Site-Specific Biomechanical Properties of Human Meniscus - Importance of Collagen and Fluid Nonlinearities

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Introduction: Meniscus distributes joint loads as well as stabilizes the knee joint during joint motion [1]. Meniscal tissue is adapted to joint loads by depth- and site-specific variation in composition and structure, e.g., in collagen fibril organization [2]. However, site-specific mechanical characteristics of the major tissue constituents (collagen, proteoglycan, fluid), and especially mechanical nonlinearity caused by these constituents, are not known. The aims of this study were to characterize fibril-reinforced nonlinear poroelastic mechanical properties of human meniscus in indentation testing and to ascertain whether the mechanical properties show site-specific variations.

Methods: Menisci (lateral and medial, n=26) (Fig. 1a) were obtained from the left knee joint of 13 human cadavers (mean age 53.5 years) and stored at -25°C until the experiment. Research was approved by National Agency of Medicolegal Affairs, Helsinki, Finland (permission 1781/32/200/01). Menisci were imaged with CT. Prior to the mechanical tests, menisci were thawed in water bath at room temperature (21°C) and then placed in an isotonic phosphate buffered saline (PBS) (pH 7.4) with enzyme inhibitors ethylenediaminetetraacetic acid (EDTA, VWR International, Radnor, PA, USA) and benzamidine HCl (Sigma-Aldrich Co., St. Louis, MO, USA). The measurement sites at the anterior, middle and posterior regions of meniscus (Fig. 1b) were marked with ink. Then, the thickness of the meniscus tissue at each measurement site was measured along the direction of indentation using a digital caliper. The whole meniscal tissue was then glued to the bottom of the measuring chamber filled with PBS. The measuring chamber was aligned to obtain perpendicular indenter-meniscus contact. The used steel indenter was cylindrical with a plane-ended tip (Ø=1.19 mm). Mechanical testing was conducted by using a custom-made material testing system [3] equipped with a high precision load cell (model 31/AL311AR, Honeywell, Columbus, OH, USA; resolution: 0.005 N) and an actuator (PM1A1798, Newport Corporation, Irvine, CA, USA; resolution: 0.1 µm). Using indentation stress-relaxation testing, four steps (5% of meniscus thickness/step) with a 100%/s strain rate and a <10 Pa/min relaxation criterion were applied. Indentations were carried out at the pre-marked locations (anterior, middle and posterior; Fig. 1b). In order to find out if the analyses of the mechanical properties of menisci can be simplified by axisymmetric model, a 3D geometry and finite element mesh of meniscus (Fig. 1b,c) were created from CT images and compared to an axisymmetric model (Fig. 1d) of a representative sample. Maximum reaction forces between the models (poroelastic) were comparable (<5% difference), thus, the axisymmetric analysis was preferred (simpler, faster) for further analysis. Then, the fibril-reinforced poroelastic material model was modified to include nonlinear characteristics of collagen [4, 5]. This novel nonlinear property for meniscus was based on our earlier experimental study [5]. Each site-
specific axisymmetric finite element model was fitted to the corresponding experimental curve by minimizing the mean square error between the simulated and experimental force curves (Fig. 1e). The optimized model parameters were the non-fibrillar matrix modulus ($E_m$), initial fibril network modulus ($E_o$), strain-dependent fibril network modulus ($E_ԑ$), initial permeability ($k_o$) and strain-dependent permeability factor ($M_k$).

**Results:** The non-fibrillar matrix modulus, initial fibril network modulus and initial permeability were lower than those typically reported for cartilage [4, 7], while the nonlinear properties in collagen fibrils and permeability ($E_ԑ$ and $M_k$) were within the range of values found earlier for cartilage [7, 9]. In lateral meniscus, only the non-fibrillar matrix modulus $E_m$ was significantly different between anterior and middle locations ($p<0.05$, Fig. 2a). There were no other site-dependent differences in any other model parameters of lateral meniscus (Fig. 2b-e). In medial meniscus, the nonlinear properties due to collagen fibrils and fluid ($E_ԑ$ and $M_k$) showed significant variations between locations (Fig. 2c, e). The strain-dependent fibril network moduli $E_ԑ$ between anterior and middle locations and between middle and posterior locations were significantly different ($p<0.05$). Also the strain-dependent permeability factor $M_k$ between anterior and middle locations was significantly different in lateral meniscus ($p<0.05$).

