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Torniainen, Jari E

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Estimating Mechanical Properties of Bovine Knee Ligaments and Tendons with Near Infrared Spectroscopy

Jari E. Torniainen, Aapo Ristaniemi, Lauri Stenroth, Juha Töyräs
Biophysics of Bone and Cartilage Research Group, Department of Applied Physics, University of Eastern Finland
jari.torniainen@uef.fi

Abstract: In this study, mechanical properties of bovine knee ligament and tendon samples were estimated using near infrared spectroscopy (NIRS). Properties related to sample stress-relaxation characteristics were found to be suitable for NIRS-based estimation.

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1. Introduction

Arthroscopic evaluation of the connective tissues of the knee (cartilage, meniscus and ligaments) is an important diagnostic technique for examining knee injuries and providing supplementary information about the tissue condition during repair surgeries. An estimate of the tissue condition is typically obtained through a visual inspection with an arthroscope and manual palpation with a metal hook. The evaluation of the tissue is based on the subjective interpretation of the orthopedic surgeon, thus impairing the reliability of the method.

Arthroscopy can be improved by utilizing near infrared spectroscopy (NIRS) for real-time analysis of tissue condition. NIRS is capable of non-destructively determining the chemical composition of biological samples. As mechanical properties of connective tissues correlate with their chemical composition, NIRS can characterize the overall condition and health of the tissue. Effectiveness of NIRS has already been demonstrated for cartilage [1] and meniscus [2] but not for ligaments. Quantified evaluation of ligament condition through NIRS could significantly improve comprehensive diagnostics of joints, such as knee and shoulder.

In this study, we tested NIRS for evaluating mechanical properties of bovine knee ligaments and tendons. NIRS and viscoelastic mechanical properties were measured from 40 samples. Variable selection methods were used to determine which mechanical variables can be reliably predicted with NIRS using partial least-squares regression (PLSR) models.

2. Materials and Methods

The four main ligaments (anterior cruciate, posterior cruciate, lateral collateral and medial collateral) and patellar tendon were extracted from eight bovine knee joints (age 12-24 months). Ligaments and tendons were cut into smaller dumbbell-shaped sample pieces in orientation parallel to the fascicles (12 mm in length, 2 mm wide and thick) using a custom-made punch. Subsequent NIRS and biomechanical measurements were performed with the samples submerged in phosphate buffered saline solution supplemented with enzyme inhibitors.

NIR absorption spectra were measured with a spectrometer (AvaSpec-NIR256-2.5-HSC, λ = 1000-2500 nm, resolution = 6.4 nm, Avantes BV, Apeldoorn, Netherlands) and a custom arthroscopic NIRS probe. Detector integration time was set to 20 ms and the acquired signal was averaged 100 times. Device was calibrated prior to each measurement using a standard reflector (Spectralon SRS-99, Labsphere Inc., North Sutton, USA). Measurements were taken from the surface of the sample at three equispaced locations. Absorption spectra were smoothed using a Savitzky-Golay filter (3rd order polynomial, filter window 39.3 nm) and the second derivative of the smoothed spectrum was used for the statistical analysis.

Viscoelastic properties of the samples were obtained from uniaxial stress-relaxation, sinusoidal and ultimate strength tests. The samples were fixed to a custom mechanical testing apparatus using custom grips. Force and displacement were measured and stress and strain were calculated based on sample dimensions. In total, 49 variables were produced by the mechanical testing. Full description of the mechanical variables is omitted here but an interested reader is directed to [3] for more details.
3. Statistical Analysis

Statistical analysis aimed to reduce the full set of mechanical variables to a subset which can be reliably predicted using NIRS. This process consisted of three steps: leave-one-out (LOO) analysis, spatial sensitivity analysis and five-fold cross-validation. The LOO analysis rejected mechanical variables where over 15% of individual samples were classified as outliers (Cook’s distance over 4/N, where N is the number of samples [4]). Spatial sensitivity analysis compared the PLSR model performance between three adjacent NIRS measurement sites. Variables where the median coefficient of determination over the three sites was below 50% were rejected. Finally, PLSR models were generated for the remaining variables using five-fold cross-validation. Models where the median cross-validated Pearson correlation coefficient was below 0.30 were rejected.

4. Results

Out of the 49 variables, 10 were selected for final analysis. The variables rejected during the elimination process were: 25 in LOO analysis, 9 in spatial sensitivity and 5 in cross-validation. Final PLSR models with calibration and cross-validation parameters are listed in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Components</th>
<th>$r_{\text{calibration}}$</th>
<th>RMSE_{calibration}</th>
<th>$r_{\text{CV}}$</th>
<th>RMSE_{CV}</th>
</tr>
</thead>
<tbody>
<tr>
<td>peak to equilibrium stress ratio at 4% strain</td>
<td>2</td>
<td>0.92</td>
<td>0.17</td>
<td>0.60</td>
<td>0.33</td>
</tr>
<tr>
<td>peak stress at 6% strain</td>
<td>2</td>
<td>0.89</td>
<td>0.30</td>
<td>0.56</td>
<td>0.34</td>
</tr>
<tr>
<td>equilibrium stress at 6% strain</td>
<td>2</td>
<td>0.89</td>
<td>0.29</td>
<td>0.56</td>
<td>0.34</td>
</tr>
<tr>
<td>peak to equilibrium stress ratio at 2% strain</td>
<td>2</td>
<td>0.90</td>
<td>0.23</td>
<td>0.56</td>
<td>0.32</td>
</tr>
<tr>
<td>equilibrium stress at 4% strain</td>
<td>2</td>
<td>0.89</td>
<td>0.26</td>
<td>0.55</td>
<td>0.32</td>
</tr>
<tr>
<td>peak stress at 4% strain</td>
<td>2</td>
<td>0.89</td>
<td>0.30</td>
<td>0.53</td>
<td>0.33</td>
</tr>
<tr>
<td>equilibrium stress at 2% strain</td>
<td>2</td>
<td>0.87</td>
<td>0.26</td>
<td>0.51</td>
<td>0.32</td>
</tr>
<tr>
<td>peak to equilibrium stress ratio at 8% strain</td>
<td>1</td>
<td>0.75</td>
<td>0.18</td>
<td>0.51</td>
<td>0.39</td>
</tr>
<tr>
<td>peak to equilibrium stress ratio at 6% strain</td>
<td>3</td>
<td>0.97</td>
<td>0.16</td>
<td>0.48</td>
<td>0.33</td>
</tr>
<tr>
<td>hysteresis percentage at 0.5 Hz</td>
<td>1</td>
<td>0.73</td>
<td>0.17</td>
<td>0.43</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Nine variables of the final models were obtained from the stress-relaxation part of the mechanical testing protocol. Models describing the ratio between peak and equilibrium stresses were retained for all four stress-relaxation steps (strains 2% - 8%), making it the most robust property for NIRS-based evaluation. Additionally, a single variable from the sinusoidal testing part corresponding to the hysteresis percentage at 0.50 Hz was also retained. Both peak-equilibrium stress ratios and sinusoidal hysteresis percentages relate to the viscous energy dissipation capabilities of the tissue. These viscoelastic properties change during a prolonged cyclic loading-unloading and can, therefore, act as indicators of decay or ultimate failure in the tissue [5]. Sensitivity towards the evaluation of these properties suggest that NIRS could be a viable diagnostics tool for determining ligament (and tendon) condition during arthroscopy.

References