Ultrastructure Comparison between Fetal and Mature Porcine Anterior Cruciate Ligament Bundles

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Human ACL

Pig ACL

Gross Anatomy
Background

Human Anterior Cruciate Ligament of knee joint is composed of 2 bundles: anteromedial (AM) and posterolateral (PL) fibrous bundles and each bundle composed of cells and extracellular matrix in which Type I collagen is the major collagen.

Collagen provides the major resistance to mechanical forces, and may be expected that the collagen content and organization would be reflected in the mechanical properties of the structure.

Diameter and density of collagen structure are known to correlate with fibril functions.

Background

Prior studies have shown that the ultimate tensile strength of fibrils is greater in larger fibrils, while the interfibrillar binding, increases in the smaller fibrils.

It also indicated that collagen fibrils may have 2 different forms

(I) Heterogeneous large fibrils, parallely tightly packed, subjected to tensile stress along their axis
: Found in highly tensile structures such as tendons, ligaments and bone

(II) Homogeneous small fibrils, helically arranged, resisting multidirectional stresses
: Found within highly compliant tissues such as blood vessel walls, skin and nerve sheaths

Background

Functionally, the ACL provides stability to the knee by restraining anterior displacement of the tibia with respect to the femur and by providing resistance to rotational loading throughout the range of motion of the knee.

Due to the different functional roles of the two bundles, they would have distinct matrix composition and ultrastructure that would change during maturation.

The **gross appearance** of the ACL in human fetuses have 2 distinct bundles (AM and PL) which was similar to the adult ACL. On the contrary, differences are evident **histologically**.

There also differences in **ultrastructure** between both bundles of human adult ACL.

In mature porcine, ACL is composed of 3 bundles: **anteromedial, intermediate and posterolateral bundles**. However, there is no study of ultrastructural comparison between piglet and mature pig ACL.

Suzuki et al. ORS 2012.
Purpose

To evaluate and determine the age related ultrastructural changes between the anteromedial bundle (AMB), the intermediate bundle(IMB) and the posterolateral bundle (PLB) of the ACL in mature porcine and between fetal and mature pig ACL
Materials

• **5 knees of pig fetuses**
  - 8 weeks gestation
  - Same litter
  - 5 knees → 5 samples

• **5 knees of mature pigs**
  - Same age
  - 5 knees → 5 x 3 (AM, IM, PL)
  = 15 samples
Materials

- Transmission Electron Microscope (TEM) (JEM-1011 Electron Microscope)
- Acceleration voltage: 80 kV
- Magnification: 80,000x
- Randomly took 10 micrographs/specimen
- Chose 5 micrographs/specimen
Materials

- MetaMorph® Software
- Collagen fibrils measurement
Materials

• Created a 5x5 grids in each micrograph

• Randomly chose 5 from 25 grids from each micrograph for the measurement region
Materials

- **Ultrastructure**: Collagen fibrils
  - Collagen fibril diameter distribution
  - Mass average diameter (MAD)
    - MAD is defined as the diameter of the collagen fibril that contains the average mass present in the ACL
    - Correlated with mechanical strength
  - Collagen fibril index (CFI)
    - CFI is the percentage of area covered by collagen fibrils and represents the collagen- to- noncollagen ratio in the extracellular matrix.

\[
MAD = \frac{\sum_{i=1}^{N} n_i d_i^3}{\sum_{i=1}^{N} n_i d_i^2}
\]
Results

- Mature pig: AM bundle fibrils

AM bundle fibrils were arranged mostly in the longitudinal or unidirectional.
Results

• Mature pig: AM bundle fibrils

AM bundle were included both large and small diameter collagen fibrils, tightly packed together.
Results

• Mature pig: PL bundle fibrils

PL bundle fibrils were aligned in **multidirection**
Results

- Mature pig: PL bundle fibrils

PL bundle fibrils were only **small and uniform ones**, tightly packed together.
Results

- Mature pig: IM bundle fibrils

IM bundle fibrils were aligned in the multidirection
Results

• Mature pig: IM bundle fibrils

IM bundle were included small diameter collagen fibrils
Results

- Piglet : ACL

Piglet ACL bundle fibrils were arranged in the longitudinal of unidirection
Piglet ACL bundle fibrils were only small and uniform ones, not tightly packed together.
Comparison

