Short communication

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

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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)
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, Minnesota, 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. 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.

Conventional ultrasound data were acquired with an iE33 ultrasound system (Philips 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. 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.)](image1.png)

![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.](image2.png)

![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.](image3.png)
from the entire accumulated lateral displacement, and defined as local peaks in the median displacement curve (Fig. 3). Strain was derived from the accumulated displacements using a 2D least square strain estimator (Kallel and Ophir, 1997; Lopata et al., 2009b) with kernel size of $10 \times 1.25$ mm.

Median strain curves were calculated from all grid points inside the ROI over the loading cycle and resampled at 20 Hz. Correlation between the strain values derived from DIC and ultrasound techniques were computed and expressed with their coefficient of determination ($R^2$). Agreement between the techniques were expressed in Bland-Altman plots and the difference values were compared to assess statistical differences (Student T-test). Additionally, ultrasound data and corresponding strain maps were qualitatively evaluated and judged on their resemblance with the results of the DIC technique.

3. Results

Displacement and strain maps as obtained with the two ultrasound techniques were similar and showed close resemblance.
with the reference DIC technique (Fig. 4). The displacement and strain maps show limited variation across the depth of the ligament, indicating that ligaments deform rather homogenously in this direction. However, the strain is more inhomogeneous along the longitudinal direction (y-axis) and the DIC measurements reveal that the largest strain is present near the insertion sites. The use of a high frequency ultrasound system resulted in B-mode images with much finer speckle appearance compared to the conventional system. Yet, the image view is smaller and therefore a smaller part of the ligament is visualized.

Strain values from conventional ultrasound techniques showed very strong correlation with DIC measurements, with a linear coefficient value of $R^2 = 0.93$. The correlation between high frequency ultrasound based and DIC based strain values showed reasonably strong correlation, with a linear coefficient value $R^2 = 0.71$. The correlation between ultrasound based and DIC based strain values for all specimens is depicted in Fig. 5a. Bland-Altman plots depict the agreement of the ultrasound based methods to DIC and are shown in Fig. 5b. Conventional ultrasound underestimates the strain with differences ranging from $-1.1\%$ to $0.13\%$ strain with an average of $-0.49\%$. The mean difference by the high frequency method was significantly ($p = 0.035$) larger with an average difference of $-0.67\%$ strain. The estimated local strain from both ultrasound and DIC techniques was lower than was applied globally (grip to grip).

4. Discussion

In this study we evaluated the use of high frequency ultrasound strain estimation of axially loaded collateral ligaments ex vivo and compared its performance to a conventional ultrasound method. The results demonstrate that conventional ultrasound based strain estimations show very strong correlation with strain values derived from DIC. The correlation obtained in this study is in accordance with earlier findings in strain imaging of tendons (Chernak Slane and Thelen, 2014; Okotie et al., 2012). Whereas, high frequency ultrasound strain estimation shows lower performance and only reasonable correlation with DIC measures.

When studying superficial structures, one obvious strategy to increase spatial resolution is to use a high frequency system. An increase in frequency decreases wavelength and consequently decreases both beam width (lateral resolution) and pulse duration (axial resolution), at a cost of penetration depth (Shung, 2006). As a result of increased spatial resolution, a better performance of high frequency ultrasound systems seems evident in strain imaging, but in this study this approach was unexpectedly found to be inferior to conventional ultrasound based measurements. A possible explanation for this phenomenon might be the smaller elevational beam width, which makes the technique more sensitive to out-of-plane motion, resulting in tracking errors. DIC measures revealed out-of-plane motion (transverse to loading direction) ranging between
0.87 mm and 1.36 mm throughout the loading cycles. Out-of-plane motion is a fundamental challenge and is the inherent limitation of 2D imaging to evaluate 3D tissue motion. Another limitation in ultrasound strain imaging is the lower resolution and lack of phase information in the lateral direction. These challenges might be resolved using additional methods, such as 3D ultrasound imaging techniques (Carvalho et al., 2017; Gijbelsbertse et al., 2017), displacement compounding (Fekkes et al., 2016; Hansen et al., 2010) or transverse oscillation (Jensen and Munk, 1998).

The estimated strain of the ligaments was lower than was applied globally. The ligament length could be prone to measurement errors leading to false global reference values. Furthermore, strain may differ spatially (Chernak Slane and Thelen, 2014; Haraldsson et al., 2005) and DIC measures indeed shows that strain varied locally with the highest strain values present near the insertion sites (Fig. 4). Therefore, local (mid-substance) ligament strain was directly compared with matching ROI sizes between ultrasound and DIC methods. It is important to note the different imaging planes between the ultrasound and DIC data, which could have contributed to the variability in strain estimation between the methods. In case of the lateral collateral ligament the variability between the surface and internal strain is expected to be lower, but this assumption may be invalid when studying structures with more complex anatomical/structural shape and deformation (e.g., cruciate ligaments). Moreover, this study addresses a simple ex vivo loading scenario and many difficulties remain prior to clinical translation, including additional challenges in data acquisition as a result of complex anatomy and loading condition that occur in an in vivo situation (Slane et al., 2017b). In a well-controlled tensile test the material would remain more in plane than practically happens and additional studies should be designed for a more informed assessment of optimal in vivo strain measurements in the lateral collateral ligaments.

In conclusion, ultrasound strain imaging is feasible in ex vivo lateral collateral ligaments. The use of high frequency ultrasound does not seem beneficial compared to conventional ultrasound strain imaging. Despite the fact that high frequency ultrasound results in more detailed images that could help in diagnosis (e.g., detect small tears), the technique is more prone to errors that might be caused by out-of-plane motion which is also expected to occur (considerably) during in vivo strain measurements (Slane et al., 2017b).

Conflict of interest statement

The authors declare that they have no financial or personal relationships with other people or organizations that could appropriately influence (bias) this work.

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