QUANTITATIVE ANALYSIS OF TENDON ECM DAMAGE USING MRI

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ABSTRACT

There is a growing demand for non-invasive methods to diagnose tendon injuries and monitor the healing processes of their repair. One particular target is to assess the quality of tendon tissue, which requires imaging modalities, such as Magnetic Resonance Imaging (MRI), that capture structural features of the extracellular matrix (ECM). However, to date there has been limited understanding of the physiological source of intratendinous MRI signal. This paper presents a novel image analysis method, based on low level features, which capture the intrinsic structural properties in order to identify ECM damage. More specifically, continuous intrinsic dimensionality (cid), based on local image descriptors and derived from the monogenic signal, is used to examine the disruption of 1D structures. The damage measure is summarized using confidence values derived from the bi-modality of local non-parametric probability density functions. Areas of normal and disrupted ECM are detected on MR images of healthy and damaged samples.

Index Terms— tendon MRI, monogenic signal, intrinsic dimensionality, NP windows

1. INTRODUCTION

The high prevalence of tendon injuries and disorders is largely attributable to sporting activities, resulting in severe pain and potentially debilitating consequences. Diagnosing these injuries and monitoring their repair is challenging; current clinical methods provide limited structural, and hence functional, information relating to the tissue quality. Therefore, there is a growing demand for specific in vivo imaging methods, for example MRI, to assess tendon quality.

To develop such methods, it is paramount to understand the source of the MR signal, and relate this back to the underlying physiology. This in turn requires ex vivo validation of the MR acquisition and image analysis methods used to assess tendon tissue quality, before the imaging tool can be exploited in vivo. This paper presents the application of sophisticated image analysis methods for interpreting images of normal and damaged tendon extracellular matrix (ECM) images ex vivo; taking into account the important structural and functional properties of the tissue.

The transmission of forces generated by muscles to bones is governed by the ultrastructure of the tendon ECM, predominantly collagen (∼70 % dry weight) and proteoglycans. Normal tendon sections stained with safranin-orange reveal fascicular bundles, bound together by epitenon connective tissue, containing tightly packed and cramped collagen fibres (Fig. 1). The crimp waveform, a characteristic of normal tendon, can be seen in the longitudinal sections.

 Clinically, intratendinous signal is often diagnosed as damage when a high signal intensity region appears against the dark background. However, the use of specialised MRI protocols, such as ultra-short echo time (UTE) [1], magic angle MRI [2] and ultra-high field (7 T) MRI [3], have demonstrated the potential of MRI to generate intratendinous signal relating to intact structures. Therefore, concurrent methods are required for image analysis and interpretation, relating to underlying physiology, and potentially clinically applicable.

Tendon damage can be modeled by enzyme-induced ECM disruption; papain and trypsin digest ECM proteins, resulting in ECM disorganisation comparable to acute injury or tendinopathy, or ECM reorganisation comparable to tendon repair. It has been demonstrated that high field (7 T) MRI (Fig. 2) and microscopy techniques can distinguish between normal and digested tendon samples [3]. These images demonstrate that visual assessment of tendon injury using MRI is challenging, and that instead quantitative analysis methods are required for relating the MRI signal to the underlying physiology. In this paper, tendon is modelled as a hierarchical organization of linear entities (i.e. fascicles), as demonstrated by earlier work [1], microscopy (Fig. 1), and MRI (Fig. 2). Disruption of this idealistic scenario is believed to represent damage. To this end, low level image analysis methods are applied that enable this type of assessment.

One way to identify the linearity, i.e. 1Dness of a local image patch is by its intrinsic dimensionality. This informs whether a given pixel is part of an i0D, i1D or i2D structure, where i0D denotes constant, i.e. homogeneous image patch; i1D signals are concentrated along a line across the origin of the Fourier space, and quantify signal variance along one dimension, e.g. lines; and i2D signals have an energy spectrum spread throughout the frequency space, e.g. corners.

Real 2D signals are a mixture of i0D, i1D and i2D. As a result, the definition of hard thresholds between these three classes is difficult. The continuous formulation of the intrinsic dimensionality (cid) [4] (ci0D, ci1D, ci2D) is currently the best available tool that

Fig. 1. Tendon sections stained with safranin-orange reveal fascicular structures. (a) and (b) Longitudinal sections. (c) Transverse section. Scalebar is 1 mm.
addresses the above limitations with the use of a probabilistic framework, which is based on an appropriate structural representation. It is required that this representation is insensitive to illumination and contrast changes. One structural descriptor that satisfies this criterion, similarly to 1D signal processing, is contained in the local phase. In 2D and higher dimensions this is derived from the analog of the 1D analytic signal, called the monogenic signal, that is constructed with the Riesz transform, i.e. the generalized Hilbert transform.

