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The knee meniscus: structure-function, pathophysiology, current repair techniques, and prospects for regeneration

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Abstract

Extensive scientific investigations in recent decades have established the anatomical, biomechanical, and functional importance that the meniscus holds within the knee joint. As a vital part of the joint, it acts to prevent the deterioration and degeneration of articular cartilage, and the onset and development of osteoarthritis. For this reason, research into meniscus repair has been the recipient of particular interest from the orthopedic and bioengineering communities. Current repair techniques are only effective in treating lesions located in the peripheral vascularized region of the meniscus. Healing lesions found in the inner avascular region, which functions under a highly demanding mechanical environment, is considered to be a significant challenge. An adequate treatment approach has yet to be established, though many attempts have been undertaken. The current primary method for treatment is partial meniscectomy, which commonly results in the progressive development of osteoarthritis. This drawback has shifted research interest towards the fields of biomaterials and bioengineering, where it is hoped that meniscal deterioration can be tackled with the help of tissue engineering. So far, different approaches and strategies have contributed to the *in vitro* generation of meniscus constructs, which are capable of restoring meniscal lesions to some extent, both functionally as well as anatomically. The selection of the appropriate cell source (autologous, allogeneic, or xenogeneic cells, or stem cells) is undoubtedly regarded as key to successful meniscal tissue engineering. Furthermore, a large variation of scaffolds for tissue engineering have been proposed and produced in experimental and clinical studies, although a few problems with these (e.g., byproducts of degradation, stress shielding) have shifted research interest towards new strategies (e.g., scaffoldless approaches, self-assembly). A large number of different chemical (e.g., TGF-β1, C-ABC) and mechanical stimuli (e.g., direct compression, hydrostatic pressure) have also been investigated, both in terms of encouraging functional tissue formation, as well as in differentiating stem cells. Even though the problems accompanying meniscus tissue engineering research are considerable, we are undoubtedly in the dawn of a new era, whereby recent advances in biology, engineering, and medicine are leading to the successful treatment of meniscal lesions.

Keywords

Knee Meniscus; Meniscus Pathology; Meniscal Repair; Tissue Engineering; Scaffolds

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1. Introduction

Six decades ago, the discovery that removing the meniscus from the knee joint—then commonly seen as the sole technique for treating sports-related injuries—resulted in the deterioration of articular cartilage and the gradual development of arthritis, radically changed the approach for treating meniscus-related problems [1]. In 1982, partial meniscectomy was suggested as an alternative to complete meniscectomy [2], while the first published account of a meniscus transplant dates back to 1989 [3]. These studies are landmarks in understanding the anatomical and functional utility of the knee meniscus, and have since resulted in numerous investigations into different treatment approaches.

The current prevailing trend in repairing meniscus-related lesions is to maintain the tissue intact whenever possible [4-6]. However, the inability of surgeons to restore the tissue—both anatomically and functionally—in cases of complex or total traumatic lesions continues to present challenges. The simultaneous inability to delay the progressive development of osteoarthritis presents a similar motivation to search for new therapeutic avenues.

This review will cover current knowledge regarding anatomical and biochemical characteristics of the knee meniscus, and discuss the tissue's biomechanical and functional properties. The review will also address the causal pathologies precipitating the need for meniscus treatment, and the effectiveness of current tissue repair methods, among different age groups. Finally, current therapeutic developments in repairing the meniscus will be discussed, focusing especially on the field of tissue engineering. Within this topic, special emphasis will be placed on advances in scaffolds and scaffold-free approaches to regenerate meniscal tissue. Finally, perspectives for the future of meniscus repair will be given.

2. Structure and Function of the Knee Meniscus

2.1 Meniscus Anatomy

The knee joint contains the meniscus structure, comprised of both a medial and a lateral component situated between the corresponding femoral condyle and tibial plateau (Figure 1) [7]. Each is a glossy-white, complex tissue comprised of cells, specialized extracellular matrix (ECM) molecules, and region-specific innervation and vascularization. Both menisci are critical components of a healthy knee joint [7-12]. The main stabilizing ligaments are the medial collateral ligament, the transverse ligament, the meniscofemoral ligaments, and attachments at the anterior and posterior horns (Figure 2) [8]. The meniscofemoral ligaments, also known as the Humphrey and Wrisberg ligaments, connect the posterior horn of the lateral meniscus to a location near the insertion site of the posterior cruciate ligament on the medial femoral condyle. Though only 46% of people have both of these ligaments, 100% of people have at least one of them [8]. The meniscus surface appears smooth upon both gross inspection and microscopically [9]. Human medial and lateral menisci have distinctly different dimensions: lateral menisci are approximately 32.4-35.7 mm in length and 26.6-29.3 mm wide, while medial menisci are 40.5-45.5 mm long and 27 mm wide [10, 11]. Though both menisci are roughly wedge-shaped and semi-lunar, lateral menisci display greater variety in size, shape, thickness, and mobility than medial menisci [12, 13]. Lateral menisci also cover a larger portion of the tibial plateau (75-93% laterally) in comparison to medial menisci (51-74% medially) [13].

Vascularization in this tissue is of high relevance. From prenatal development until shortly after birth, the meniscus is fully vascularized. Afterwards, however, vascularization appears to subside. At 10 years of age, vascularization is present in around 10-30% of the meniscus, and at maturity the meniscus contains blood vessels and nerves only in the peripheral 10-25% of the tissue [13]. Subsequently, two distinct regions of the meniscus can be

distinguished: the outer, vascular/neural region (red-red zone), and the inner, completely avascular/aneural region (white-white zone). These two areas are separated by the red-white region, which presents attributes from both the red-red and white-white regions (Figure 3). Critically, the healing capacity of each area is directly related to blood circulation, leaving the white region susceptible to permanent post-traumatic and degenerative lesions [14].

2.2 Biochemical Content

Regarding composition by wet weight, the meniscus is highly hydrated (72% water), with the remaining 28% comprised of organic matter, mostly ECM and cells [15]. In general, collagens make up the majority (75%) of this organic matter, followed by GAGs (17%), DNA (2%), adhesion glycoproteins (<1%), and elastin (<1%) [15, 16]. The above proportions might vary depending on age, injuries, and other pathological conditions [17, 18].

Although collagen is the main fibrillar component of the meniscus, different collagen types exist in varying quantities in each region of the tissue. In the red-red zone, collagen type I is predominant, at approximately 80% composition by dry weight, with other collagen variants (type II, III, IV, VI, and XVIII) present at less than 1%. In the white-white zone, collagen makes up 70% of the tissue by dry weight, of which 60% is collagen type II and 40% is collagen type I [19]. Aside from collagen, another fibrillar component is elastin: a combination of mature and immature elastin fibers has been found in very low concentrations (<0.6%) in the adult meniscus. Elastin's exact biochemical and functional importance in the meniscus has yet to be determined [9, 18, 20].

