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Interactions of meniscal cells with extracellular matrix molecules

Towards the generation of tissue engineered menisci

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Menisci are one of the most commonly injured parts of the knee. Conventional surgical interventions are often associated with a long-term increased risk of osteoarthritis. Meniscal tissue engineering utilizes natural or synthetic matrices as a scaffold to guide tissue repair or regeneration in three dimensions. Studies have shown that a diverse cellular response can be triggered depending on the composition of the surrounding extracellular matrix (ECM) components. As such, attempts have been made to replace or repair meniscus defects using tissue grafts or reconstituted ECM components prepared from a multitude of tissues. This commentary summarizes the most recent data on the response of meniscal cells to ECM components, both in vivo and in vitro, and focuses on their potential roles in meniscal repair and regeneration. We also discuss our recent investigations into the interactions of meniscal cells and a self-assembled biomimetic surface composed of meniscal ECM molecules. The biological effects conferred by the biomimetic surface, in terms of cell adhesion, proliferation, gene expression profiles and matrix synthesis, were evaluated. Finally, some suggested directions for future research in this field are outlined.

Key words: meniscus, tissue engineering, extracellular matrix, cell-matrix interactions, chondroitin sulfate, collagen

Abbreviations: TGFβ, transforming growth factor-beta; BMP-2, bone morphogenetic protein-2; PDGF, platelet-derived growth factor; IGF-1, insulin-like growth factor-1; FGF, fibroblast growth factor; ECM, extracellular matrix; GAGs, glycosaminoglycans; Sox-9, (sex determining region Y)-box 9 transcription factor

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The meniscus is a C-shaped fibrocartilaginous tissue positioned within the knee joint that plays a chondroprotective role through load bearing, shock absorption and joint lubrication.[1-3] Normal human menisci is composed of 72% water, 22% collagen and 0.8% glycosaminoglycans (GAGs), with chondroitin-6-sulfate being the major GAG constituent.[4] In contrast to articular cartilage, where the predominant collagen is type II, collagen I is expressed throughout the meniscus and accounts for >90% of its collagen content.[5] Collagen II, on the other hand, is only detected in the inner region. A small quantity of collagen III, -IV, -V and -VI is also found within the meniscus.[5-7] It is suggested that interactions among collagen, proteoglycans and water accounts for the ability of meniscus to resist compressive load.[3] The cellular components of meniscal tissue include elongated fibroblast-like cells located at the outer vascular region of the tissue and round fibrochondrocytes that are interspersed within the middle and inner region.[8,9] These cells are responsible for synthesizing and maintaining the ECM components, especially the collagen.[10,11] A schematic diagram of the cellular and matrix components of the meniscus is shown in **Figure 1**.

Menisci are one of the most commonly injured parts of the knee, accounting for a total number of 850,000 surgeries performed in the United States each year.[12] Although conventional surgical interventions, including meniscectomy and suture fixation, appear to relieve pain symptom in the short term, long-term follow-ups suggest that these treatments are associated with an increased risk of osteoarthritis.[13-15] The bottom line is that none of these procedures initiate repair or regeneration of the injured meniscus. This situation is worsened by the fact that the inner two-thirds of the meniscus is avascular.[6,16] Without serum-derived factors to trigger the normal healing process and a provisional scaffold for cell migration

