Collagen expression in various degenerative meniscal changes: an immunohistological study

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ABSTRACT

Purpose. To examine changes in acid mucopolysaccharides and collagen expression during meniscal degeneration, tearing, and repair, using menisci excised from knee joint surgeries.

Methods. Menisci excised from 23 patients aged 15 to 80 years who underwent meniscal surgery for flap and bucket handle tears (n=11) and total knee arthroplasty (TKA) for osteoarthritis (n=12) were examined histologically. Staining images were converted to greyscale images to measure the mean grey levels, which indicated densitometry. Comparisons were made between acutely injured menisci and menisci with and without degeneration (from patients with osteoarthritis) in terms of acid mucopolysaccharides, collagen types I, II, and III expression.

Results. In menisci with no degeneration, acid mucopolysaccharides, collagen types I and II were expressed throughout the entire meniscus except for the circulating area. Collagen type III was intensely expressed at the exterior peripheral border and on the surface. During progression of meniscal degeneration, the expression of acid mucopolysaccharides increased, and the expression of collagen types I, II, and III decreased. In acutely injured menisci, collagen types II and III disappeared first, followed by collagen type I, resulting in the abrogation of fibre construction.

Conclusion. In normal menisci, acid mucopolysaccharides and collagen types I, II, and III were well-balanced, and meniscal function was maintained. When the limits of repair were exceeded, the meniscus tissue deteriorated owing to the disappearance of collagen types II and III and a decrease in collagen type I, resulting in the abrogation of meniscus fabric construction.

Key words: cartilage; collagen; glycosaminoglycans; medial collateral ligament, knee

INTRODUCTION

Both meniscectomy and meniscal suturing for injured menisci yield good results. Nonetheless,
osteoarthritic changes may occur after meniscectomy owing to the loss of meniscal function including alterations in load distribution, impact absorption, and articular sliding and stabilisation.\textsuperscript{3,7,10} The loss of meniscal function may lead to mechanical wearing of the articular cartilage and degeneration of menisci.\textsuperscript{3,6,10} Intra-articular injection of mesenchymal stem cells for osteoarthritis and repair of injured menisci has been studied.\textsuperscript{11,12} When menisci lose normal function, regeneration may dilapidate.\textsuperscript{4,10,13–17} This study examined changes in acid mucopolysaccharides and collagen expression during meniscal degeneration, tearing, and repair, using menisci excised from knee joint surgeries.

**MATERIALS AND METHODS**

Menisci excised from 23 patients aged 15 to 80 years who underwent meniscal surgery for flap and bucket handle tears (n=11) and total knee arthroplasty (TKA) for osteoarthritis (n=12) were examined histologically.

Paraffin sections of the menisci were prepared and stained with haematoxylin and eosin (H&E), Alcian blue, and immunohistochemical staining using the LSAB+HRP (DakoCytomation) method and anti-collagen types I, II, and III antibodies. The specimens were deparaffinised with xylene, and hydrated in a descending series of ethanols. After being soaked in Proteinase K, the specimens were rinsed repeatedly with Tris-buffered saline with Tween 20 (TBST) and then blocked. After being incubated with the anti-collagen types I, II, and III antibodies and rinsed with TBST, the specimens were labelled using biotin-labelled secondary antibodies. Then, after being rinsed with TBST and soaked in peroxidase-labelled streptavidin solution, the specimens were rinsed again with TBST and soaked in 3,3’-diaminobenzidine-tetrahydrochloride solution. They were then rinsed with sterile water, before being counterstained with haematoxylin, rinsed under running water, dehydrated with an ascending series of ethanols, penetrated with xylene, and embedded in paraffin. The tissues were then examined under a light microscope.

Staining images were converted to greyscale images to measure the mean grey levels, which indicated densitometry. Comparisons were made between acutely injured menisci and menisci with and without degeneration (from patients with osteoarthritis) in terms of acid mucopolysaccharides, collagen types I, II, and III expression (Table).

**RESULTS**

In menisci with no degeneration, collagen type I expression varied from subject to subject, whereas collagen types II and III expression was similar. Collagen types I and II were expressed throughout the entire meniscus (except for the peripheral vascular area) and intensely expressed in the circumferential and radial fibres (Fig. 1). Collagen type III was intensely expressed at the exterior peripheral border, on the meniscal surface, and along the vessels in the vascular area of the meniscus. However, it was not expressed in any of the internal regions of the meniscus that expressed collagen type II. Acid mucopolysaccharides were consistently observed on the surface of the meniscus and along the vessels in the vascular area of the meniscus, as well as in the internal regions of the meniscus that expressed collagen type II.