Proteoglycans are heavily glycosylated molecules that constitute a major component of the meniscus ECM [21]. These molecules are comprised of a core protein which is decorated with glycosaminoglycans (GAGs). The main types of GAGs found in normal human meniscal tissue are chondoitin-6-sulfate (60%), dermatan sulfate (20-30%), chondroitin-4-sulfate (10-20%), and keratin sulfate (15%) [15]. Aggrecan is the major large proteoglycan of the meniscus while biglycan and decorin are the main small proteoglycans [22]. Regional variation of these molecules has also been observed, with the inner two-thirds containing a relatively higher proportion of proteoglycans than the outer one-third [22]. Their main function is to enable the meniscus to absorb water, whose confinement supports the tissue under compression [22, 23]. Adhesion glycoproteins are also indispensible components of the meniscus matrix, as they serve as a link between ECM components and cells. The main adhesion glycoproteins present in the human meniscus are fibronectin, thrombospondin, and collagen VI [18, 24].

2.3 Meniscus Cells

During early development, all meniscus cells present the same cellular morphology—in terms of both size and shape—with no regional variations. However, later in development, morphologically and phenotypically-distinct cells appear, which also vary in terms of number and topographic localization (Figure 3) [25]. Ghadially et al. [9] suggested categorization of meniscus cells according to their shape and the presence or absence of territorial matrix. Under this classification method, chondrocytes, fibroblasts, or intermediate cells exhibiting characteristics of both were identified. Today, the characterization of meniscus cells appears somewhat controversial in the literature, with a number of different terms being used (i.e., fibrocytes, fibroblasts, meniscus cells, fibrochondrocytes, and chondrocytes) [25]. Regardless of the varying terminology used, it is apparent that outer zone cells have an oval, fusiform shape and are similar in appearance and behavior to fibroblasts. Thus, they may be described as fibroblast-like cells. These cells also display long cell extensions, which facilitate communication with other cells and the

extracellular matrix. The matrix surrounding these cells is mainly comprised of type I collagen, with small percentages of glycoproteins and collagen types III and V present [26, 27]. In contrast, cells in the inner portion of the tissue appear more round and are embedded in an ECM comprised largely of type II collagen intermingled with a smaller but significant amount of type I collagen and a higher concentration of GAGs than in the outer region. This relative abundance of collagen type II and aggrecan in the inner region is more reminiscent of hyaline articular cartilage. Therefore, cells in this region are classified as fibrochondrocytes or chondrocyte-like cells [27, 28]. A third cell population has also been recognized in the superficial zone of the meniscus. These cells possess a flattened, fusiform morphology and are absent of cell extensions. It has been suggested that these cells are possibly specific progenitor cells with therapeutic and regenerative capabilities [29]. In summary, cell phenotype and ECM composition render the outer portion of the meniscus akin to fibrocartilage, while the inner portion possesses similar, but not identical, traits to articular cartilage.

2.4 Biomechanical and Functional Properties

The meniscus withstands many different forces such as shear, tension, and compression. It also plays a crucial role in load-bearing, load transmission, shock absorption, as well as lubrication and nutrition of articular cartilage [16, 30-33]. These multiple and complex functions require a specialized form. Since the tissue is wedge-shaped, it proves highly adept at stabilizing the curved femoral condyle during articulation with the flat tibial plateau [17, 34, 35]. During everyday activity, axial tibiofemoral forces compress the menisci. The wedge shape of the meniscus and its horn attachments serve to convert the vertical compressive forces to horizontal hoop stresses (Figure 4). At the same time, shear forces are developed between the collagen fibers within the meniscus while the meniscus is deformed radially [17, 32, 36].

The biomechanical properties of the knee meniscus are appropriately tuned to withstand the forces exerted on the tissue. Many studies have helped to quantify the properties of the tissue both in humans and in animal models (Tables 1 and 2). According to these studies, the meniscus resists axial compression with an aggregate modulus of 100-150 kPa [37]. The tensile modulus of the tissue varies between the circumferential and radial directions; it is approximately 100-300 MPa circumferentially and 10-fold lower than this radially [38]. Finally, the shear modulus of the meniscus is approximately 120 kPa [38].

The contact forces on the meniscus within the human knee joint have been mapped. It has been calculated that the intact menisci occupy approximately 60% of the contact area between the articular cartilage of the femoral condyles and the tibial plateau, while they transmit >50% of the total axial load applied in the joint [6, 39, 40]. However, these percentages are highly dependent on degree of knee flexion and tissue health. For every 30° of knee flexion, the contact surface between the two knee bones decreases by 4% [41]. When the knee is in 90° of flexion the applied axial load in the joint is 85% greater than when it is in 0° of flexion [40]. In full knee flexion, the lateral meniscus transmits 100% of the load in the lateral knee compartment, whereas the medial meniscus takes on approximately 50% of the medial load [36].

Conversely, of notable interest are the changes in contact area and contact force following partial or total meniscectomy. Paletta et al. [42] investigated the biomechanical effects of total removal of the lateral meniscus in 10 cadaveric knees and reported a 50% decrease in total contact area, resulting in a 235-335% increase in peak local contact load. In a similar study, Kurosawa et al. [43] noted that following total meniscectomy the tibiofemoral contact area decreased by approximately 50%, therefore leading to an overall increase in contact

forces by 2-3 times. Correspondingly, partial (16-34%) meniscectomy has been shown to lead to a >350% increase in contact forces on the articular cartilage [44].

3. Meniscus Pathophysiology

In the United States, meniscal lesions represent the most common intra-articular knee injury, and are the most frequent cause of surgical procedures performed by orthopedic surgeons [45, 46]. The mean annual incidence of meniscal lesions has been reported to be 66 per 100,000 inhabitants, 61 of which result in meniscectomy [47, 48]. Men are more prone to such injuries than women, with a male to female incidence ratio between 2.5:1 and 4:1, and overall incidence peaking at 20-29 years of age for both sexes [47, 49, 50]. Meniscal lesions are most commonly found in the right knee [47] and occur in all age groups, with the main etiological and pathophysiological factors varying and being highly dependent upon the patient's age [46, 51].