AM bundle

PL Bundle

IM Bundle
### Comparison

#### AM bundle

<table>
<thead>
<tr>
<th>Mature Pig</th>
<th>Diameter (nm)</th>
<th>CFI (%)</th>
<th>MAD (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>281.88 ± 44.29</td>
<td>96.03 ± 2.73</td>
<td>356.21 ± 50.59</td>
</tr>
<tr>
<td>PL</td>
<td>167.67 ± 39.68</td>
<td>72.15 ± 12.85</td>
<td>196.20 ± 53.00</td>
</tr>
<tr>
<td>p-value</td>
<td>0.007 *</td>
<td>0.027 *</td>
<td>0.006 *</td>
</tr>
</tbody>
</table>

#### PL Bundle

<table>
<thead>
<tr>
<th>Mature Pig</th>
<th>Diameter (nm)</th>
<th>CFI (%)</th>
<th>MAD (nm)</th>
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</tr>
<tr>
<td>p-value</td>
<td>0.076</td>
<td>0.039 *</td>
<td>0.032 *</td>
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</table>

#### IM Bundle

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<tr>
<td>p-value</td>
<td>0.496</td>
<td>1</td>
<td>0.951</td>
</tr>
</tbody>
</table>
Comparison

AM bundle

PL Bundle

IM Bundle

Piglet ACL
Collagen diameter distribution

- Piglet
- AM
- PL
- IM
Collagen diameter distribution
### Comparison

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<td>Mean ± SD</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Piglet</strong></td>
<td>40.62 ± 10.11</td>
<td>0 *</td>
</tr>
<tr>
<td><strong>AM</strong></td>
<td>96.03 ± 2.73</td>
<td></td>
</tr>
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*Significant difference.*
Comparison

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<tr>
<th>Piglet ACL</th>
<th>IM bundle</th>
</tr>
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<tbody>
<tr>
<td><img src="image1.png" alt="Piglet ACL Image" /></td>
<td><img src="image2.png" alt="IM bundle Image" /></td>
</tr>
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Discussion

- Piglet ACL bundle
  - Collagen fibril diameter distribution
  - Piglet is unimodal diameter distribution

**Hypothesis:** There is no difference in ultrastructure between each ACL bundle of fetal porcine.
Discussion

- **Between Mature Pig**: AM, PL, IM
  - **Fibril alignment**
    - AM is unidirection alignment >> unidirectional stress
    - PL and IM are multiidirection alignment >> multidirectional stress
  - **Collagen fibril diameter distribution**
    - AM is a bimodal diameter distribution
    - PL and IM are unimodal diameter distribution
  - **MAD and CFI**
    - AM > IM = PL
  - **Tensile strength**
    - MAD is well correlated with mechanical strength.
    - The AMB could be assumed to be stronger than the PLB and IMB

**Hypothesis**: There are differences in ultrastructure between each ACL bundle of mature porcine ACL.
Discussion

• **Between Piglet and Mature pig AM,PL,IM**
  
  • *Fibril alignment*
    • Piglet is unidirection fibril alignment >> unidirectional stress
    • AM is unidirection fibril alignment >> unidirectional stress
    • PL and IM are multiidirection alignment >> multidirectional stress

• **Collagen fibril diameter distribution**
  • Piglet is unimodal diameter distribution
  • AM is a bimodal diameter distribution
  • PL and IM are unimodal diameter distribution
Discussion

- **Between Piglet and Mature pig AM, PL, IM**
  - **MAD and CFI**
    - AM > IM = PL > Piglet
  - **Tensile strength**
    - MAD is well correlated with mechanical strength.
    - The AMB could be assumed to be stronger than the PLB and IMB and they are all stronger than the Piglet bundle

**Hypothesis:** There were changes during maturation in the ultrastructure of ACL bundles from fetal to mature porcine
Conclusion

- From our study, we could not find the ultrastructural difference in piglet ACL bundles. However, there were differences in collagen fibril diameter distribution, CFI and MAD between each ACL bundles of mature porcine ACL.
- There are differences in Collagen fibril distribution, CFI and MAD between Piglet ACL bundles and Mature pig ACL bundles.
- There were changes during maturation in the ultrastructure of ACL bundles from fetal to mature porcine.
Limitation

• Piglet ACL bundles were too small and could not distinguish between each bundles

• We could not compare between each bundle of piglet and mature pig ACL

• Due to the small size of piglet ACL, It was difficult to orientate and dissect the specimen in order to get the mid portion of the bundle
Thank you so much for your attention

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