Tissue-Engineered Meniscal Constructs

Andrew J. Schoenfeld, MD, William J. Landis, PhD, and David B. Kay, MD

Abstract

The medial and lateral menisci play important roles in knee biomechanics, kinematics, and stability. Unfortunately, these structures are prone to damage and, because of a tenuous blood supply, have great difficulty healing. Many interventions have been proposed for treatment of damaged meniscal tissue, but most surgical options are fraught with difficulties, from continued osteoarthritic degeneration to potential for disease transmission. The field of tissue engineering has made wide inroads into constructing meniscal tissue. Investigations involving collagenous tissue, meniscal fibrochondrocytes, chondrocytes, synthetic scaffolds, and gene therapy have all been reported in the literature. Despite these advances, however, more work needs to be done, including incorporating concepts and applications from other engineering disciplines, to potentiate the possibility of a tissue-engineered meniscus that approximates native tissue. In particular, the histologic, morphologic, and biomechanical properties of tissue-engineered meniscal constructs must be better understood to facilitate this goal.

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The medial and lateral menisci, the 2 fibrocartilaginous disks located on the outer margins of the tibial plateau, play a vital role in direct load transmission and in the restraint mechanism of the human knee. Unfortunately, these structures are also the most likely to be injured and the least likely to heal. Meniscal tears, cysts, and degeneration are evident in every demographic, from young athletes injured on the playing field to the elderly disabled by complications of disk thinning and deterioration. These problems, coupled with the limited blood supply which impedes healing in most of the meniscus, have compelled the orthopedic and sports medicine community to continue to examine possible new means of restoring meniscal function and reducing pain.[1]

Meniscal repair is considered only when the peripheral third or "red-red zone" of the tissue is injured.[2] Repair can also be considered in certain select patient populations, such as elite athletes and adolescents, when tears have occurred in the partially vascularized "red-white" zone. Meniscectomy, either complete but more commonly partial, is the treatment of choice for all other pathologies involving the menisci. This surgical option, however, disrupts the load-bearing capabilities of the knee joint and significantly increases patient risk for osteoarthritis. At the same time, it seems suboptimal for young athletes and others for whom there is a strong incentive to avoid long-term immobilization, rehabilitation, and the degenerative aspects associated with meniscectomy.

Given these limitations in treatment for meniscal tears, researchers have proposed new and revolutionary models for repairing, reconstructing, and replacing the meniscus in a manner conducive to restoring its native structure and function. These new approaches have included creating artificial meniscal prostheses, reconstituting meniscal defects with collagen scaffolds, and tissue-engineering the human meniscus.

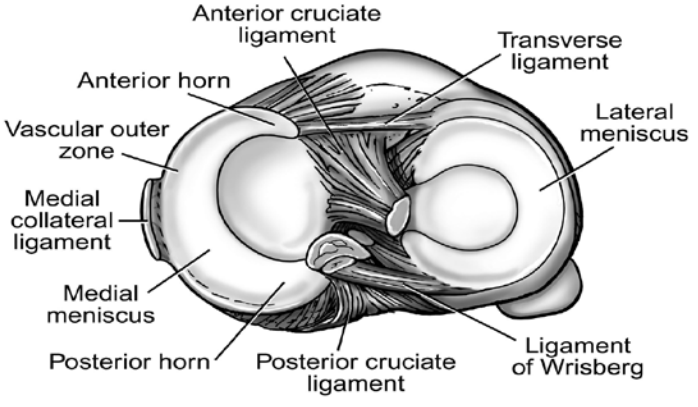

Figure 1. Intra-articular axial section through the human knee joint. Lateral and medial menisci are illustrated with their important attachments. Illustration by the Cleveland Clinic Foundation Medical Illlustration Team.

In this article, we review the latest tissue-engineering advances toward development of meniscal constructs, and we describe the unique anatomical, histologic, and biomechanical parameters that challenge researchers trying to construct human menisci in the laboratory.

Anatomy and Histology

The human medial and lateral menisci are crescent-shaped wedges of fibrocartilaginous tissue between the articular surfaces of the femur and tibia (Figure 1). Meniscal blood supply is predominantly derived from the medial and lateral geniculate arteries by means of a perimeniscal capillary plexus.[1-3] Depth of vascular penetration is 10% to 30% of the width of the medial meniscus and 10% to 25% of the width of the lateral meniscus.[1-6] The meniscus is divided into thirds. The peripheral third, termed the red-red zone for its rich vascular supply, has the most potential for healing. The middle third, or the red-white zone, has a substantially reduced healing capability. In the inner third, or the white-white zone, there is minimal healing potential.[1-6]

The menisci are anchored to several other intra-articular knee structures, including the medial collateral ligament, the meniscofemoral ligaments, the transverse ligament, and the anterior and posterior horns. The 2 meniscal horns are the sites of attachment to the meniscal plate; these regions have additional blood supply and innervation.[3]

Microscopically, the meniscus has 2 main zones—superficial and deep. In these zones, the fibrochondrocyte is the predominant cell type. Fibrochondrocytes are so named because they resemble chondrocytes phenotypically, but they also establish and develop a fibrous territorial matrix.[4,7,8] Morphologically, fibrochondrocytes differ from each other on the basis of their location in one zone or the other. In the superficial zone, the cells are oval or fusiform with sparse cytoplasm; in the deep zone, they are round or polygonal with abundant endoplasmic reticulum and other cytoplasmic organelles. Microvascular endothelial cells and myofibroblasts also exist in small amounts in meniscal tissue.