3.1 Meniscus Tears in Young People

In young patients, sports-related (football, basketball, soccer, baseball, and skiing in particular) injuries are the most common cause of meniscal lesions, accounting for more than 1/3 of all cases [47, 49]. The underlying mechanism of these injuries usually involves cutting or twisting movements, hyperextension, or actions of great force [12]. Meniscal tearing during these sports is accompanied by anterior cruciate ligament (ACL) tearing in >80% of cases [52-58]. Most patients report an acute onset of sharp pain following a twisting injury with the knee flexed and the foot planted on the ground [59, 60]. Meniscal tears resulting from vehicle accidents are also associated with increased incidences of meniscus lesions in this particular age group [47].

Classification of meniscal injuries occurs depending on location, thickness, and resulting stability [6, 12, 61]. Thus, tears in the peripheral vascularized portion are denoted as red-red tears, in the middle third portion as red-white or white-white tears, and in the inner avascular portion as white-white tears. According to the depth of the tear, injuries are observed as partial or full thickness, of which full thickness injuries can be further categorized as stable or unstable. Another means of classifying meniscal injuries is based on tear pattern [6, 62]. This way, one can distinguish between different types of meniscal tears—the most important being vertical/longitudinal (including bucket handle), flat/oblique, radial/transverse, and horizontal/complex (including degenerative) [63]. The above categorization is highly relevant when deciding upon the most appropriate and effective therapy. Studies have shown that there is also a significant difference in tear pattern between stable knees and those with concurrent ACL lesions [64-66].

Diagnosing meniscal malfunction is greatly dependent upon both the experience and insight of a physician. A detailed patient history, a thorough physical exam, and modern imaging techniques can help guide the process towards reaching diagnostic consensus. Starting from the patient's history, an accurate description of the injury's acquisition can set the ground for suspected meniscal tearing. Patient complaints concerning pain, swelling, or ‘locking’, and diagnostic characteristics during physical examination (joint effusion, joint line tenderness), should be taken under serious consideration [12, 67]. The main tests, which will need to be conducted each time a patient's knee presents with any of the above findings, are joint line palpation, the flexion McMurray test, the Apley's grind test, and the Thessaly test [68-70]. Imaging modalities that need to be applied when diagnosing such injuries are X-ray and MRI [12].

3.1.1 Peripheral Meniscal Tears—Numerous techniques have been described and applied regarding surgical meniscus repair in the peripheral (vascular) zone. Though such

techniques undergo continuous development, surgical treatment approaches can be classified under four main categories: inside-out, outside-in, and all-inside arthroscopic techniques; and open repair [5, 6, 71]. These techniques have been extensively described in the literature, along with their accompanying complications and appropriate rehabilitation programs, and will therefore not be largely discussed in the present review.

A substantial amount of research has focused on the efficacy and reliability of these repair techniques for achieving anatomical and functional restoration of the meniscus. In general, there is an ever-increasing amount of literature supporting current meniscus repair techniques for treating tears in the vascular zone. Functionally successful outcomes in young individuals with stable knees are fairly frequent, with success rates varying from 63% to 91% [45, 57, 72-76]. However, more long-term follow-up studies need to be conducted so as to robustly confirm such evidence, and exclude the possibility of long-term degenerative deteriorations of the articular cartilage and meniscus.

3.1.2 Avascular Zone Meniscal Tears—Tears in the avascular zone of the meniscus are generally more complex and broad, and are often associated with a poor prognosis following repair. Improving the healing process in this type of injury is an ongoing challenge for clinicians and researchers. Several different therapeutic approaches have been brought forward, with a variety of reported results (Table 3). The most notable of these new approaches are: the use of parameniscal synovial tissue, trephination of the peripheral meniscus rim with suture of the meniscus tear, creation of vascular access channels, and use of mesenchymal stem cells or growth factors [77, 78].

Despite these results, none of the above techniques has been a recipient of general acceptance and application. Therefore, the main strategy for treating such tears is partial meniscectomy, with its related long-term degenerative implications for articular cartilage [79, 80]. The lack of acceptance and clinical application of the above methods is mainly due to a deficiency in long-term follow-up studies and replication of results, which could confirm these findings in a greater number of clinical cases. In future studies, evaluation of articular cartilage in a long-term follow-up is of special importance. In addition to this, investigation of the biomechanical properties of healed meniscus in experimental and clinical studies is essential.

3.2 Meniscal Tears in Older People and Children

In general, meniscal lesions occur frequently in middle-aged and elderly patients. Tears encountered in patients belonging to this age group usually result from long-term degeneration. Such meniscal lesions lead to joint swelling, joint line pain, and mechanical blocking [81, 82]. The reported prevalence of meniscal lesions in patients with clinical and radiographic findings of osteoarthritis is 68-90% [83-85]. This high correlation creates a series of diagnostic problems, mainly concerning the identification of the main pathology in a symptomatic knee. Therefore, on some occasions, symptoms that may be due to a pathological cause (such as osteoarthritis) may be attributed by the physician to the presence of a meniscal tear in MRI, while on other occasions, symptoms which may result from trauma (such as a meniscal tear) may be attributed to osteoarthritis. This has obvious repercussions on the choice of proper therapy. For example, treatment of meniscal tears with partial meniscectomy is rather unlikely to reduce symptoms caused by osteoarthritis.

Regarding the successful application of meniscus repairs in older people, less promising findings have been reported as compared to patients in younger age groups [72, 81]. The main reason behind such unfavorable results is the degenerative etiology surrounding meniscal tears in such patient groups, as well as the declining vascularization of the aging meniscus. Barrett et al. [81] reported only a small percentage (6%) of repairable meniscal

tears in this special aged patient group. In general, the current preferred intervention for the majority of surgeons is meniscectomy, either partial or total, depending on the degree of meniscal damage.

An increased incidence in meniscal tearing has recently been observed in skeletally-immature children [86, 87]. The main causative factor behind this increased incidence is the growing participation of children in highly-demanding athletic activities. Simultaneously, the expansion in health services focused on child pathology, and the extensive use of highly specialized imaging techniques such as MRI, have aided in contributing to these diagnoses.

Meniscal lesions in children are different than those in adult patients. In children, the vast majority of cases (>71%) are isolated meniscal lesions [88-90]. The main mechanism of meniscal tearing in children is sports-related twisting of the knee. In a small percentage of these cases, a common predisposing factor is a discoid meniscus [91]. Diagnosis is dependent upon the presence of a complete medical history for the patient and a clinical examination. If a meniscal tear is suspected following clinical examination, the application of an imaging technique should be pursued. Nonetheless, the sensitivity and specificity of MRI for diagnosing meniscal lesions in children is considerably less than that for adults [92, 93].