**Discussion:** For the first time, nonlinear, fibril-reinforced and poroelastic mechanical properties of human meniscus were determined using experimental indentation testing and computational modeling. Especially, a formulation for the nonlinearity of collagen fibrils [5] was applied here first for meniscus. Our results reveal that the mechanical nonlinearity of meniscus under indentation was related to both collagen fibrils and fluid. These nonlinearities were in the range reported for cartilage (e.g., [4]). The medial meniscus exhibited site-specific differences in the parameters related to nonlinear properties ($E_ԑ$ and $M_k$). The highest values were found at the anterior horn. The site-specific variations may relate to physiological loading, i.e., the anterior horn of medial meniscus is subjected to high stresses at the end of the stance phase of gait [8]. Therefore, stiffening of the meniscal tissue, as a result of nonlinearities linked to collagen and fluid, may protect meniscus from excessive strains. Furthermore, medial meniscus is less mobile than lateral meniscus during knee joint loading [6]. This may lead to more time- and site-dependent contact with femoral cartilage, while more mobile lateral meniscus may rather move along with the femoral condyle. In the lateral meniscus, only the non-fibrillar matrix (equilibrium) modulus showed site-specific variations (anterior vs. middle). This could be potentially due to greater load-bearing by the middle than anterior meniscus during static loading (e.g., standing). The initial fibril network modulus and initial permeability (parameters defined at contact) showed no statistically significant site-specific differences. Possibly, the most superficial layer of meniscus is relatively homogeneous, and thus, exhibits highly similar response to the applied load in all locations.

**Significance:** The present results show that intact human medial meniscus exhibits site-specific differences in the nonlinear mechanical properties, closely related to collagen and fluid. In lateral meniscus, only the nonfibrillar matrix showed site-specific variations in the mechanical characteristics. This information can be useful for computational knee joint models, addressing e.g. knee joint disorders, and when designing meniscal implants.
Fig. 1: A CT image of lateral meniscus (a). A 3D geometry and finite element mesh created from CT images; A=anterior, M=middle, P=posterior meniscal regions (b), and a zoomed portion (red circle in b) around the indenter (c). An axisymmetric finite element mesh (d) used for analyses (<5% difference in reaction forces from 3D geometry). A representative example of a stress-relaxation test with an optimized finite element (FE) model curve (e).

Fig. 2: Optimized values (Mean ±SD) of the non-fibrillar matrix modulus (a), initial fibril network modulus (b), strain-dependent fibril network modulus (c), initial permeability (d) and strain-dependent permeability factor (e) of lateral and medial menisci. Statistical analysis was conducted using a non-parametric Wilcoxon signed rank test (*p<0.05). A=anterior, M=middle, P=posterior.

Table 1: Optimized values (anterior, middle, posterior combined) of the fibril-reinforced nonlinear poroelastic parameters of human meniscus (Mean±SD).

<table>
<thead>
<tr>
<th></th>
<th>$E_m$  (MPa)</th>
<th>$E_r$  (MPa)</th>
<th>$E_p$  (MPa)</th>
<th>$k_r$ ($10^{12}$ m²/Ns)</th>
<th>$M_e$ (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral meniscus (n=32)</td>
<td>0.07±0.04</td>
<td>0.06±0.09</td>
<td>9.32±6.23</td>
<td>0.69±0.13</td>
<td>12.31±10.70</td>
</tr>
<tr>
<td>Medial meniscus (n=26)</td>
<td>0.08±0.03</td>
<td>0.06±0.10</td>
<td>14.58±10.85</td>
<td>0.88±0.07</td>
<td>13.14±7.76</td>
</tr>
<tr>
<td>All samples (n=58)</td>
<td>0.07±0.04</td>
<td>0.06±0.10</td>
<td>11.68±8.93</td>
<td>0.88±0.11</td>
<td>12.69±9.43</td>
</tr>
</tbody>
</table>

No significant differences between lateral and medial meniscus (p>0.05) (non-parametric Wilcoxon signed rank test).