Meniscal fibrochondrocytes secrete an extracellular matrix composed primarily of collagen and elastin fibers.[3,7,8] Collagen accounts for 60% to 70% of the extracellular dry weight, but content decreases as fibrochondrocytes age. Collagen types I, II, III, V, and VI are all present in the meniscus, but type I collagen is overwhelmingly predominant.[5] Structurally, the organization of type I collagen in the meniscus is uniquely reflective of the function of the tissue. The 3 layers recognized in the meniscus have different organizational patterns of collagen fibers (Figure 2). The outer layer contains randomly oriented type I collagen fibers; the middle or lamellar layer contains fibers with a more parallel orientation and radial fibers at peripheral ends that are continuous with the anterior and posterior ligamentous horns[6]; and the deep layer contains circumferentially oriented type I fibers and small amounts of radially oriented tie fibers. The circumferential fibers of the deep layer are also contiguous with the anterior and posterior horns.[7]

Compression in meniscal tissue and maintenance of tissue hydration are principally the result of the presence of proteoglycans. The avascular two-thirds of the meniscus produce more proteoglycans than the peripheral vascular region does, though the glycosaminoglycan composition of the proteoglycans remains the same throughout the tissue. The proteoglycans in normal human meniscus consist of 40% chondroitin-4-sulfate, 10% to 20% chondroitin-6-sulfate, 20% to 30% dermatan sulfate, and 15% keratin sulfate.

Biomechanical Properties of the Meniscus

The unique geometry and histologic structure of the meniscus endow it with properties that enable it to withstand the complex combinations of flexion, extension, rotation, and translation that occur at the tibiofemoral joint during normal motion.[9] Biomechanical testing has demonstrated that, with compression at the knee joint, the meniscal surface gives rise to a vertical force and a radially oriented component of compressive force that outwardly displace the menisci.[3,5-7,10] The rigid meniscal attachments at the anterior and posterior horns limit the extent of such

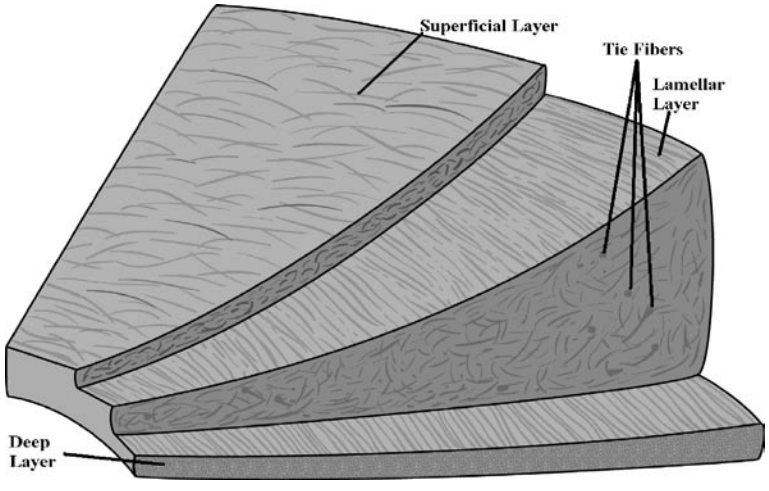

Figure 2. Schematic of the human meniscus illustrates the differing organizational patterns of meniscal collagen fibers. The superficial layer is composed of randomly oriented collagen fibers; the lamellar layer consists of fibers with a more parallel orientation with radial tie fibers providing contiguity with the anterior and posterior ligamentous horns; and the deep layer consists of circumferentially oriented type I fibers arranged in a highly ordered fashion. Illustration by the Cleveland Clinic Foundation Medical Illlustration Team.

outward displacement and, in turn, produce circumferentially directed forces and tensile hoop stresses that are dissipated throughout the meniscal tissue. The large tensile, compressive, and shear stresses that the meniscus withstands under normal physiologic conditions are distributed within the tissue by the circumferentially oriented collagen fibers located in the deep layer of the meniscus.

The biomechanical properties of the meniscus have been well summarized.[3,5-7,9] Joshi and colleagues[11] measured the compressive aggregate modulus of human menisci at 0.22 MPa. Thus, human meniscal tissue is weaker than articular cartilage, which has a compressive aggregate modulus of 0.70 MPa.[6] Tissakht and Ahmed[12] found that the menisci are able to resist circumferentially oriented loads better than radially oriented loads. Fithian and colleagues[6] determined that the tensile modulus of the medial meniscus ranges from 94 to 160 MPa, while the tensile modulus of the lateral meniscus ranges from 160 to 295 MPa.