Meniscus pathology in children has received fairly limited attention in the literature with regards to repair techniques. Most of the studies in this field deal with patient groups comprised mostly of adults, with children representing a small portion of cases and with fairly short follow-ups [94-98]. In general, most of these studies report that the overall success rate for meniscal repair in children appears analogous to that observed in adults, especially for cases of isolated tears [96-98].

4. Cell Sources for Tissue Engineering the Knee Meniscus

4.1 Autologous Cells

One of the leading questions in tissue engineering is whether the engineered tissue should be an exact replica of the native tissue, or whether it should merely carry out its main functions. Several researchers argue that the development of a biomimetic replica of the native meniscus necessitates the use of a biodegradable scaffold seeded with native cells that will produce the same fibrocartilaginous ECM [99-101]. However, this approach exhibits several limitations. Two surgical interventions would be required of a patient: a biopsy to obtain autologous meniscal cells, and a second procedure to implant the tissue-engineered meniscus. Moreover, tissue scarcity and current techniques yield only a limited number of isolated cells, of which only cells from the inner part of the meniscus produce sufficient matrix GAGs [102, 103]. To tackle these issues, research has moved to the fairly simple expansion of autologous meniscal cells in monolayer culture. However, monolayer expansion of meniscus cells leads to significant downregulation of ECM gene expression [104]. Similarly, some approaches have included the use of autologous chondrocytes for meniscal tissue engineering, as they have proven to produce more GAGs and collagen II compared to meniscal cells after expansion, although they too undergo differentiation [99, 105, 106].

Though the development of functional tissue-engineered meniscus constructs has advanced, important problems still persist. The limited ability of cell isolation for large-scale constructs is a notable example. Furthermore, the dedifferentiation of cells after expansion, as well as the possibility of autologous cells already being in either a degenerated state, or in an age-related disease state, deem their utility in tissue engineering questionable [107]. For these reasons, a wide variety of cell sources may be considered for meniscus tissue engineering.

4.2 Allogeneic and Xenogeneic Cell Sources

The realization of the fact that the isolation of a sufficient number of healthy, undifferentiated meniscal cells extracted from an injured meniscus ranges from difficult to impossible, and the need for alternative cell sources, has led many researchers to use allogeneic cells for meniscus tissue engineering [25]. The first trials in this direction have been largely based on the positive healing outcomes of allogeneic articular, auricular, and costal chondrocytes in lesions in the avascular zone of the meniscus, in a large animal model study [108]. In another study, both autologous and allogeneic chondrocytes were seeded in a degradable scaffold and implanted in 17 pigs to repair previously inflicted bucket-handle meniscal tears [109]. After 12 wks, the authors found that both allogeneic and autologous-based scaffolds were capable of promoting healing when compared to the control group. The fact that no statistically significant therapeutic outcome was found between the two cell-based implants is of importance, as it suggests that the use of allogeneic cells is feasible [109].

In terms of the use of xenogeneic cells in tissue engineering, increasing amounts of research seem to strongly encourage their use [110-112]. In a study by Ramallal et al. [110], the investigators created cartilage defects in the femoral condyle of 30 rabbits and subsequently tried to repair the injury by suturing a periosteal flap to the articular cartilage, while also infusing cultured pig chondrocytes into the defect void. After 24 wks, the authors reported the appearance of articular cartilage neotissue, which integrated with the native tissue, and an overall lack of measurable immune response. In another study, researchers used four different types of cells: allogeneic chondrocytes, MSCs, fibroblasts, and human umbilical cord blood (hUCB) stem cells, and embedded them in PLA scaffolds to repair cartilage defects in a rabbit model [111]. Although they found better results when using allogeneic MSCs, they reported no immune response when using xenogeneic hUCB stem cells, motivating further investigations in this area. The results from these studies point towards the possibility of using xenogeneic cell sources in meniscus tissue engineering, which is further supported by unpublished data from our lab based on both *in vitro* and *in vivo* studies.

4.3 Human Embryonic Stem Cells

Lately, an increasing interest in the use of stem cells for regenerating destroyed or degenerative tissue (such as articular cartilage, meniscus, intervertebral disc, TMJ disc, and heart muscle) has been shown [113-117]. Stem cells can play an important role in rectifying meniscal damage through their ability to differentiate and regenerate tissue, and through their ability to produce cytokines and growth factors [118]. Human embryonic stem cells (hESCs) have proven to be an emerging cell source for fibrocartilage tissue engineering [117]. Some of the main characteristics which make this cell source ideal for tissue engineering are pluripotency and unlimited proliferative capacity [119, 120]. Attempts towards tissue-engineering the meniscus using this cell source are still in early phases. A main step in this direction was made by Hoben et al. [121], who investigated hESCs' differentiation potential into fibrochondrocyte-like cells, and characterized the resulting differentiated cells. In this study, hESCs were cultured with growth factors (TGF-β3, BMP-2, BMP-4, BMP-6, PDGF-BB, sonic hedgehog protein), and/or primary cells (chondrocyte or fibrochondrocytes) for 3 wks. Following this time, their ability to produce GAGs and collagen types I, III, and VI was assayed, along with the presence of certain surface markers (CD105, CD44, SSEA, PDGFRa). Following comparison of these treatments, results showed that the combination of TGF-β3 with BMP-4 yielded embryoid bodies positive for collagen type I, II, and VI with 6.7 and 4.8-fold increases in GAG and collagen, respectively. Also, co-culture with fibrochondrocytes led to 9.8-fold increases in collagen II production. Results from this study point to the suitability of hESCs for meniscal

tissue engineering and highlight at least 3 effective strategies to create hESC-derived fibrocartilage [121].

4.4 Adult Stem Cells

While the usage of hESCs in meniscal tissue engineering remains at a preliminary stage, many studies have focused on using mesenchymal stem cells (MSCs) as a potential cell source. MSCs are multipotent progenitor cells of stromal origin whose main source is adult bone marrow, although they may be isolated from other tissues in both adults and fetuses [122-125]. The large scientific interest surrounding these cells is due to two main abilities. First, MSCs have been observed to differentiate into many terminally-differentiated cells which synthesize mesenchymal tissue (i.e., cartilage, bone, ligaments, muscle, fat, dermal, and other connective tissue), and can therefore be used to engineer mesenchymal-derived tissue [126]. Second, MSCs secrete a large variety of immunoregulatory molecules, and contribute to the healing process of injured tissue by providing paracrine trophic mediators [118].