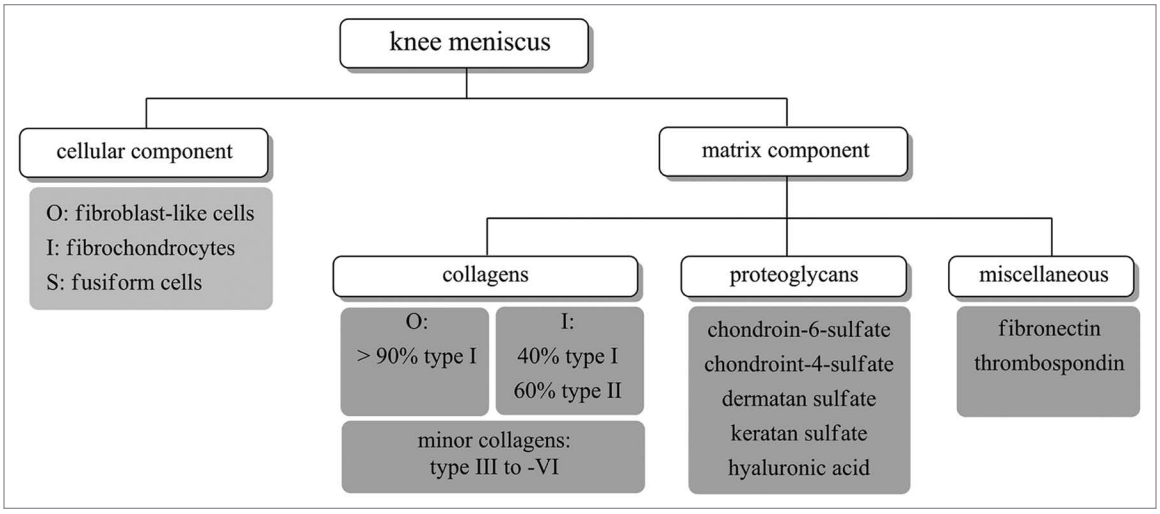

Figure 1. A schematic diagram showing the cellular and matrix components found in knee menisci. O, outer one-third of the tissue; I, inner two-thirds of the tissue; S, superficial zone.

into the defect site, cells within this region are deprived of their intrinsic reparative response to injury.[17,18]

Meniscal tissue engineering utilizes natural or synthetic matrices as a scaffold to guide tissue repair or regeneration in three dimensions. This can be attained via recruitment of cells from adjacent tissues (the meniscal remnant and the synovial membrane), or generation of neo-fibrocartilage in vitro by growing cells in the scaffold prior to implantation. Regardless of the strategies employed, a permissive microenvironment for cell attachment, proliferation and matrix synthesis should be created. Numerous studies have demonstrated the positive effect of growth factors (TGFβ, BMP-2, PDGF, IGF-1, FGF, etc.)[19-22] low oxygen tension[22,23] and cell-cell interactions[24,25] on meniscal tissue regeneration.

Growing evidence indicates that, through its dynamic interactions with growth factors and degradative enzymes, the ECM microenvironment plays an essential role in regulating cell behaviors (reviewed in refs. 26–28). As such, attempts have been made to replace or repair meniscus defects using tissue grafts, including periosteal membrane,[29] small intestinal submucosa,[30] tendon[31] and decellularized meniscus.[32,33] The main drawback with all of these tissue grafts is that their initial material properties (e.g., strength, geometry, architecture) cannot be tailored to match those of native meniscal tissue. Despite the fact that cell repopulation was evident in most of these cases, the repair process was often incomplete, with continual progression of degenerative changes and graft shrinkage being reported in some cases. The possibility of disease transmission and tissue shortage also limits their applicability. Alternatively, ECM molecules can be extracted from a multitude of tissues and reconstituted according to the desired requirement, thus allowing examination of their individual effects on cell behavior. These molecules are typically presented to cells in the form of three-dimensional (3D) scaffolds, and to a lesser extent, as surface coatings or in a soluble (free) form.

Several candidate ECM molecules, mostly constituting the matrix of the native meniscus, are of interest for meniscal tissue engineering (**Table 1**). Among these is hyaluronic acid (HA), a non-sulfated GAG component found in the meniscus.[34] Improvement in meniscal healing with intra-articular injection of HA solution has been documented in both rabbit and canine models.[35,36] The exact mechanism for this therapeutic effect remains unclear, although the suppression of local nitric oxide production and proteoglycan degradation appear to play a role.[37,38] In vitro, the solubilized HA exerted a mitogenic response on human meniscal cells in a dose-dependent manner without altering their appearance and chondroitin sulfate secretion.[11] In contrast, ovine fibrochondrocytes grown on a HA-based scaffold showed a decrease on growth rate over a 4-week period.[39] In our experience, poor cell attachment and proliferation are also consistently observed in rat meniscal cells that are seeded on a bare HA surface. Although no direct comparison is available, it is tempting to speculate that the way in which the HA molecules are presented to the cells might affect the cellular response and hence be related to the reported discrepant effects on meniscal cell behavior.

The most extensively investigated matrix molecule in meniscal tissue engineering thus far is collagen I, owing to its abundance in the tissue and ease of reconstitution. Besides its major role as a structural protein to maintain meniscal integrity, collagen also provides binding sites for adhesion molecules involved in cell attachment or in the organization of the ECM.[3,7] Walsh et al.[29] reported neo-fibrocartilage formation in a rabbit partial meniscectomy model using a collagen I sponge pre-seeded with bone marrow-derived mesenchymal stem cells (bmMSCs). Without pre-seeding with bmMSCs, the collagen sponge was repopulated only with fibroblastic cells after six weeks. Degenerative changes were still present in both experimental groups, indicating that the mechanical properties of these constructs were overall suboptimal.

