvic organs. The CL is subjected to repeated loads over time *in vivo*, especially after the levator ani muscle is damaged during vaginal delivery. These loads are likely to cause an increase in the tissue's length over time, compromising the support function of the CL and contributing to the development of POP.

In a recent review article, we summarized the current knowledge of the mechanical properties of female reproductive organs and supporting connective tissues, presenting the results of experimental studies that characterized the nonlinear elastic and viscoelastic responses of these tissues (Baah-Dwomoh et al., 2016). *Ex vivo* uniaxial tensile tests of supportive ligaments were conducted (Reay Jones et al., 2003; Moalli et al., 2005; Vardy et al., 2005; Martins et al., 2013; Rivaux et al., 2013; Chantereau et al., 2014) and mechanical quantities, such as the ultimate strength and tensile modulus were reported for the CL (Tan et al., 2015). *In vivo* uniaxial tests were also performed to measure stiffness and repeated force-relaxation of USL/CL complexes in women affected by POP (Smith et al., 2013; Luo et al., 2014). Clearly, the *in vivo* tests produced the most physiologically relevant mechanical data but, due to ethical considerations and limited time in the operating room during testing, the tests only lasted a few minutes. Both the USL and CL are membrane-like and experience loads in multiple directions over long time intervals and thus *ex vivo* planar biaxial tests can offer a more complete description of their mechanical behavior. Using *ex vivo* planar biaxial methods, more recently, the Authors characterized the elastic, stress relaxation (Becker and De Vita, 2015), and creep (Tan et al., 2016) properties of the USL/CL complex (Fig. 1).

In this study, we investigate the effects of repeated equi-biaxial loads on the mechanical properties of swine CLs. The swine is selected as an animal model due to histological similarities that exist between the CL in swine and the CL in humans (Gruber et al., 2011; Tan et al., 2015, 2016). *Ex vivo* testing is a valuable alternative method to *in vivo* testing for exploring the time dependent behavior of CL since changes in mechanical properties can be assessed over longer time intervals. More specifically, the creep properties are evaluated after three 1200 s long equi-biaxial loads are applied along the main *in vivo* loading direction of the CL and the direction perpendicular to this one. While the CL specimens are loaded, accurate strain maps are obtained using the Digital Image Correlation (DIC) method. This study extends our limited knowledge about the time-dependent mechanical behavior of the CL, providing insight into the effect of repeated loading on the supportive function of CL within the pelvic floor. The findings could suggest new treatment strategies for PFDs and cervical cancer.

2. Materials and methods

2.1. Specimen preparation

This study was conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at Virginia Tech. Four adult (3–4 year-old, approximately 450 lbs) domestic swine were obtained from a slaughterhouse (Gunnoe Sausage Co, Goode, VA). The CLs were harvested from the swine using techniques detailed in our previous study (Tan et al., 2015). They were hydrated with phosphate-buffered saline solution (PBS, pH 7.4, Fisher Scientific, USA) and then frozen at – 20 °C. They were thawed at room temperature and cut into approximately 3 × 3 cm^2 specimens (Fig. 2(a)–(b)). A total of 24 specimens were used for mechanical testing.