Different strategies for using autologous connective tissue progenitors in MSC-based tissue engineering have been described in the literature. An approach that has been investigated by many researchers is *in situ* activation of the migration, proliferation, and differentiation of local MSCs. This can be achieved by the transplantation of an acellular scaffold [127] or by the local administration of growth factors such as VEGF, which activates these MSC functions [128, 129].

Another strategy is the local administration of autologous MSCs to replenish the population of local cells which has been diminished due to trauma, degeneration, tissue defects, or compromised vascularity. Currently, many surgeons use this method to transplant bone marrow-derived stem cells (BMSCs) for bone healing applications due to its high value and low risk and cost [130]. This strategy has been the center of much interest as a therapeutic approach for rehabilitating meniscal lesions. Some of the main techniques utilized with this approach include the creation of vascular access channels and vascular tunnels by trephination or rasping in the vascular region of the meniscus. This allows the influx of blood—and subsequently MSCs—into the damaged avascular area [77, 78, 131, 132]. Other techniques use vascularized synovial flaps or fibrin clots, based on the same rationale [133-138]. Results of these techniques appear to conflict in the existing literature.

The transplantation of expanded or modified autologous MSCs is another approach in MSC-based tissue engineering. The first attempt of this took place in 2005, when Izuta et al. [139] used autologous BMSCs from green fluorescent protein transgenic rats; these were isolated, expanded in monolayer culture, and then transplanted into meniscal defects inflicted in the avascular zone. After 8 wks follow-up, the investigators found that MSCs could survive and proliferate in the meniscal tears while also developing an extensive extracellular matrix, aiding the healing process in the avascular meniscus [139]. Similarly, investigators in another study reported that autologous BMSCs injected into meniscal wounds of eight canines improved healing [140]. In the same light, studies have proven the use of MSCs seeded onto scaffolds for meniscus tissue engineering as a rather effective one [141, 142]. Another study showed that undifferentiated MSCs, as opposed to precultured cells, display a more potent healing response [143]. Here, the investigators studied the therapeutic value of autologous MSCs in meniscal tissue lesions in a rabbit model by comparing their action to that of platelet-rich plasma and autologous BMSCs. More specifically, they created circular meniscal punch defects (2mm), and either left the resulting gap intact, or covered it with hyaluronan-collagen composite matrices using one or none of the above cell categories. Of notable importance is that some of the stem cell matrices were precultured in chondrogenic medium for 14 days prior to transplantation. Twelve wks after transplant the researchers

concluded that the non-precultured autologous MSCs led to integrated meniscus-like repair tissue, while the precultured MSCs led only to partial integration [143].

Undoubtedly, the expansion of MSCs *in vitro* has significant advantages and disadvantages. The primary advantage of expansion is an increase in cell number. Disadvantages include possible cell infection during culture, as well as decreased capacity for proliferation prior to implantation [144-146]. Finally, another danger not extensively reported on in the literature is the development of tumor-like abnormalities following implantation of precultured autologous MSCs with mutations or epigenetic changes [130]. This appears to be a relatively unexplored topic, showing the need for more studies.

Finally, another strategy utilizing MSCs is *ex vivo* tissue differentiation/generation, with subsequent transplantation of this tissue. Research using this approach is currently being conducted in the authors' lab to generate fibrocartilaginous tissues, including meniscus tissue. The main challenges in this field coincide with the main challenges of tissue engineering in general: development of functional tissue mirroring the composition of native tissue, which will satisfactorily integrate with the host, and which will allow long-term preservation of cell viability and meniscus function.

5. Scaffolds for Tissue Engineering the Knee Meniscus

Scaffolds for tissue engineering the meniscus may be categorized into four broad classes: synthetic polymers, hydrogels, ECM components, or tissue-derived materials. Synthetic polymers are materials that do not exist in the body, at least not in polymer form. Hydrogels are hydrophilic colloids capable of holding large amounts of water, and may be derived from natural or synthetic sources. ECM component scaffolds are comprised of whole materials formed primarily from a component macromolecule of natural matrix, such as collagen or hyaluronan. Finally, tissue-derived materials include decellularized ECM and other significant components or byproducts of living tissue such as small intestinal submucosa. Importantly however, these four categorizations are not mutually exclusive, and rather serve as a broad guide to appreciate significant differences in properties among scaffolds. Hybrids and composites between these materials also exist. Since cell-seeded polymers consistently outperform acellular scaffolds in terms of regenerative capacity [101, 147, 148], this section will primarily focus on studies examining the capabilities of scaffolds incorporating cells.

The ideal meniscus construct will excel in three criteria: mechanics, bioactivity, and logistics (Table 4). Since heterogeneous loading of the meniscus occurs every day *in vivo*, appropriate mechanical properties, tissue anisotropy, geometry [149], and lubrication are requirements of the mechanics criterion. Any implanted meniscus construct will also need to display sufficient bioactivity. This means maintenance of cell phenotype, induction of ECM synthesis, lack of immunogenicity, and capacity for host-tissue integration. Finally, the logistics of a successful construct must not be unwieldy: supply, processability, sterilization, and eventual surgical implantation must all be practical.

5.1 Synthetic Polymer Scaffolds

Synthetic polymers, such as polyurethane (PU), polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA), and polylactic co-glycolic acid (PLGA), hold several advantages, including fabrication under a variety of methods, near-limitless supply, and the potential to achieve appropriate pore size, fiber size, mechanical properties, and scaffold geometry. These advantages are countered by a central weakness—minimal intrinsic biomimetic and bioactive properties. By contrast, the mechanical properties of some more highly bioactive scaffolds, such as small intestinal submucosa, are considerably less than that of some synthetic polymers [158-160]. The lack of inherent biological support among

synthetic scaffolds has motivated the exploration and use of many synthetic scaffolds as acellular meniscus prostheses, which provide some biomechanical functionality, as well as modest tissue regeneration, when implanted [150-157].

Tissue engineering has spurred recent advances in synthetic polymer scaffolds that emphasize and build upon the advantages stated above. Methods for generating mechanical anisotropy within synthetic cartilage scaffolds are one example. This characteristic is essential, since loading of the meniscus *in vivo* is highly non-uniform [38, 39]. It has been demonstrated that fibers in PCL scaffolds may be preferentially aligned by the use of a rotating collection platform during electrospinning [161]. When evaluated mechanically, these scaffolds exhibited a 33-fold change in tensile moduli if tested in the direction parallel versus perpendicular to fiber alignment [161]. Aligned scaffolds can also subsequently promote cell and ECM orientation [162-165]. It has been found that aligned PCL scaffolds seeded with meniscus cells and cultured over 10 wks display a 7-fold greater increase in tensile modulus in the direction of alignment than corresponding non-aligned scaffolds [162]. Importantly, collagen per DNA was not statistically different between aligned and non-aligned scaffolds, suggesting differential organization of the existing ECM [162], although some studies have also reported that scaffold alignment serves to increase matrix deposition [166, 167]. Regardless of the underlying mechanism, scaffold orientation appears to have a beneficial effect.