The organization of collagen fibers in its matrix makes the meniscus anisotropic in response to dynamic shear stresses. The meniscus is 20% to 30% stiffer when shear forces are directed perpendicular to its deep collagen fiber bundles than when forces occur parallel to them.[6] Increasing torsional force across the meniscus increases the stiffness of the tissue while energy dissipation remains constant—a direct result of the elastic stiffening of the meniscal collagen-proteoglycan matrix. Under physiologic loading conditions, however, increasing shear strain causes a reduction in the elastic stiffness of the meniscus while energy dissipation is increased.[6] The meniscal response to shear, especially in parallel with collagen fiber bundles, has been suggested as an important factor in development of the vertical and horizontal cleavage tears commonly found in this tissue.

Similar to the shear stress properties of the meniscus, the tensile modulus, tensile strength, and ultimate strain of meniscal tissue all depend heavily on the collagen ultrastructure inherent in the tissue and on the proteoglycan content within the matrix. Collagen fiber orientation is largely responsible for the anisotropic nature of the meniscus and is the major factor in load bearing in tension. Proteoglycans, meanwhile, provide the meniscus with its ability to resist compression.

The Movement Toward Tissue Engineering

The idea of tissue-engineering a meniscal construct stemmed from the difficulties that orthopedic surgeons experience in treating patients with tears or other meniscal pathology. Because of the avascular nature of the inner two-thirds of the meniscus, tears and other damage in this region are not amenable to healing and can result in debilitating pain, locking, and progressive osteoarthritis. The meniscus initially was thought to be a vestige of leg muscle and was excised whenever injured.[13] Fairbank[14] demonstrated that this practice inevitably leads to further osteoarthritic degeneration of the knee joint and eventually to the partial meniscectomy advocated as a viable alternative to meniscal excision. Although arthroscopic partial meniscectomy may also lead to additional osteoarthritic degeneration of the knee, and may not be ideal, it is still the standard of care for complex, irreparable meniscal tears, even in pediatric patients, adolescents, elite athletes who wish for an early return to sports, and trauma patients with severe meniscal disruption.

More recently, Stone[15] proposed aggressive salvage of meniscal tissue in all patients younger than 50. Suture, meniscal arrows, fibrin sealants, and laser welding all can be used to promote healing in the vascular zone of the tissue.[16] Tears in the avascular zone of the meniscus require vascular induction in the form of trephination or formation of vascular access channels. Radial needling of the meniscal tissue allows for introduction of blood supply and clot formation. The clot, in addition to the fixation device (for example, suture, arrows), stabilizes the meniscal tear and provides a rudimentary scaffold for cellular ingrowth and repair. These repair approaches have been shown to be of some benefit to the meniscus, but they remain inappropriate for complex meniscal tears and menisci devitalized or degenerated to an advanced degree.

"Attempts have been made to replace severely damaged or otherwise irreparable menisci with various meniscal prostheses..."

Limited application of meniscal repair led to the advancement of meniscal transplantation. In a recent study, investigators found a 10-year graft survival rate of 70% and significant improvement in symptoms in patients who received viable meniscal allografts.[17] However, complexity of meniscal transplant surgery, paucity of donor tissue, continued progression of osteoarthritis after transplantation, and potential for disease transmission all constrain this treatment approach. Possibility of disease transmission is particularly concerning, as modalities for sterilizing meniscal allografts substantially increase the failure rate of the tissue.[2,5,18]

Attempts have been made to replace severely damaged or otherwise irreparable menisci with various meniscal prostheses, including tendon grafts,[19] polytetrafluoroethylene, and Dacron.[20] In 2003, Kobayashi and colleagues[21] developed polyvinyl alcohol hydrogel

(PVA-H) for meniscal replacement. It was proposed that the viscoelastic properties of PVA-H would allow it to approximate the meniscus more closely in terms of compression and stress-relaxation properties. In laboratory testing, the viscosity of PVA-H was found to be lower than that of normal menisci, and no prosthesis has proved to be mechanically equivalent to meniscal tissue. On the basis of these findings, Kobayashi and colleagues concluded that "transplantation of regenerated meniscus induced from autograft by tissue engineering will become the most effective therapy in the future."[21]

> **"The polymers typically degrade over time and leave behind the consolidated tissue that they helped to support."**

Tissue-Engineered Meniscal Constructs

The first steps toward a tissue-engineered meniscus derived from research into regeneration of meniscal defects in vivo. Arnoczky and colleagues[22] were the first researchers to suggest that fibrin clots could be used to enhance meniscal repair techniques. They hypothesized that a fibrin clot would provide an ideal environment for meniscal healing—as a scaffold for blood vessel and cellular ingrowth in a milieu rich in growth factors and cytokines. Their research results led to a variety of studies eventuating in the design, development, and promotion of multiple permanent and biodegradable synthetic materials for use as scaffolds to heal defects in meniscal tissue.