In menisci with degeneration, tear formation and fibrillation at the injury site was detected by H&E staining. Using immunohistochemical staining, at the injury site, expression of collagen types I, II, and III (particularly type I) was decreased, and acid mucopolysaccharides expression slightly increased (Fig. 1). In mildly degenerative menisci, collagen type II expression was upregulated as part of the reparative process. In severely degenerative menisci, collagen types I, II, and III tended to be absent, and acid mucopolysaccharides expression was preserved with various degrees of attenuation. Collagen type II expression tended to increase in mildly degenerative

<table>
<thead>
<tr>
<th>Meniscus</th>
<th>Acid mucopolysaccharides</th>
<th>Collagen type I</th>
<th>Collagen type II</th>
<th>Collagen type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>No degeneration (n=2)</td>
<td>20.13</td>
<td>151.08</td>
<td>83.77</td>
<td>83.27</td>
</tr>
<tr>
<td>Degenerative (n=6)</td>
<td>29.83±8.78</td>
<td>20.13±13.30</td>
<td>32.22±22.99</td>
<td>22.92±13.27</td>
</tr>
</tbody>
</table>
menisci and decrease in severely degenerative menisci.

In acutely injured menisci, hyalinisation, myxoid degeneration, and metaplasia were detected at the ruptured site by H&E staining. Using immunohistochemical staining, expression of collagen types II and III were markedly downregulated and often absent in the hyalinising regions. Collagen type I was present, but its expression had various degrees of attenuation. In the regions of myxoid degeneration and necrosis, collagen types I, II, and III were all absent, and fabric construction was abrogated (Fig. 2). Collagen types II and III were expressed in the cartilaginous metaplastic area, where reparative changes occurred in the avascular area.

In acutely injured and degenerative menisci, acid mucopolysaccharides expression was the same or slightly higher, and collagen types I, II, and III expression was markedly lower, compared with menisci with no degeneration (Table).

**DISCUSSION**

Degeneration of the articular cartilage and meniscal injuries are the main pathological manifestations of osteoarthritis, which can be caused by genetic predisposition, bone metabolism, and hormone abnormality.\(^{5,18-21}\) In older patients with meniscal injuries who have few osteoarthritic changes on radiographs, the degenerative changes in their articular cartilage are often mild, whereas patients with meniscal injuries secondary to decline in

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**Figure 1**  Menisci excised from patients with osteoarthritis showing (a) no, (b) mild, (c) severe, and (d) very severe degeneration.
meniscal quality have more severe articular cartilage damage. In young patients with no meniscal injuries who undergo 2-stage reconstruction for an anterior cruciate ligament injury, articular cartilage defects are sometimes noted to be filled with regenerated cartilage. Therefore, menisci have great effects on injuries, degenerative changes, and reparation of articular cartilage in the knee joints.

Articular cartilage consists of 2% cartilage cells and an abundant extracellular matrix, which is composed of 70% water, 20% collagen, and 10% cellular components. Collagen type II is the main type of collagen in articular cartilage, but collagen types VI, IX, X, XI, etc are also present. The meniscus is a fibrocartilage and contains an abundant extracellular matrix. Collagen type I is most commonly found in the meniscus, which also contains collagen types II and III and acid mucopolysaccharides. There have been many morphological and histochemical studies of menisci.4,10,13-15,23,24

Meniscal injury may involve ruptures, degeneration, reparation, and a combination of these.4,13,15 Ruptures involve collagen fibre bundle disruption. Degeneration involves a decrease in cell density, cell loss, abnormal bundle formation, and necrosis. Reparation involves cell coverage of the tissue surface, granulatative changes accompanied by neoangiogenesis at the injury site, cell proliferation in the avascular region, and cartilaginous metaplasia by proliferating cells. These tissue changes can be detected histologically using H&E staining, but variations in collagen expression associated with such tissue changes remain unknown. In our study, immunohistological examinations detected attenuated collagen type I expression at meniscal tear sites. The lack of fibre bundle clarity was caused by the disappearance of collagen types II and III and a decrease in collagen type I. Moreover, myxoid degeneration and necrosis were induced by a reduction in fibre construction owing to the disappearance of collagen types I, II, and III.

In our study, in menisci with no degeneration, acid mucopolysaccharides and collagen types I, II and III were in a good balance, and meniscal function was maintained. In degenerative menisci from patients with osteoarthritis, the expression of acid mucopolysaccharides was increased, and the expression of collagen types I, II and III decreased. If degeneration is mild, the meniscus can be repaired (mainly via upregulation of collagen types II expression), and its function can be maintained. If the limit of repair is exceeded, the meniscal tissue deteriorates secondary to the disappearance of collagen types II and III and a decrease in collagen type I, resulting in abrogation of fabric construction. This suggest that abnormalities of collagen metabolism play a role in osteoarthritis.

In the ruptured site of the acutely injured meniscus, collagen types II and III disappeared first, followed by collagen type I, resulting in the abrogation of fibre construction. If a tear occurs in the peripheral vascular area, collagen types I, II, and III can be produced by the remaining meniscal cells or via the invasion of undifferentiated mesenchymal cells, full recovery of meniscal function may be resulted. However, if a tear occurs in the avascular portion, no collagen is produced via the invasion of undifferentiated mesenchymal cells, and therefore meniscal function of the ruptured fragment cannot be maintained, as most meniscal cells have disappeared. If any meniscal
cells remain, only minimal collagen types II and III are produced, resulting in poor results from meniscal suturing.

DISCLOSURE

No conflicts of interest were declared by the authors.

REFERENCES