Recent work has also demonstrated that scaffolds may be physically woven to further increase their compressive, tensile, and shear properties and to introduce scaffold anisotropy [168, 169]. In this strategy, a custom-built weaving loom was used to produce anisotropic PGA or PCL scaffolds with mechanical characteristics generally on the same order of magnitude as native articular cartilage [168, 169]. These woven scaffolds may be combined with hydrogels to make composites capable of supporting seeded articular chondrocytes or adipose-derived stem cells [168, 169]. Although these studies focused on engineering articular cartilage, they would also be highly relevant for recapitulating the mechanical properties and anisotropy of the knee meniscus.

Other recent advances focus on making synthetic polymers more biomimetic and bioactive. One group of investigators recently reported the fabrication of a peptide scaffold sensitive to matrix metalloproteinase-2 (MMP-2) degradation [170]. Although this scaffold has not yet been applied to meniscus tissue engineering, it may greatly aid in coupling cell-mediated matrix remodeling to scaffold degradation. Since native components of the ECM are nanoscale molecules, nanofibrous scaffolds may also help to coax cells to behave as they do in native matrix. One study comparing PLLA nanofiber and microfiber scaffolds reported increased production of sulfated GAGs, cartilage link protein, collagen II, and aggrecan by bovine chondrocytes seeded in the nanofiber scaffolds [171]. Some synthetic polymers may also inherently provide a more biomimetic environment than hydrogels for meniscus cells. A comparison of meniscus cells cultured in PGA or agarose scaffolds over 7 wks reported 2 to 6-fold higher cell numbers, 2 to 4-fold higher GAG production, and 3-fold greater collagen production in the PGA scaffolds [172]. The authors concluded that cell proliferation and ECM synthesis may be reduced when meniscus cells are forced to assume a round morphology in highly hydrophobic agarose, since meniscus cells display a morphology and phenotype that is representative of elongated fibroblasts as well as chondrocytes [173, 174]. This conclusion is corroborated by other studies of fibroblast functionality in gels, where proliferation diminished as cell spreading was restrained [175, 176].

Despite the recent advances described above, the main disadvantage of synthetic polymer scaffolds still lies in facilitation of the development of a functionally-robust matrix prior to scaffold degradation *in vivo*. Future research in this direction is needed. Integration of

synthetic polymer tissue constructs with neighboring host tissue also remains an issue to tackle. Finally, fine-tuning of synthetic polymers, so as to promote joint lubrication and to prevent tissue wear and tear at bone-cartilage interfaces, also represent avenues for further research.

5.2 Hydrogel Scaffolds

Hydrogels have also been investigated for use as meniscus scaffolds. Hydrogels can be synthetic materials such as poly N-isopropyl acrylamide (PNIPAAm), or natural materials such as alginate. The physical properties of hydrogels are largely influenced by their water content, which is often >90%. Hydrogels are also versatile—they may be crosslinked through various methods [177-179], reversibly gelled [180], and patterned with cells [181-184] and growth factors [185, 186]. Many hydrogels can also be synthesized from readily available reagents. However, hydrogels may hamper meniscus cell phenotype by preventing encapsulated cells from assuming their characteristic spread fibroblastic morphology [172, 173, 175]. Additionally, the mechanical properties of hydrogel scaffolds are not as easily manipulated as in synthetic polymers. Finally, hydrogels such as polyvinyl alcohol (PVA) [187, 188] and fibrin [189, 190] have been investigated as acellular meniscus materials, but these will not be focused on in this review.

Much research has focused on utilizing the versatile chemistry of hydrogels to create more biomimetic structures. The chemical functionalization of hydrogels is one strategy that has been pursued to create a more native microenvironment for cells. Hybrid hydrogels (chitosan-alginate-hyaluronan) have been conjugated with the adhesive arginine-glycine-aspartic acid (RGD) polypeptide and cultured with articular chondrocytes over 1 to 2 wks to show higher collagen and GAG content over unconjugated controls [191]. Hydrogels have also been functionalized to undergo proteolytic degradation by MMPs [192-194]. Both of these approaches are highly relevant to meniscus tissue engineering, but further work is needed to explore these scaffolds in conjunction with meniscus cells. Hydrogel co-cultures may also be created by spatial patterning of different cell types [182-184], using insoluble adhesion molecules or sequential photopolymerization. Fibroblasts have been co-cultured with BMSCs in this manner [183], although the diverse meniscus cell subpopulations have not. This method is one way in which the regional cellular variation of the meniscus may be replicated.

Another principal advantage of hydrogels is their ability to reversibly gel in response to environmental factors such as temperature, pH, electric field, ultrasound, or salt concentration [180]. This has allowed for the development of “smart” biomaterials whose design allows for responses according to the environment, which is favorable for tissue engineering because injectable scaffolds that solidify in the body can be produced. Building on this concept, Chen et al. [195] have produced temperature-sensitive chitosan-hyaluronan-PNIPAAm hybrid gels which maintain meniscus cell viability, encourage native matrix synthesis, and reversibly solidify in response to temperature. Though an injectable knee meniscus hydrogel is minimally invasive and enticing, a major limiting factor lies in the scaffold's insufficient mechanical properties after solidification. This deficiency may potentially be modulated through increased hydrogel crosslinking, but some crosslinking methods have been shown to affect cytotoxicity and cellular metabolism [196, 197].

Other tissue engineers have taken advantage of various methods to create cell-seeded hydrogel scaffolds that accurately represent the complex geometry of the meniscus. One method combines the imaging capabilities of computed tomography or MRI with robotic printing to automate creation of a geometrically-accurate model of the meniscus [177, 198]. Alginate scaffolds seeded with bovine meniscus cells in high density (50 million/mL) in this manner displayed a high geometrical fidelity to the target shape, high cell viability, and

some properties similar to native tissue (50% of aggregate modulus, 33% of GAG content, but 2% of hydroxyproline content), though tensile properties were not examined [198]. However, tissue grown in this manner was highly heterogeneous, possibly due to limited transport in the fairly large construct. Subsequent work demonstrated the use of a magnetic stir bar “mixing bioreactor” to produce constructs with a larger degree of homogeneity, higher equilibrium and tensile moduli, and greater ECM deposition, although detrimental effects were observed at higher stirring intensities [199]. This work underscores the necessity of, and opportunities and challenges associated with, bringing together diverse tools (imaging modalities, processing methods, bioreactors) to successfully tissue engineer the knee meniscus.