This paper presents a quantitative method for assessing ex vivo ovine tendon ECM quality from MR images. The proposed analysis exploits the direct connection between MR image and underlying biological structure, and is based on the observation that the extent of damage is quantifiable using the bi-modality of the local non-parametric probability density function (PDF) from carefully selected image descriptors. These measures include, however, are not limited to, c1D and local orientation (LO) from the monogenic signal, and represent meaningful quantities related to the underlying linearity and regularity of the fascicular structures and ECM. We proceed as follows: Section 2 describes image acquisition and analysis; Section 3 presents our results through Fig. 3 and 4; finally we provide an outlook of the possible clinical application of the presented method. The input images, observations and results are illustrated in Fig. 2, 3, and 4, respectively.

2. METHODS

This section provides a brief overview of the sample preparation and image analysis methodology.

2.1. Image Acquisition

Cylindrical tendon samples of approximately equal dimensions (diameter ~5 mm and length ~10 mm) were cut from extracted ovine flexor tendons. The treated samples were incubated in digestion buffer containing papain or trypsin enzyme at 37°C for 18 hours (Fig. 2 e-f). Control samples were incubated in Phosphate Buffered Saline (PBS) for 18 hours (Fig. 2 a-b). In plane resolution is 0.2×0.2 mm².

MR imaging was carried out using a 300 MHz horizontal bore 7 T magnet using a transmit receive quadrature birdcage coil of 55 mm diameter at room temperature. T₁-weighted imaging along the longitudinal axes of the tendon samples was performed using a fast spin echo (FSE) pulse sequence with TR 0.1 s and TE 0.0125 s. Corresponding samples were imaged using near infra-red multiphoton laser scanning microscopy to confirm the protein damage at the level of the ECM (see [3] for microscopy results).

2.2. Image Analysis

Local descriptors of low level features are extracted from the monogenic signal [5], which is the generalization of the 1D analytic signal to 2D and higher dimensions using the Riesz transform. As compared to the 1D analytic signal which allows the derivation of an energy and an independent phase term, a second phase term which encodes geometric orientation is also possible. Denote the bandpassed 2D input signal at scale s as \(b(\mathbf{r}, s)\), where \(\mathbf{r} \in \mathbb{R}^2\). Then, the monogenic signal is defined as \(M(b(\mathbf{r}, s)) = (b(\mathbf{r}, s), \mathbf{R}(b(\mathbf{r}, s)))\), where \(\mathbf{R}\) denotes the Riesz transform, and its components in 2D are denoted as \((r_1(\mathbf{r}, s), r_2(\mathbf{r}, s)) = (\text{Re}(\mathbf{R}(b(\mathbf{r}, s))), \text{Im}(\mathbf{R}(b(\mathbf{r}, s))))\). Local energy (LE), phase (LP) and orientation (LO) are local descriptors of the image that enable separation of the intensity dependent energy from structural phase information and orientation of the underlying geometry:

\[
LE : \mathcal{E} = \sqrt{b(\mathbf{r}, s)^2 + r_1(\mathbf{r}, s)^2 + r_2(\mathbf{r}, s)^2}
\]

\[
LP : \psi = \arctg \left( \frac{b(\mathbf{r}, s)}{\sqrt{r_1(\mathbf{r}, s)^2 + r_2(\mathbf{r}, s)^2}} \right)
\]

\[
LO : \phi = \arctg \left( \frac{r_2(\mathbf{r}, s)}{r_1(\mathbf{r}, s)} \right).
\]

The bandpassed signal \(b(\mathbf{r}, s)\) is achieved using the Mellor-Brady filter, which in 1D is defined as: \(f(r) = \frac{1}{\pi} \frac{\beta}{r^2 + \beta^2}\), where \(r\) is the distance from the centre of the filter, while \(\alpha\) and \(\beta\) are design parameters such that \(\beta \ll \alpha\) and ensures sharp cut off in the spatial domain. \(A\) and \(B\) are scalar values chosen to guarantee a zero mean value; \(\alpha = 2\) and \(\beta = 0.25\), while \(A\) and \(B\) are adjusted to these at each scale.