Stone and colleagues,[23] expanding on the notion of Arnoczky and colleagues,[22] proposed using collagen scaffolding instead of fibrin clots. The collagen network, they theorized, would allow for initial ingrowth of meniscal cells and progressive degradation as meniscal regeneration occurred. Their work was reinforced by subsequent clinical results reported by Stone and colleagues[23,24] and Rodkey and colleagues,[25] who used collagen meniscal implants to regenerate meniscal tissue in human subjects. By means of knee arthroscopy, these researchers inserted collagen meniscal implants into patients with medial meniscal injuries. Their approach yielded new fibrocartilage matrix on histologic examination and excellent clinical improvement in all study patients.[25] This work demonstrated not only that it is possible to use tissue-engineered constructs in the clinical setting but also that meniscal replacement was amenable to arthroscopic surgery. Limitations to using collagen constructs for meniscal repair on a large scale include the need for the outer rim of the meniscus to be in place for fixation, the commonly occurring shrinkage of collagen implants, and the complexity of the suturing technique required to secure the constructs.[26]

In 2005, Steadman and Rodkey[26] described their short-term success in using tissue-engineered collagen implants. They followed 8 patients who had received a partial meniscal replacement with a type I collagen scaffold manufactured from bovine Achilles tendon. After a mean follow-up of approximately 6 years, they found that patients who received a collagen meniscal implant had significant short-term improvement in pain and in activity scores. Furthermore, relook arthroscopy and biopsy demonstrated 69% defect filling, 170% increase in amount of meniscal tissue, and inhibition of further cartilage degeneration.[26] Although this study seems to support the feasibility and survivability of tissue-engineered implants in the human knee joint, the collagen implants that were used are not suitable for replacement in the event of severe meniscal injury or total meniscectomy.

The success that Stone and colleagues,[23,24] Rodkey and colleagues,[25] and Steadman and Rodkey[26] had with collagen constructs led to development of biodegradable synthetic polymers as scaffolds for meniscal ingrowth and ultimately to tissue-engineering of entire menisci. Synthetic polymers, which may be generated from various polyesters, degrade by means of normal hydrolysis over variable periods of time.[2,27] Polymer scaffolds perform the same function as that of the fibrin clots and collagen constructs described here. The polymer network forms a mesh that allows mechanical attachment of cells and promotes the cellular proliferation and matrix production that ultimately result in tissue re-creation. The polymers typically degrade over time and leave behind the consolidated tissue that they helped to support. It should be noted that the polymer scaffolds break down into nontoxic byproducts—carbon, hydrogen, and oxygen.[2,4,27]

Besides being readily degradable, synthetic polymers can be modified to various strengths, degradation rates, shapes, and pore sizes—all of significant benefit. Pore size of biodegradable scaffolds is the primary factor in extent of meniscal and other cell ingrowth.[27] Klompmaker and colleagues[28] concluded that, for complete ingrowth and incorporation of meniscal cells into a biodegradable scaffold, macropore size of the polymer must be in the range of 100 to 150 μm.

Several experiments have been conducted with different synthetic polymer scaffolds. The most popular polymers are polyglycolic acid (PGA), poly-L-lactic acid (PLLA), polyurethane, and poly-ϵ-caprolactone (PCL).[2,3,15,29] A typical procedure for producing PCL (as an example of these polymer scaffolds) was initially described by de Groot and colleagues[30] in 1997. Subsequent animal research with these polymer scaffolds helped identify the optimal mechanical properties that facilitate ingrowth in a native joint environment.[2,4,24,27-29] One finding is that, by varying the initial compressive modulus of the synthetic matrix, modifications can be made in the ingrowth rate and morphology of resultant tissue. In this regard, Setton and colleagues[5] reported 150 kPa as being the minimal compressive modu-

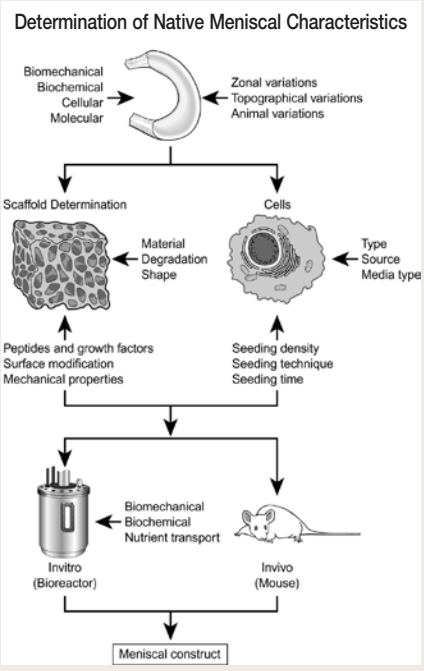

Figure 3. Algorithm for tissue-engineering meniscal constructs. Original artwork adapted, with permission, from Sweigart MA, Athanasiou KA. Toward tissue engineering of the knee meniscus. *Tissue Eng*. 2001;7(2):111-129. Schematic provided by the Cleveland Clinic Foundation Medical Illustration Team.