Hydrogels represent a versatile class of tissue engineering scaffolds, but their mechanical properties (especially in tension) and bioactivity (especially in promoting meniscus cell phenotype and ECM synthesis) need to be improved. Cell-adhesive hydrogels have been created, and these may help with cell spreading and phenotype issues [176]. Other research has been directed towards ECM molecule hydrogels, yielding studies on elastin-like polypeptide [200, 201] and collagen-mimetic peptide [202, 203] hydrogels. Further research into these approaches may combine the bioactivity of ECM molecules with the versatility of hydrogel scaffolds.

5.3 ECM Component Scaffolds

ECM component scaffolds are materials formed primarily from a macromolecule abundant in native matrix. Examples include collagen meniscus implants or hyaluronan scaffolds. Combinations of these molecules may also be used (i.e., collagen-GAG scaffolds or scaffolds containing multiple types of collagen). Collagen scaffolds in particular are amenable to several fabrication and processing methods, including nanofiber electrospinning, anisotropic deposition, and crosslinking. Because of these methods, ECM scaffolds may possess strength comparable to synthetic scaffolds. As far as bioactivity, ECM scaffolds would logically constitute a natural environment for seeded cells. Yet, although these scaffolds are made of natural matrix, they may not completely recapitulate the cell microenvironment (i.e., the collagen VI pericellular matrix, collagen IX crosslinks, etc.). In addition, some other scaffold materials such as silk have also been shown to more robustly promote matrix deposition compared to collagen scaffolds [204, 205]. Yet, in general, ECM component scaffolds are more intrinsically biomimetic than synthetic and hydrogel materials.

Since meniscus cells normally rest in a dense network of collagen and GAG molecules, scaffolds made from these components would logically provide a natural environment for the regeneration of meniscus tissue. Interestingly, not all ECM molecules are equally effective. An early study showed that GAG-collagen II matrices promoted more meniscus cell proliferation, more GAG deposition, and less contraction versus GAG-collagen I matrices [206]. Other researchers have shown that aggrecan surfaces are more effective in encouraging meniscus cell ECM deposition than collagen I surfaces [104]. HYAFF-11 is another ECM component scaffold, made by modifying the glucoronic acid groups in hyaluronan. A study comparing meniscus cells seeded in HYAFF-11 and collagen scaffolds (types I, II, and III) found no differences in GAG and collagen I synthesis [207]. Thus, a variety of results have been observed when comparing the efficacy of different ECM component scaffolds. More research into these ECM component scaffolds and combinations of these scaffolds is needed.

Scaffolds, and especially ECM component scaffolds, can exert a strong effect on seeded cells through the microenvironment they provide. A recent investigation developed a hybrid scaffold consisting of chitosan, hyaluronan, chondroitin-6-sulfate, collagen I, and collagen II

molecules [208]. Rat meniscus cells passaged in monolayer underwent conventional dedifferentiation, but those subsequently cultured in these hybrid scaffolds underwent partial redifferentiation over 1 wk. Results from RT-PCR demonstrated the upregulation of collagen I, collagen II, and aggrecan, although not to the levels seen prior to passage [208]. Although these results are exciting in that they show scaffolds may induce redifferentiation of previously dedifferentiated meniscus cells, further studies are needed to characterize the matrix deposited in these systems, especially over longer culture periods.

From a clinical perspective, ECM scaffolds have received perhaps the most attention of all scaffold categories, due to the use of collagen meniscus implants. The collagen meniscus implant is a surgical mesh composed of bovine collagen type I, crosslinked with aldehydes, and molded in the shape of the lateral or medial menisci [209]. A multi-center clinical trial of collagen meniscus implants showed greater tissue restoration 1 year after operation compared to partial meniscectomy; and activity levels also rose in chronic sufferers of meniscal problems 7 years after implantation [210]. Smaller non-randomized trials report positive patient outcomes over longer periods, with losses in pain and higher activity levels documented [211, 212]. Despite these results, significant scientific and clinical drawbacks associated with the collagen meniscus implant exist. The implant is not an option for patients who have undergone total meniscectomy. In addition, once implanted, scaffold degradation and shrinkage as well as shape incongruency remain significant issues [213, 214]. The technical difficulty of suturing the implant also limits its use [213, 214]. Finally, this acellular scaffold's primary mode of healing is thought to be through host cell migration and subsequent synthesis of meniscus matrix, yet results in sheep have indicated more developed healing if collagen meniscus implants are seeded with autologous fibrochondrocytes [101]. In this work, seeded constructs were significantly larger than unseeded constructs or resection controls after 3 wks of implantation. In addition, histology showed greater ECM deposition and lower cellularity in seeded constructs, suggesting accelerated matrix remodeling [101]. This lends credit to cell-based tissue engineering. Lastly, as of this writing, the FDA approval granted to the collagen meniscus implant in 2008 has been rescinded, and the device has been removed from clinical use.

In general, ECM component scaffolds display a mix of desirable traits between mechanics, bioactivity, and logistics. This category may hold the most promise amongst scaffold-based approaches for meniscus tissue engineering. However, the technology and use of these materials is still relatively new, and the efficient incorporation and development of suitable replacement tissue within ECM scaffolds *in vivo* and *in vitro* remains a topic for more investigation. The introduction of appropriate lubrication, and the modulation of ECM component scaffold degradation kinetics, also present opportunities for further research in functional tissue engineering.

5.4 Tissue-Derived Scaffolds

Tissue-derived materials comprise the final category of scaffolding currently being investigated for engineering the knee meniscus. Tissue-derived materials include processed whole tissue such as small intestinal submucosa (SIS), decellularized tissue or ECM (dECM), and silk. The hypothesis of using such materials is similar to that of using ECM components: they constitute a natural environment for cell seeding, migration, and ECM deposition. Though geometric fidelity and bioactivity of these scaffolds can be high, they must be procured from natural tissue, and thus supply is problematic. In addition, some decellularization and processing protocols compromise the mechanical integrity of these tissues.

Several investigations have demonstrated the comparatively high bioactivity of processed whole tissue scaffolds. A study of passaged and seeded canine chondrocytes in SIS and

PLGA scaffolds, implanted in athymic mice, reported that sulfated GAG and hydroxyproline content was higher in the SIS scaffolds, although collagen II was present only in PLGA scaffolds [158]. SIS has also demonstrated superiority to other tissue-derived meniscus scaffolds. In a fairly recent study, three dermis isolates (human, fetal bovine, and crosslinked porcine) were compared against two small intestine isolates (porcine and crosslinked porcine) in a rat model [215]. Canine meniscal cells, synoviocytes, tendon fibroblasts, and bone marrow progenitor cells were seeded in co-culture in all five scaffolds, and porcine small intestine (principally the non-crosslinked scaffolds) displayed the greatest capacity for encouraging retention, infiltration, and viability of these cells [215].

