INTRODUCTION
Engineered tendon scaffolds require enough mechanical strength, appropriate pore size and porosity for temporary mechanical support, cell infiltration and survival (Butler et al, 2000; Whitlocka et al, 2007). Meanwhile, tendon extracellular matrix (ECM) components play the important roles in modulating the biological activities of tenocytes or tendon stem cells (Bi et al, 2007). Few artificial and native scaffolds have been developed to satisfy the above criteria. In order to develop a novel scaffold to possess the sufficient mechanical strength, efficient cell seeding area and natural ECM micro-architecture of native tendons, we plan to investigate the scaffold made from acellular tendon slices. In present study, we determined the mechanical characteristics of native tendon slices to identify the minimum thickness of tendon slice which has the elemental mechanical characteristics of native tendon. We hypothesized that there would be mechanical alterations with varying thicknesses of native tendon slices.

METHODS AND PROCEDURES
Eight hind limbs were obtained from 4 dogs, which were euthanized for Institutional Animal Care and Use Committee approved studies. Three bundles of each Achilles tendon (AT) were dissected from one hind limb. Each bundle was fixed on a cryostat (Leica CM1850, Germany) with Tissue-Tek® optimal cutting temperature compound (Sakura Finetek USA, Inc., USA), and sliced with varying thickness of 100, 200, 300, 400, and 500 µm in succession. AT bundles (n=12) and AT slices (n=60) were used in present study. The ends of the AT bundles and slices were wrapped with saline-soaked sandpaper, and mounted into custom made grips on an uniaxial load frame (MTS 312, MTS Co., MA) for failure testing. Cross-sectional area and the distance between the grips were measured prior to testing with digital calipers. Bundles and slices were pre-loaded to 0.2 N, and then stretched to failure at a rate of 12 mm/min. Samples were kept moist during testing with PBS. Ultimate tensile stress (UTS), failure strain, and elastic modulus were calculated from the load and displacement data obtained from the test.

RESULTS
Typical stress-strain curves for one AT bundle and representative slices are shown in Figure 1. For the thicknesses of more than 300µm, the toe regions were apparent, followed by linear regions, which continued until ultimate failure. However, there seemed to be no toe region for 100µm thick slice.
The mean UTS and modulus values exhibited a gradual rise with increasing thickness (Table1). For 300µm thick slice, the UTS and modulus reached mean values of 70 and 77 per cent of AT bundles, respectively. Also, the failure strain of 300µm thick slice was remarkably higher than that of 100 or 200µm thick slice, reaching a mean value of 87 per cent of native AT bundles.

<table>
<thead>
<tr>
<th>Thickness (µm)</th>
<th>UTS (MPa)</th>
<th>Failure strain (%)</th>
<th>Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>17.5 (10.1)</td>
<td>9.3 (2.9)</td>
<td>230.3 (99.2)</td>
</tr>
<tr>
<td>200</td>
<td>27.3 (10.5)</td>
<td>9.3 (2.4)</td>
<td>351.9 (87.6)</td>
</tr>
<tr>
<td>300</td>
<td>40.9 (11.6)</td>
<td>16.0 (2.2)</td>
<td>375.2 (113.1)</td>
</tr>
<tr>
<td>400</td>
<td>42.1 (14.4)</td>
<td>16.4 (2.0)</td>
<td>378.8 (85.0)</td>
</tr>
<tr>
<td>500</td>
<td>55.1 (11.8)</td>
<td>16.5 (5.8)</td>
<td>468.8 (76.6)</td>
</tr>
<tr>
<td>AT bundle</td>
<td>58.3 (11.2)</td>
<td>18.3 (4.5)</td>
<td>484.7 (88.6)</td>
</tr>
</tbody>
</table>

Table1. Mechanical properties of AT slices and AT bundles (n=12 for each group).

**DISCUSSION**

The tendon is a fibrous connective tissue, consisted predominantly of type I collagen fibres. The collagen fibrils follow a natural periodic crimp, which is planar with respect to the alignment of the tendon structure. The dimension of fibres with crimp waveform is approximately 10-50µm. When the slice was cut with thickness of 100-200µm, there would be more or less damage to the crimp structure and some fibrils were likely to be broken off. Macroscopically, the slice with 100 or 200µm exhibited weak mechanical strength. This is mainly caused by adjacent fibre sliding and without the initial crimp straightening. In contrast, the slices with more than 300µm, equivalent to the fascicle level (50-400µm), retained most of the crimp structure and collagen fibrils. The mechanical properties of these slices are determined by the straightening of cramped collagen fibres and subsequent fibre slipping with increasing load. These slices showed a standard stress-strain curve similar with native AT bundles.

**SUMMARY**

This study demonstrated that mechanical characteristics of slices from AT bundles depended largely on the thickness of the slice. The slice with the thickness of 300 µm seemed to be the thinnest slice to keep the essential mechanical characteristics of the AT bundle. These findings are fundamental to the selection of tendon slices as engineered tendon scaffolds.

**ACKNOWLEDGEMENTS**

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**REFERENCES**