Direct Seeding of Constructs With Cells: The Model of Ibarra and Colleagues

In 1998, shortly after successes were achieved with synthetic biodegradable polymers, Ibarra and colleagues[29,34] directly seeded constructs with cells to constitute menisci. The researchers were successful in creating meniscal tissue in an athymic nude mouse model (Figure 3). In their study, immature bovine fibrochondrocytes were expanded in vitro before being seeded onto PGA scaffolds. The constructs were then implanted into the athymic mice. After 12 weeks of incubation, the tissue-engineered constructs were still in their original shape and resembled meniscal tissue morphologically as well as histologically. Some specimen areas showed matrix architecture almost identical to that of normal meniscal tissue. Meniscal fibrochondrocytes were found embedded in a collagen-rich extracellular matrix, and histologic staining revealed collagen and proteoglycans in abundance. Despite the structural similarities, however, biomechanical testing of the meniscal constructs demonstrated that the tissue had only 40% of the compressive properties of normal meniscus.[34]

Ibarra and colleagues[29,34] showed that meniscal fibrochondrocytes can be isolated from meniscal tissue, expanded in culture, and then loaded onto polymer scaffolds to create artificial constructs that reasonably resemble native meniscus. They also showed that meniscal tissue can develop in an environment divorced from the biomechanical strains and forces applied to the normal meniscus by its attachments within the knee joint. Meniscal tissue was developed under minimal forces—that is, only those applied by the skin and subcutaneous fascia in the dorsal skin pouch of the host mice.[29] Ibarra and colleagues[34] proposed that the basis for such an important finding could be the intrinsic ability of the meniscal cells to form specific tissue structures in a predetermined fashion.

lus of synthetic scaffolds—a result confirmed in 1996 by Klompmaker and colleagues,[31] who demonstrated the importance of compressive modulus in transforming fibrous tissue into fibrocartilage when using a scaffold as a meniscal prosthesis.

Building on these findings, Heijkants and colleagues[32] developed a biodegradable scaffold from PCL and 1,4-butanediisocyanate and 1,4-butanediol. This new synthetic matrix for meniscal ingrowth combined high intrinsic porosity with a desirable compressive modulus for meniscal tissue. After 6 months of maturation within the knee joints of beagles, the constructs were harvested, and full ingrowth of meniscal cells was noted. The resultant tissue was meniscal in nature, and its compression behavior was comparable to that of a native meniscus.

Most recently, Tienen and colleagues[33] examined the performance of polyesterurethane scaffolds after insertion into human cadaveric knee joints. Although the biodegradable scaffolds approximated the behavior of native meniscal tissue, these constructs showed less excursion than native menisci and contributed to increased joint laxity. These findings led Tienen and colleagues to conclude that synthetic polymer scaffolds required further optimization to achieve better initial function in the knee joint.[33]

In 1998, Ibarra and colleagues directly seeded polymer scaffolds with cells. Their interesting work is described in the Box above.

The model of Ibarra and colleagues[29,34] has been replicated with PGA and PGA/PLLA polymers. Both constructs, once seeded with meniscal fibrochondrocytes, have successfully been used in the repair of meniscal defects in animal studies.[29,34] Furthermore, PGA/PLLA polymers that were formed in the shape of a normal meniscus and seeded with fibrochondrocytes retained that shape and generated new, meniscus-like tissue when transplanted into the knees of sheep.[27]

Peretti and colleagues[35] developed similar meniscal constructs by seeding devitalized meniscal scaffolds with chondrocytes and transplanting them into the dorsal pouches of athymic nude mice. These constructs were placed into 4-mm bucket-handle incisions, and, after 14 weeks, gross inspection and histologic analysis demonstrated obliteration of the interface between the meniscal tissue and the construct. To a lesser extent, pluripotential fibroblasts and mesenchymal stem cells were also identified as potential candidates for use in meniscal constructs.[2] As fibroblasts will not synthesize meniscal fibrocartilage de novo, and stem cells require complex differentiation, these cell lines are rendered less attractive than articular chondrocytes or meniscal fibrochondrocytes as cellular sources for tissue-engineering the meniscus.[2]

Much research has subsequently been directed to using various growth factors, cytokines, and nutrient media to

prepare meniscal cells or their derivatives for seeding onto construct scaffolds. In this context, Webber and colleagues[36] found 2 cell types, polygonal and fusiform, derived from meniscal cells cultured in monolayer. The authors assumed the polygonal cells were chondrocytes and thought fusiform cells to be fibroblasts. Nakata and colleagues[37] described 3 distinct lines of human meniscal cells in monolayer culture: elongated fibroblast-like cells, polygonal cells, and round chondrocyte-like cells. The fibroblast-like cells and polygonal/chondrocyte-like cells were suggested to represent the morphologically distinct cell types described in the superficial and deep zones of the meniscus. Alternatively, Almarza and Athanasiou[10] hypothesized that the polygonal cells may actually have been dedifferentiated chondrocytes, having characteristics different from those of their progenitor line in vivo.