Apart from these examples, collagen-based copolymers have also been investigated. In the 1990s, the group of

Table 1. The response of meniscal cells to ECM components

ECM components	Cell source	In vitro or in vivo	Treatments	Time	Results	References
Soluble (free) form						
Hyaluronic acid (HA)	-	Rabbit model with a cylindrical meniscal defect	1% solution of HA was injected into the joint once per week.	6 weeks	• Increased rate of healing. • The cell population changed from fibroblast-like cells to chondrocyte-like cells.	35
HA	-	Rabbit model with anterior cruciate ligament (ACL) deficiency	The HA was injected into the joint once per week.	5 weeks	• Stimulated collagen remodeling in peripheral zone.	36
HA	Human meniscus	In vitro	Passage 0 (P0) cells were cultured as monolayers in DMEM/F12 with 10% FBS overnight before treated with the solubilized HA.	5 days	• Enhanced cell proliferation in a dose-dependent manner. • Maintained the cellular morphology • No effects on the chondroitin sulfate production.	11
Scaffolds						
Fibrin clot	-	Canine model with avascular meniscal defect	Circular defects (2 mm in diameter) were filled with an exogenous fibrin clot that had been prepared from the animal.	6 months	• Formation of tissue that is morphologically similar to the reparative tissue in the vascular meniscal defects.	18
Fibrin gel	Autologous fibrochondrocytes	Lapine model with full thickness defect	2-week in vitro culture of cells before transplantation.	4 weeks	• Cell proliferation with matrix synthesis • Neo-tissue formation.	54
Collagen I (allogenic devitalized meniscus)	Ovine fibrochondrocytes	In vitro	Cells at P3 were seeded onto the allograft and maintained in DMEM with 10% FBS and 1% penicillin and streptomycin.	4 weeks	• Clusters of elongated fibroblast-like cells.	55
Collagen I	-	Rabbit model with partial medial meniscectomy	The defect (6–7 mm in length) was filled with a collagen I sponge.	24 weeks	• Repopulated with fibroblast-like cells. • Presence of tendonous collagen fibers.	29
Collagen I (CMI, Regen Biologics, USA)	Autologous fibrochondrocytes	Sheep model with meniscectomy	3-week in vitro culture of cells before transplantation.	12 weeks	• Enhanced vascularization, tissue remodeling and ECM production. • Graft shrinkage: - Pre-seeded: ~22% - Non-seeded: ~44%	56
Collagen I (Menaflex CMI, Regen Biologics, USA)	-	Human patients with partial medial meniscectomy	The implant was sutured to the meniscus remnant with non-absorbable and an inside-out technique.	1-year relook	• Neo-fibrocartilage formation. • 45–58% defect filling. • No apparent graft shrinkage. • No chondroprotective effect on patients with the newly injured knee.	43

Abbreviations: DMEM/F12, Dulbecco's modified Eagle's/Nutrient Mixture F-12 culture medium; FBS, fetal bovine serum; CMI, collagen meniscus implant.

Stone et al.[40,41] fabricated a copolymeric collagen scaffold that facilitated cell migration, proteoglycan synthesis and regeneration of meniscus in dogs. The scaffold was then used as a prototype for the development of the ReGen Biologics Menaflex® Collagen Meniscus Implant (formerly CMI®), which is the only commercially available graft for medial meniscus replacement following partial meniscectomy. It is composed mainly of bovine collagen I (>97%) with the remaining components being GAGs (chondroitin sulfate and hyaluronic acid).[42] The randomized clinical trial revealed 45–58% of the defect being filled after one year, with the formation of biomechanically competent neo-fibrocartilage in patients with

