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Short communication

Strain imaging of the lateral collateral ligament using high frequency and conventional ultrasound imaging: An *ex-vivo* comparison

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ABSTRACT

Recent first attempts of *in situ* ultrasound strain imaging in collateral ligaments encountered a number of challenges and illustrated a clear need for additional studies and more thorough validation of the available strain imaging methods. Therefore, in this study we experimentally validated ultrasound strain measurements of *ex vivo* human lateral collateral ligaments in an axial loading condition. Moreover, the use of high frequency ultrasound (>20 MHz) for strain measurement was explored and its performance compared to conventional ultrasound. The ligaments were stretched up to 5% strain and ultrasound measurements were compared to surface strain measurements from optical digital image correlation (DIC) techniques. The results show good correlations between ultrasound based and DIC based strain measures with R^2 values of 0.71 and 0.93 for high frequency and conventional ultrasound, subsequently. The performance of conventional ultrasound was significantly higher compared to high frequency ultrasound strain imaging, as the high frequency based method seemed more prone to errors. This study demonstrates that ultrasound strain imaging is feasible in *ex vivo* lateral collateral ligaments, which are relatively small structures. Additional studies should be designed for a more informed assessment of optimal *in vivo* strain measurements in collateral knee ligaments.

1. Introduction

Ultrasound is a widely used imaging modality to assess tendon and ligament abnormalities. Traditional ultrasound techniques are directed towards assessing tissue structure or geometry. However, the biomechanical properties of ligaments may change as a consequence of injury or disease. Quantitative ultrasound imaging techniques can be used to evaluate soft tissue biomechanics. Ultrasound strain imaging is such a technique that can quantify tissue deformation (Ophir et al., 1991) and in the musculoskeletal field it has been mostly applied in tendons such as the Achilles and patellar tendons (Bogaerts et al., 2016; Chimenti et al., 2016; Slane et al., 2016, 2017a; Slane and Thelen, 2014).

Within the musculoskeletal field, there are many potential clinical applications of this technique that have not been fully explored. For example, knee instability may be a result of ligamentous insufficiency (*e.g.*, tear, attenuation) and is a major cause of early total knee arthroplasty failures. Also for patients suffering from varus or valgus knee deformities, the strains in the collateral ligaments may not be physiological. In order to quantify these abnormalities ultrasound strain imaging can be a potential technique to assist surgeons and researchers.

First attempts of *in situ* ultrasound strain imaging in the collateral ligaments by Slane and coworkers encountered a number of challenges in both data acquisition and processing (Slane et al., 2017b). Their study illustrated a clear need for additional work, particularly relating to the collection of ground-truth data and reliable image methodologies for *in vivo* measurements. The small size of the ligaments also complicates data processing and requires more thorough validation studies of the available strain estimation methods. Slane et al. suggested to explore the usage of a higher resolution ultrasound transducer, as the higher spatial resolution of these systems could visualize more structural detail in these small tissues and consequently improve the characterization of ligament displacement and strain. In this paper we analyzed the effects of the latter suggestion and performed high frequency (>20 MHz)

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ultrasound strain measurements and compared it to strain estimations using conventional (~7 MHz) ultrasound. The performance of both techniques was analyzed in an *ex vivo* experiment with strain values obtained with an optical Digital Image Correlation (DIC) technique serving as a reference.

2. Methods

Six fresh-frozen cadavers (78 ± 11 years), without signs of hard and soft tissues injuries were received from the Anatomy Department of Radboudumc with a permission statement for experimental use. The cadaver legs were dissected by an orthopedic surgeon and the lateral collateral ligaments were obtained with bone attachments intact at the insertion sites of the ligament. To facilitate surface strain estimation as obtained with DIC measurements, the ligaments were stained with a methylene blue solution to create a dark background and sprayed with an white oil-based paint to create highly contrasted speckles (Lionello et al., 2014; Luyckx et al., 2014). To provide a better grip during testing, each bone part was embedded in bone cement (polymethylmethacrylate), see also Fig. 1.

The specimens were submerged in water, with the ends fixed in custom build grips (proximal end in upper grip) of a tensile testing machine (MTS system corporation, Minesota, USA). The grips allowed free motion of the ligament, except for the axial direction (*i.e.,* rotation and translation perpendicular to the axial loading direction). Specimens were preloaded to 10N to reduce slackness and their initial length was measured (using the image data from the DIC method), followed by a preconditioning step of 10 cycles of axial stretching to 5% at a frequency of 1 Hz (Ristaniemi et al., 2017). Axial stretching was accomplished by displacement driven elongation of the initial measured length of the ligament. After preconditioning, the ligaments were subjected to two trials that both consisted of 5 sinusoidal cycles of axial stretching to 5% at a frequency of 1 Hz. For each trial a different ultrasound system was used to obtain raw ultrasound radio-frequency (RF) data. For one of the trials ultrasound data were acquired using a conventional ultrasound transducer (center frequency ~7 MHz), and for the other trial a preclinical system with a high resolution transducer (center frequency ~20 MHz) was used.