With regard to media used to enhance the population of cells for tissue engineering, Nakata and colleagues[37] found that fibroblast-like meniscal cells predominated in cultures using Dulbecco's modified Eagle's medium. Cultures that used a mixture of Ham's nutrient mixture F-12 and Dulbecco's modified Eagle's medium, however, resulted in the production of meniscal cells that retained their morphologic features.[37] Furthermore, introduction of hyaluronan increased proliferation of the meniscal cells without inhibiting chondroitin production or altering cell morphology.[27]

Transforming growth factor β (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1) and fibroblast growth factor (FGF) have all been proposed as factors in increasing meniscal cell proliferation and proteoglycan production before and during cell seeding and incubation on a polymer scaffold.[2,4,27,29] Tanaka and colleagues[38] and Collier and Ghosh[39] demonstrated that TGF-β increases the proteoglycan synthesis of meniscal fibrochondrocytes in a dose-dependent manner. Bhargava and colleagues[40] investigated the effect of PDGF-AB, hepatocyte growth factor (HGF), and bone morphogenic protein 2 (BMP-2) on DNA synthesis. They found that all 3 factors positively affected DNA replication in meniscal cells. Webber and colleagues[36] achieved similar results using FGF and human platelet lysate.

Recently, Pangborn and Athanasiou[41] demonstrated the advantage of TGF-β1 over IGF-1, PDGF-AB, and bFGF in stimulating growth and matrix production in fibrochondrocytes attached to PGA scaffolds. These findings were reinforced by the research of Imler and colleagues,[42] who also reported the inhibitory effects of static compression on tissue-engineered meniscal constructs. Specifically, static compression inhibited collagen synthesis and matrix production irrespective of the growth factor present. It also affected the biosynthetic capabilities of meniscal constructs in a dose-dependent manner.

Several researchers have explored the possibility of inducing tissue differentiation of cells by means of genetic engineering. Hidaka and colleagues[43] used adenovirus vectors encoding for HGF to stimulate blood vessel infiltration and formation in cell-seeded PGA constructs, and Martinek and colleagues[44] used viral vectors to transfect allograft menisci with lacZ, luciferase, and green fluorescent protein genes. The latter authors found that gene expression persisted for 4 to 8 weeks, and they speculated that viral transfer of specific growth factor genes has the potential to improve the regeneration and healing capabilities of meniscal allografts as well as autografts.[43,44]

Future Directions

Other more recent investigations hold particular promise for further advances in tissue-engineering the human meniscus. Alhadlaq and Mao[45] showed the possibility of mesenchymal stem cell differentiation in vitro and its potential application in the field. More importantly, Isogai and colleagues[46] and Landis and colleagues[47] achieved particular success with engineering phalanges and small joints in the laboratory environment. By suturing together various polymer scaffolds seeded with different cells, these researchers tissue-engineered distal interphalangeal joints complete with articular cartilage and tendon insertions.[46,47] Such a technique could readily be applied to the human meniscus, whereby the morphologically distinct cells of the different layers are cultured, isolated, and seeded on individual polymers, subsequently to be sutured together to replicate the native meniscal architecture.

Clearly, tissue engineering as applied to the human meniscus is in its infancy. Despite major advances in the past 8 to 10 years, more work needs to be done, particularly with regard to the effect of meniscal constructs on knee joint osteoarthritis and the quantitative measurement and assessment of their biomechanical properties. Building on the data and advances of earlier research studies, and introducing techniques that have produced positive results in other fields of tissue engineering, it is now readily possible to envision the successful assembly of meniscal constructs that more closely approximate native tissue molecularly, biochemically, structurally, and biomechanically.

"...introduction of hyaluronan increased proliferation of the meniscal cells without inhibiting chondroitin production or altering cell morphology.[27]"

Summary

The effects of aging, trauma, and aggressive athletic activity can take their toll on the meniscal structures of the human knee. Because of the poor reparative function of meniscal tissue, most of its injuries, wear, and damage result in further degeneration and an accelerated development of osteoarthritis, even with surgical intervention. Traditional methods of meniscal repair have now been questioned as to their effectiveness, and alternative approaches are being considered. In this respect, tissue engineering may be a possible means of developing viable meniscal constructs that can be used to replace impaired menisci and prevent progressive articular destruction.

The field of tissue engineering has expanded rapidly, particularly over the past decade, and has led to important discoveries in use of synthetic scaffold materials, cell culture methodology, and gene therapy to enhance cell growth and tissue production. For the meniscus, additional work is needed to characterize the unique properties of the native tissue and to identify the molecular, biochemical, structural, and biomechanical properties of current tissue-engineered meniscal constructs. Once these inherent features of both native and engineered meniscal tissue are determined, distinct design protocols for advanced tissue-engineered constructs can be instituted that will ultimately enable these artificial menisci to become viable therapeutic implants.