Table 1 (continued). The response of meniscal cells to ECM components

ECM components	Cell source	In vitro or in vivo	Treatments	Time	Results	References
Collagen I-GAG	Bovine fibrochondrocytes	In vitro	Cells at P5 were seeded onto the scaffold and maintained in DMEM/F12 containing 10% FBS, 1% penicillin/streptomycin and ascorbic acid.	3 weeks	• Dense cell population at the margins. • The cell number remained unchanged. • Increased GAG production (3-fold). • Shrinkage (54%)	44
Collagen II-GAG					• Cells distributed throughout the scaffold. • Enhanced cell proliferation. • Increased GAG production (7-fold). • Shrinkage (12%).	
Collagen II/I,III (Geistlich Biomaterials, Switzerland)	Ovine meniscal cells	In vitro	Cells at P2 were seeded onto the scaffold and maintained in DMEM/F12 containing 10% FBS, 1% penicillin/streptomycin and ascorbic acid.	4 weeks	• No cell proliferation. • Predominance of polygonal shape cells. • Cell migration into the scaffold. • Collagen I and GAG production. • Absence of collagen II production. • More cells were found in the collagen II layer.	39
HA (Hyaff-11, Fidia Advanced Biopolymers, Italy)					• Similar results as above, except a decrease in cell number.	
Surface coatings						
Collagen I or aggrecan	Bovine knee fibrochondrocytes	In vitro	• Primary and passaged (P1-P4) cells were seeded on tissue culture plate coated with the ECM molecule. • Maintained in DMEM/F12 containing 10% FBS, 1% penicillin/streptomycin and ascorbic acid.	1 day	Both coatings: • downregulate collagen I gene expression. • upregulate COMP gene expression. • could not reverse loss of collagen II gene expression. • have no significant effects on aggrecan gene expression.	52
Collagen I/II (at a 2:3 ratio)	Rat meniscal cells	In vitro	• Cells at P3 were seeded on coverslips coated with a precursor coating of hyaluronic acid/chitosan multilayers with covalently immobilized ECM molecules. • Maintained in DMEM/F12 containing 10% FBS and 1% penicillin/streptomycin.	2 weeks	• Enhanced cell proliferation. • Upregulation of aggrecan gene expression. • No significant effects on collagen II gene expression.	46
Collagen I/II and chondroitin-6-sulfate					• A slower cell proliferation rate. • Upregulation of aggrecan, Sox-9 and collagen II gene expression. • Increased GAG production.	

Abbreviations: DMEM/F12, Dulbecco's modified Eagle's/Nutrient Mixture F-12 culture medium; FBS, fetal bovine serum; CMI, collagen meniscus implant.

prior meniscal repair.[43] The chondroprotective effect of the implant, however, was absent in recently injured patients.

Mueller et al.[44] employed various porous collagen-GAG (chondroitin sulfate) matrices as a scaffold to investigate their effect on the growth and biosynthetic activity of seeded calf meniscal cells. Compared to the collagen I-GAG scaffold, the collagen II-GAG matrix displayed greater scaffold integrity and produced higher cell proliferation, as well as GAG production, consistent with a previous finding using canine articular chondrocytes.[45] In a collagen-based composite sponge, it was also observed that more cells were attached to the collagen II layer compared to the collagen I/III layer.[39] Whether these effects are the result of direct cell-matrix interactions remains to be resolved. Regardless, the differential responses of meniscal cells in these matrices comprised of different collagen types are highly likely to be related to the difference in the structural properties of the resultant scaffold.

We have recently demonstrated that primary rat meniscal cells rapidly dedifferentiated during monolayer expansion on standard tissue culture plastic; the cells acquired a fibroblastic-like morphology and expressed high levels of collagen I, low levels of collagen II and proteoglycans, a gene expression profile opposite to that of native meniscal tissue.[46] We also showed that this process could be reverted by culturing the cells on an engineered surface that is mimetic of the native meniscal ECM microenvironment. The surface is comprised of a precursor coating of hyaluronic acid/chitosan (HA/CH) mutilayers (a bioinert surface) to which major ECM components in the avascular region of the meniscus, namely collagen I/II (at a 2:3 ratio) and chondroitin-6-sulfate, were covalently immobilized (hereafter referred to as the C6S surface) (**Fig. 2**).

In our study, the initial attachment and growth rate was slower for cells seeded on the C6S surface compared to the collagen I/II-coated surface (hereafter referred to as the COL.I/II surface). A previous study, comparing effects of various ECM molecules on adhesion of chondrocytes, ligament cells and mesenchymal stem cells, showed that both collagen I and -II coatings induced greater cell adhesion, while HA, chondroitin sulfate and aggrecan served to inhibit this behavior.[47] Enhanced cell adhesion and growth over native collagen was reported by Srivastava et al.[48] when chondroitin sulfate was incorporated into collagen films. The absence of this enhancing effect on the meniscal cells, as observed in our study, may be due to the inverse relationship between proliferation and differentiation, in which differentiation (or "redifferentiation" in our case) is usually accompanied by decreased proliferation.[49,50] On both surfaces, the cells regained their differentiated appearance, and started to lay down an extensive ECM over time.