As described in the introduction, one way of representing image pixel information is through its intrinsic dimensionality (i0d, i1d, i2d) based on the variance of its local autocorrelation functions, i.e. the well known structure tensor approach (ST) pioneered by Granlund, Bigun and Knutsson [6]. This allows the quantification of variance by averaging the outer product of first order derivatives. One of the earliest measure of ‘1diness’ based on the ST is that of coherence which is the ratio of difference and sum of the two eigenvalues. This allows the differentiation of i1d and i2d structures based on some heuristic threshold values. One well known i2d detector that uses this approach is known as the Harris or Plessey corner detector [7].

The major drawback of the methods in use currently is the fact that real signals can not be exclusively classified in the three given categories. The reason being that no higher level contextual knowledge is available, noise is present and the intrinsic nature of real signals. This raises the need for a representation that allows continuous transition between these three discrete classes. The most promising method to solve this, was put forward by Felsberg [4] who proposed a triangular representation of the continuous id (c1d)
Fig. 3. Observation: continuous intrinsic 1D (ci1D) and absolute local orientation (|LO|) based local PDFs from patches of approximately 25 × 25 pixels are bi-modal or near bi-modal (h) for normal tendon and deviates from this if damage occurs. (a-b) grayscale image of the control and damaged tendon sample (the white rectangles indicate the region to which the presented ci1D and LO maps belong), (c-d) and (e-f) are the corresponding absolute LO and ci1D, and (g-h) shows the corresponding PDFs based on the LO and cid. To aid comparison (g) contains the PDF based on LO for both the control (blue, bold) and damaged (red), while (h) presents PDFs based on ci1D with the same colour coding.

Bi-modality of these local PDFs may be tested with several methods, such as those based on some form of entropy (proposed for foreground background separation), and also the Otsu algorithm. In this paper, confidence values obtained with the Otsu method based on the NP windows PDFs are shown. Results with alternative approaches can be obtained from the corresponding author upon request.

3. RESULTS

In this section we denote the PBS image as control and the papain digested sample as damaged (Fig. 2).

The observation that normal tendon tissue MRI is associated with a bi-modal distribution, and deviation from bi-modality marks damaged regions is demonstrated in Fig. 3. Here, for demonstration purposes only the region within the white rectangle (a-b) is illustrated. The difference between the control (c,e) and damaged (d,f) sample is believed to be due to ECM damage corresponding to disruption of tightly packed fascicles, and as such to the loss of coherence in absolute LO values (d) and reduction in the number and regularity of the ci1D structures (f).

The results in Fig.4 show the calculated ‘normality maps’ as the confidence values that the Otsu algorithm can find two separate clusters of the NP windows PDFs. This is calculated for each centre pixel (excluding the ones on the boundaries) of a local patch of the entire image available, and presented in Fig. 3 (a-b). A lower confidence value reflects a low belief in bi-modality, therefore high confidence in damage (blue area), whereas healthy tissue is the opposite of this (red). LO maps and confidence values are included here only for demonstration purposes; ci1D values that rely both on structural LP coherence and LO are expected to provide a higher fidelity measure.
Fig. 4. Results: (a-b) shows the absolute local orientation (|LO|), (c-d) presents the continuous intrinsic 1D (ci1D) probability maps, both of which are calculated for the entire images presented in Fig. 3 (a-b), (e-f) tendon ‘normality’ measure based on LO and (g-h) ci1D. Higher (red) values represent ideal/healthy tendon tissue, while the lower the value (blue) the higher the damage is. Note that the control sample also contains damaged regions, for explanation see text. Black circles on (b-c) indicate suspected damaged regions according to visual inspection, while white circles (g-h) show the ones indicated by our measure.

of ‘damage’, as compared to the intensity images presented in Fig. 3 a-b. The damage observed at the periphery of the control sample may have arisen as a result of tissue handling (Fig. 4).

The biological relevance of these findings is that disrupted ECM regions are reflected by irregular banding, where the signal likely corresponds to the connective tissues surrounding the fascicles. It is therefore possible that structural irregularity at the level of the ECM can be captured using MRI.

The clinical implication for such a method is the potential assessment of tendon tissue quality in vivo, for example in diagnosing a tendon injury or monitoring repair and ECM remodelling. Future work includes validation of our analysis method applied to larger datasets ex vivo, and evaluation of the proposed imaging tool on in vivo images. Ethical approval has been obtained to acquire clinical tendon images.

4. CONCLUSION

This paper has demonstrated that low level image processing techniques that capture the intrinsic characteristics of high-field MR images are a sensible approach to quantitatively assess tendon damage through its structural changes. The presented method is a prospective tool to diagnose tendon damage and/or monitor healing after injury in the clinic. Furthermore, it provides an insightful tool for probing the underlying ECM ultrastructure, and as such advances the basic scientific understanding of investigations in this field.

5. REFERENCES