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References

1. Miller RH III. Knee injuries. In: Canale ST, ed. *Campbell's Operative Orthopaedics.* St. Louis, Mo: Mosby; 2003:2165-2338.
2. Sweigart MA, Athanasiou KA. Toward tissue engineering of the knee meniscus. *Tissue Eng.* 2001;7(2):111-129.
3. Arnoczky SP. Gross and vascular anatomy of the meniscus and its role in meniscal healing, regeneration, and remodeling. In: Mow VC, Arnoczky SP, Jackson DW, eds. *Knee Meniscus:* Basic and Clinical Foundations. New York, NY: Raven Press; 1992:1-14.
4. Arnoczky SP. Building a meniscus. Biologic considerations. *Clin Orthop.* 1999;367(suppl):S244-S253.
5. Setton LA, Guilak F, Hsu EW, Vail TP. Biomechanical factors in tissue engineered meniscal repair. *Clin Orthop.* 1999;367(suppl):S254-S272.
6. Fithian DC, Kelly MA, Mow VC. Material properties and structure–function relationships in the menisci. *Clin Orthop*. 1990;252:19-31.
7. Mow VC, Ratcliffe A, Chern KY, Kelly M. Structure and function relationships of the menisci of the knee. In: Mow VC, Arnoczky SP, Jackson DW, eds. *Knee Meniscus: Basic and Clinical Foundations*. New York, NY: Raven Press; 1992:37-57.
8. Adams ME, Hukins DWL. The extracellular matrix of the meniscus. In: Mow VC, Arnoczky SP, Jackson DW, eds. *Knee Meniscus: Basic and Clinical Foundations.* New York, NY: Raven Press; 1992:15-28.
9. Fu FH, Thompson WO. Motion of the meniscus during knee flexion. In: Mow VC, Arnoczky SP, Jackson DW, eds. *Knee Meniscus: Basic and Clinical Foundations.* New York, NY: Raven Press; 1992:75-89.
10. Almarza AJ, Athanasiou KA. Design characteristics for the tissue engineering of cartilaginous tissues. *Ann Biomed Eng*. 2004;32(1):2-17.
11. Joshi MD, Suh JK, Marui T, Woo SL. Interspecies variation of compressive biomechanical properties of the meniscus. *J Biomed Mater Res.* 1995;29(7):823-828.
12. Tissakht M, Ahmed AM. Tensile stress-strain characteristics of the human meniscal material. *J Biomech.* 1995;28(4):411-422.
13. King D. The function of semilunar cartilages. *J Bone Joint Surg Am.* 1936;18(4):1069-1076.
14. Fairbank TJ. Knee joint changes after meniscectomy. *J Bone Joint Surg Br.* 1948;30(4):664-670.
15. Stone KR. Current and future directions for meniscal repair and replacement. *Clin Orthop.* 1999;367(suppl):S273-S280.
16. Arnoczky SP, Lavagnino M. Tensile fixation strengths of absorbable meniscal repair devices as a function of hydrolysis time: an in vitro experimental study. *Am J Sports Med.* 2001;29(2):118-123.
17. Verdonk PC, Demurie A, Almqvist KF, Veys EM, Verbruggen G, Verdonk R. Transplantation of viable meniscal allograft: survivorship analysis and clinical outcome of one hundred cases. *J Bone Joint Surg Am*. 2005;87(4):715-724.
18. Wirth CJ, Peters G, Milachowski KA, Weismeire KG, Kohn D. Long-term results of meniscal allograft transplantation. *Am J Sports Med.* 2002;30(2):174-181.
19. Johnson LL, Feagin JA Jr. Autogenous tendon graft substitution for absent knee joint meniscus: a pilot study. *Arthroscopy.* 2000;16(2):191-196.
20. Messner K, Gillquist J. Prosthetic replacement of the rabbit medial meniscus. *J Biomed Mater Res.* 1993;27(9):1165-1173.
21. Kobayashi M, Toguchida J, Oka M. Development of an artificial meniscus using polyvinyl alcohol-hydrogel for early return to, and continuance of, athletic life in sportspersons with severe meniscus injury. I: mechanical evaluation. *Knee.* 2003;10(1):47-51.
22. Arnoczky SP, Warren RF, Spivak JM. Meniscal repair using exogenous fibrin clot: an experimental study in dogs. *J Bone Joint Surg Am*. 1988;70(8):1209-1217.
23. Stone KR, Rodkey WG, Webber R, McKinney L, Steadman JR. Meniscal regeneration with copolymeric collagen scaffolds: in vitro and in vivo studies evaluated clinically, histologically and biochemically. *Am J Sports Med.* 1992;20(2):104-111.
24. Stone KR, Steadman JR, Rodkey WG, Li ST. Regeneration of meniscal cartilage with the use of a collagen scaffold. *J Bone Joint Surg Am*. 1997;79(12):1770-1777.
