INTRODUCTION
Collagen fibers in ligaments and tendons enable these tissues to support tensile loads. Polarized light is a commonly used technique to describe the structure of collagen fibers in ligamentous and tendinous tissues. Immunohistochemistry and confocal microscopy are also used to visualize collagen structure, however, these techniques only allow viewing the two-dimensional form of the collagen fiber not the 3-D fiber orientation. Previous studies have suggested that the posterior ligamentous horn attachments of the menisci are exposed to both tensile and compressive loads whereas the anterior attachments are exposed to only tensile loads. This may result in differences in the orientation of collagen fibers between the two horn attachments. The goal of this study was to describe the 3-D collagen fiber structure in meniscal attachments using scanning electron microscopy (SEM).

METHODS
Six human knee samples were obtained from a national tissue bank (NDRI) with an age restriction between 45-65 yrs. Knees were dissected leaving approximately 2 inches of tibia and the menisci attached to it. Each attachment was transversely cut into 5-mm pieces (Figure 1). The samples were placed in fixative (2.5% glutaraldehyde in distilled water) for 24 hours at room temperature. After washing, the samples were immersed in a 2NaOH solution for 3-4 days and rinsed in distilled water for 2 days. Samples were incubated in a 1% tannic acid solution (buffered with 0.05M cacodylate pH 7.2) for 4-5 hours at room temperature and rinsed in distilled water for 24 hours. The samples were dehydrated in ascending concentrations of ethanol (30%, 50%, 70%, 80%, 90%, and 100%). After dehydration, each piece was immersed in liquid nitrogen and fractured using a razor blade to obtain a proper surface to be examined on the microscope. The samples were then dried using different concentrations of hexamethyldisilazane (HMDS). An 8-nm gold or platinum/palladium coating was applied to the samples to be viewed on the SEM. ANOVA was used to assess differences between attachments in crimping angle and crimping length (Figure 2d).

RESULTS

Table 1. Crimp parameters for collagen fiber orientation in human meniscal horn attachments. LA=Lateral Anterior attachment, MA=Medial anterior attachment, LP=lateral posterior attachment, MP=medial posterior attachment.

<table>
<thead>
<tr>
<th>Attachment</th>
<th>Crimp Length (μm)</th>
<th>Crimp Angle (deg)</th>
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<tbody>
<tr>
<td>LA</td>
<td>6.49 ± 2.34</td>
<td>27.00 ± 8.00</td>
</tr>
<tr>
<td>LP</td>
<td>6.91 ± 2.29</td>
<td>22.63 ± 9.71</td>
</tr>
<tr>
<td>MA</td>
<td>4.76 ± 1.95</td>
<td>19.44 ± 3.68</td>
</tr>
<tr>
<td>MP</td>
<td>3.72 ± 2.31</td>
<td>24.07 ± 3.82</td>
</tr>
</tbody>
</table>

There were no significant differences in either crimping length or crimping angle between different attachments.

DISCUSSION
The fiber bundles running parallel show that the meniscal attachments work mainly in tension carrying the hoop stress generated on the menisci as a consequence of the compressive forces. In this study it was found that some fibers run oblique to the fiber bundles, however, this could be an artifact caused by the freeze-fracturing process. Yahia et al., 1989 showed that there are two different fiber bundles, a planar and a helical. Figure 2(b) showed a planar form of the bundles in meniscal attachments, a common pattern seen in ligaments. The toe-region of stress-strain curves for ligaments and tendons is typically explained in terms of the uncrimping of collagen. Similar crimping parameters suggests a similar response in tension for small strains in meniscal horn attachments. This agrees with previous data from our lab showing that for small strain viscoelastic properties there were few differences between the attachments, and the 2D crimping wavelength was not significantly different between attachments. Fibroblast in lacunae were observed in the enthesis (Figure 2c). A dense network of fibrils between the lacunae is common in fibrocartilage of the enthesis in ligaments. The presence of fibroblast in the enthesis of ligaments is related with compressive forces elicit in the bony insertion.

This data suggests strong similarities in the collagen orientation between the anterior and posterior attachments. Therefore, when designing meniscal replacements it is likely that a single type of attachment could be utilized for both the anterior and posterior attachments. Additional work is underway to quantify the material properties of human meniscal horn attachments.

REFERENCES

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