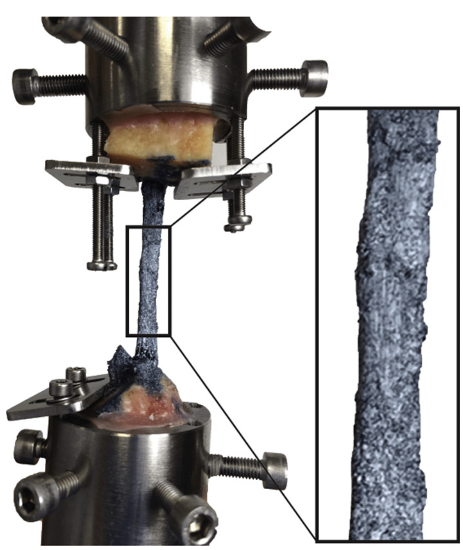

Fig. 1. Ligament preparation. The ligaments were stained with methylene blue and a white speckle pattern was applied to facilitate digital image correlation techniques. To provide good grip, the bone parts were embedded in PMMA and fixed in custom build grips. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Conventional ultrasound data were acquired with an iE33 ultrasound system (Philip Medical Systems, Bothell, USA), equipped with a L11-3 linear array transducer (footprint 39 mm, 576 lines), with a fixed echo depth of 30 mm (1250 samples) and a frame rate of 21 Hz. The high resolution ultrasound acquisition system was a Visualsonics 2100 (FUJIFILM VisualSonics Inc., Toronto, Canada) equipped with an MS250s linear array transducer (footprint 23 mm, 512 lines), with a fixed echo depth of 30 mm (6112 samples) and a frame rate of 21 Hz. As a reference, optical image sequences of the medial surface of the ligament were captured using a SPOT™ Insight 2.0 Color digital camera (SPOT TM Imaging Solutions, Michigan, USA) at a frame rate of 10 Hz (see Fig. 2).

Both ultrasound and optical image data were processed using a custom developed and validated 2D speckle tracking method (Gijsbertse et al., 2017; Lopata et al., 2009a). Displacements were calculated on an equidistant grid with 0.5 mm spacing within a ROI of 20 × 5 mm within each specimen. The centers of the ROI of the different modalities were manually aligned with each other (transducer positions were visible on the optical images). Inter-frame displacements were calculated by normalized cross-correlation of template kernels (1.2 × 0.6 mm) within larger search kernels (2.2 × 1.1 mm) in the consecutive frame. Sub-pixel displacement estimation was detected by spline fitting of the cross correlation peak. To remove outliers, the displacements were 2D median filtered (1.5 × 2.5 mm kernel). Accumulated displacement estimates over the entire loading cycle were computed from the inter-frame displacements using bilinear interpolation (Lopata et al., 2009a). To synchronize the data and minimize drift as a result of tracking errors, we tracked every stretching phase of the ligaments individually. Starting frames of the cycle were derived

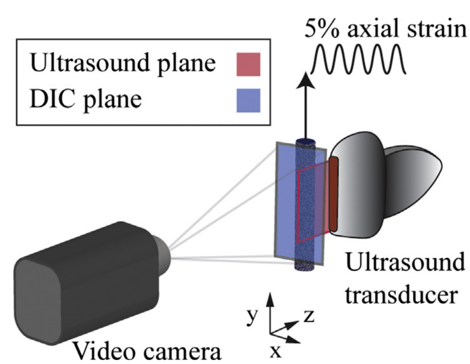

Fig. 2. Experimental set-up. The ligament is cyclically stretched to 5% strain in axial direction (displacement driven). Ultrasound and optical images were simultaneously acquired from orthogonal planes.

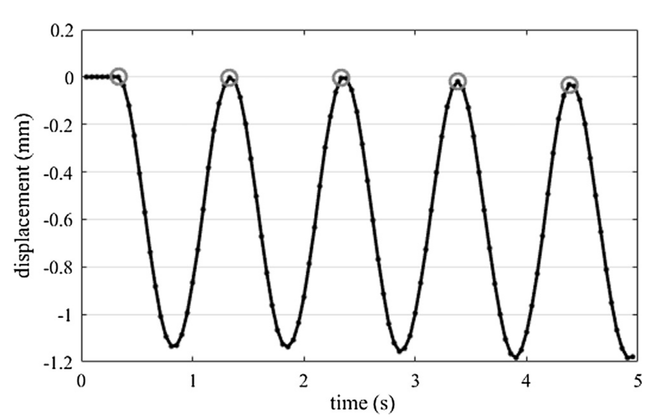

Fig. 3. Local peak detection of the accumulated lateral displacement was used to detect the starting frames for tracking every stretching phase of the loading cycle.