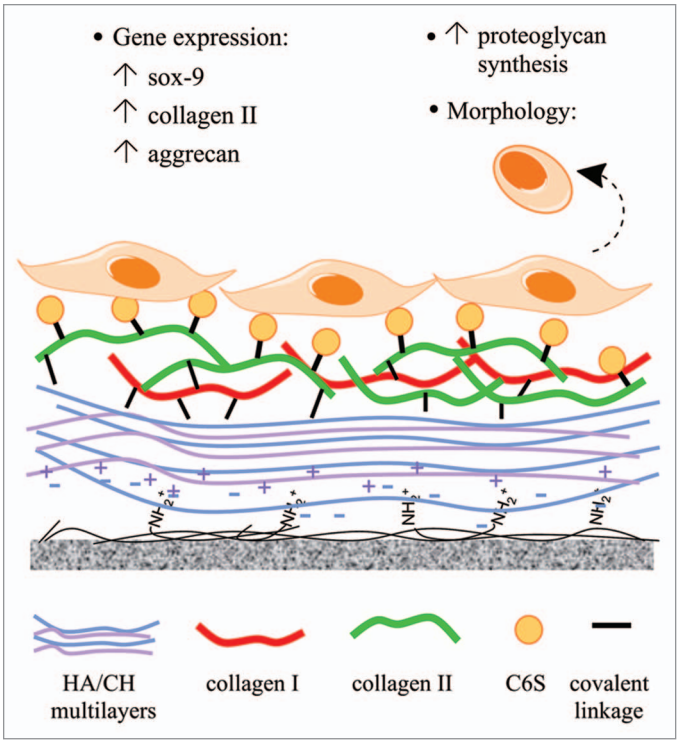

Figure 2. A schematic diagram showing the interaction of meniscal cells and a biomimetic surface comprised of a precursor hyaluronic/chitosan (HA/CH) film and major ECM molecules found in the native meniscus. C6S, chondroitin-6-sulfate.

In vitro culture of the dedifferentiated meniscal cells on the COL.I/II surface resulted in an upregulation of aggrecan gene (11-fold), although there was no significant effect on their collagen II expression levels. The inclusion of immobilized chondroitin-6-sulfate molecules on the surface (C6S surface) was found to enhance the cellular response, in which a dramatic upregulation of aggrecan (43-fold) and collagen II (8-fold) gene expression was noted. Simultaneously, these cells produced nearly three times more sulfated GAGs/DNA than on the COL.I/II surface. The upregulation of collagen II gene expression in meniscal cells by chondroitin-6-sulfate molecules could have been mediated via Sox-9 [(Sex determining region Y)-box 9] transcription factor, as an accumulation of Sox-9 mRNA preceded the expression of collagen II. In contrast, on the COL.I/II surface, where no upregulation of collagen II gene expression was detected, a low level of Sox-9 mRNA was noted throughout the experimental period. It has been reported that Sox-9 is strongly expressed in mesenchymal condensation preceding cartilage formation, as well as during chondrocyte differentiation in mouse embryos.[51] A study by Gunja et al.[52] demonstrated that collagen I and aggrecan (a chondroitin sulfate-rich proteoglycan) coatings were able to revert collagen I and cartilage oligomeric matrix protein (COMP, a chondrocytic marker), but not collagen II expression levels in dedifferentiated bovine fibrochondrocytes. Altogether, the findings of both of

these investigations highlight the possibility to modulate gene expression in meniscal cells via cell-matrix interactions.

Tissue engineering holds great promise for meniscal repair and regeneration. Compared to other musculoskeletal tissues, such as cartilage and bone, relatively little is known about the biology of meniscal cells and their interactions with the native microenvironment. A systematic investigation of the effects of ECM components on meniscal cell behavior is thus vital to gaining a greater understanding of cell-material interactions and the requisite material inputs for optimal scaffold design. Another emergent area of interest and importance is the mechanism(s) involved in the response of meniscal cells to ECM molecules. For example, Lee et al.[53] have shown that the articular chondrocyte attachment to collagen II, achieved partly via β1 integrin engagement, enhanced subsequent TGFβ-induced cell growth and proteoglycan synthesis. Much work remains to be done in this area with menisci. Last but not least, the effect of mechanical stimulation, at both macroscopic (tissue)[54,55] and microscopic (cellular) levels,[56] on meniscal cell behavior, is another important parameter to consider when developing a tissue engineered meniscus. Given the complexity of the in vivo microenvironment, an optimal outcome for meniscal tissue engineering will likely require a tailored combination of all of these microenvironmental factors.[22,23,55]

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