25. Rodkey WG, Steadman JR, Li ST. A clinical study of collagen meniscus implants to restore the injured meniscus. *Clin Orthop.* 1999;367(suppl):S281-S292.
26. Steadman JR, Rodkey WG. Tissue-engineered collagen meniscus implants: 5- to 6-year feasibility study results. *Arthroscopy.* 2005;21(5):515-525.
27. Buma P, Ramrattab NN, Van Tienen TG, Veth RPH. Tissue engineering of the meniscus. *Biomaterials.* 2004;25(9):1523-1532.
28. Klompmaker J, Jansen HW, Veth RP, Nielsen HK, de Groot JH, Pennings AJ. Porous implants for knee joint meniscus reconstruction: a preliminary study on the role of pore sizes in ingrowth and differentiation of fibrocartilage. *Clin Mater.* 1993;14(1):1-11.
29. Ibarra C, Koski JA, Warren RF. Tissue engineering meniscus: cells and matrix. *Orthop Clin North Am.* 2000;31(3):411-418.
30. de Groot JH, Zijlstra FM, Kuipers HW, et al. Meniscal tissue regeneration in porous 50/50 copoly(L-lactide/epsilon-caprolactone) implants. *Biomaterials.* 1997;18(8):613-622.
31. Klompmaker J, Veth RP, Jansen HW, Nielsen HK, de Groot JH, Pennings AJ. Meniscal replacement using a porous polymer prosthesis: a preliminary study in the dog. *Biomaterials.* 1996;17(12):1169-1175.
32. Heijkants RG, van Calck RV, de Groot JH, et al. Design, synthesis and properties of a degradable polyurethane scaffold for meniscus regeneration. *J Mater Sci.* 2004;15(4):423-427.
33. Tienen TG, Verdonschot N, Heijkants RG, et al. Prosthetic replacement of the medial meniscus in cadaveric knees: does the prosthesis mimic the functional behavior of the native meniscus? *Am J Sports Med.* 2004;32:1182-1188.
34. Ibarra C, Jannetta C, Vacanti CA, et al. Tissue-engineered meniscus: a potential new alternative to allogenic meniscus transplantation. *Transplantation Proc.* 1997;29(1-2):986-988.
35. Peretti GM, Caruso EM, Randolph MA, Zaleske DJ. Meniscal repair using engineered tissue. *J Orthop Res.* 2001;19(2):278-285.
36. Webber RJ, Harris MG, Hough AJ Jr. Cell culture of rabbit meniscal fibrochondrocytes: proliferative and synthetic response to growth factors and ascorbate. *J Orthop Res.* 1985;3(1):36-42.
37. Nakata K, Shino K, Hamada M, et al. Human meniscus cell: characterization of the primary culture and use for tissue engineering. *Clin Orthop.* 2001;391(suppl):S208-S218.
38. Tanaka T, Fujii K, Kumagae Y. Comparison of biomechanical characteristics of cultured fibrochondrocytes isolated from the inner and outer regions of human meniscus. *Knee Surg Sports Traumatol Arthrosc.* 1999;7(2):75-80.
39. Collier S, Ghosh P. Effects of transforming growth factor beta on proteoglycan synthesis by cell and explant cultures derived from the knee joint meniscus. *Osteoarth Cart.* 1995;3(2):127-138.
40. Bhargava MM, Attia ET, Murrell GA, Dolan MM, Warren RF, Hannafin JA. The effect of cytokines on the proliferation and migration of bovine meniscal cells. *Am J Sports Med.* 1999;27(5):636-643.
41. Pangborn CA, Athanasiou KA. Growth factors and fibrochondrocytes in scaffolds. *J Orthop Res*. 2005;23(5):1184-1190.
42. Imler SM, Doshi AN, Levenston ME. Combined effects of growth factors and static mechanical compression on meniscus explant biosynthesis. *Osteoarth Cart.* 2004;12(9):736-744.
43. Hidaka C, Ibarra C, Hannafin JA, et al. Formation of vascularized meniscal tissue by combining gene therapy with tissue engineering. *Tissue Eng.* 2002;8(1):93-105.
44. Martinek V, Usas A, Pelinkovic D, Robbins P, Fu FH, Huard J. Genetic engineering of meniscal allografts. *Tissue Eng.* 2002;8(1):107-117.
45. Alhadlaq A, Mao JJ. Tissue-engineered osteochondral constructs in the shape of an articular condyle. *J Bone Joint Surg Am.* 2005;87(5): 936-944.
46. Isogai N, Landis WJ, Kim TH, Gerstenfeld LC, Upton J, Vacanti JP. Formation of phalanges and small joints by tissue-engineering. *J Bone Joint Surg Am*. 1999;81(3):306-316.
47. Landis WJ, Jacquet R, Hillyer J, et al. The potential of tissue engineering in orthopaedics. *Orthop Clin North Am.* 2005;36(1):97-104.