Though processed whole tissues such as SIS display significant bioactivity and have been seen to induce some tissue regeneration, the resulting tissue mechanics may be insufficient, which subsequently compromises knee function. An early work studied unseeded porcine SIS implanted in surgically-created canine medial meniscus defects [216]. After 12 wks, improved lameness scores, less articular cartilage erosion, and some tissue growth and retention were observed over controls [216]. Similar results have also been reported in a longer-term study with SIS specimens assessed after up to 12 months of implantation, although the study also reported samples with biomechanics inferior to contralateral meniscectomy [217]. A contrasting study reported meniscal regeneration but increased articular cartilage degeneration (compared to contralateral controls) in the knee joints of goats implanted with unseeded porcine SIS [218]. The fact that tissue growth seems apparent in these studies, but inferior biomechanics and/or cartilage degeneration are observed simultaneously, highlights the possibility that this regenerated tissue is mechanically insufficient.

Other research centers on the considerable difficulty of creating tissue-derived scaffolds with appropriate pore sizes. Studies of native menisci have deemed pore sizes of 100-150 microns as appropriate for meniscus cells [219], yet cell infiltration can be highly variant through the depths of both whole processed tissue (SIS, dermis, etc.) [215] and decellularized meniscus [220], likely due to the dense matrix present even after processing. However, other recent work has achieved progress by increasing decellularized ovine menisci porosity (to a value of 80% in the outer meniscus) as well as connected pore volume, although residual DNA content was still significant and compressive properties trended lower [221].

Though decellularized tissue scaffolds are promising, several studies have reported decreased mechanical properties (especially compressive properties) due to the treatment protocols used for decellularizing tissue. Losses in GAG content are similarly reported. A variety of alternate treatment methods have been investigated. One recent study demonstrated no reaction to MHC1 and MHC2, and preservation of, or even increases in, compressive stiffness after ovine menisci were treated with a self-developed enzymatic solution [222]. Further, ovine meniscus cells were successfully cultured within these scaffolds over 4 wks. Despite these positive findings, a 3-fold GAG loss and non-uniform cell distributions were observed within the re-seeded scaffolds [222]. Decellularization of human meniscus has also been performed [223]. This investigation revealed that collagen structure within the meniscus was intact, and mechanical properties were comparable to native tissue, after a 2 wk treatment with 2% sodium dodecyl sulfate (SDS) [223]. However, the use of SDS is associated with many detrimental side effects. Other work has shown that it is possible to remove the vast majority of cellular DNA as well as the primary xenogeneic epitope galactose-α-1,3-galactose, though significant GAG loss is also observed with this procedure [224].

Despite their advantages, tissue-derived meniscus scaffolds suffer from several drawbacks. Uniform cell infiltration and preservation of mechanics and chief ECM components such as GAGs are two areas for future research. Additionally, biological and mechanical performance after recellularization and *in vivo* implantation represent areas for further exploration. Some work has been done in this regard with devitalized rat menisci re-seeded with BMSCs [220, 225]. Constructs displayed cell migration and increases in compressive stiffness over 4 wks, but collagen and GAG content was not assayed [220]. More work in this area needs to be pursued. By bringing together some of the approaches reviewed above, it may be possible to resolve these problems. However, the bottlenecks of supply, sterilization, and standardization of tissue-derived scaffolds still need to be addressed for a large-scale tissue engineering solution to be obtained.

6. Scaffoldless Self-Assembly of Tissue

The paradigm of tissue engineering has traditionally been defined as the combination of replacement cells, cell-signaling stimuli (mechanical or chemical/biochemical), and supporting scaffolds (Figure 5). Although the use of these three elements in combination comprises the classical approach to engineering replacement tissue, in recent years cell self-assembly has begun to gain recognition and support in generation of functional cartilage, fibrocartilage, vasculature, and retina [226-231]. In cartilage and fibrocartilage, the self-assembly approach supersedes the need for scaffolds by seeding cells in very high density, possibly promoting cell-cell adhesion, cell-matrix adhesion, and cell-cell signaling; and encouraging cells to rapidly develop and integrate into a matrix with quantifiable mechanical properties [226, 232, 233]. Characterization of this process in articular chondrocytes shows high expression of N-cadherin during cell coalescence, followed by collagen VI pericellular matrix synthesis, and finally collagen II and GAG ECM synthesis [233].

Obviating the need for a support scaffold conveys key tissue engineering advantages. First, the natural synthesis of and adherence to cartilage ECM as it develops bestows the most bioactive microenvironment of any approach. Second, the all-biologic construct greatly increases likelihood of integration with host tissue. Third, since no material is completely biocompatible in every use, the lack of a scaffold diminishes further contributions to an immune response. Fourth, removal of any degradation products minimizes potential toxicity and allows for greater cell viability. Fifth, stress-shielding effects exerted by scaffolds are mitigated. More robust and homogeneous mechanical stimulation is possible during tissue development, which is particularly pertinent for engineering the knee meniscus. Sixth, since self-assembled tissue has a continuous ECM, it may be more fully able to remodel itself in response to catabolic exogenous agents such as chondroitinase ABC (C-ABC).

Indeed, self-assembled constructs have been shown to respond well to treatment with exogenous agents [234-236]. A meniscus tissue engineering study using bovine meniscus cells and articular chondrocytes found that treatment with TGF-β1 and C-ABC led to a 196% increase in collagen per wet weight, a 136% increase in compressive instantaneous modulus, a 68% increase in compressive relaxation modulus, a 600% increase in circumferential tensile modulus, and a 500% increase in radial tensile modulus [232]. Circumferential and radial tensile moduli were also significantly different, mirroring prior results indicating anisotropic collagen fiber alignment in scaffoldless meniscus constructs [232, 237]. It is thought that confinement in circular agarose molds during self-assembly may allow for the development of circumferential contractile forces on coalescing neotissue, thus aiding in the development of tissue anisotropy [237].

Mechanical stimulation of scaffold-free and self-assembled tissue has also shown promising results [229, 232, 238]. Self-assembled articular cartilage has been shown to withstand hydrostatic pressures of up to 10 MPa and respond positively by increasing aggregate modulus (96%), Young's modulus (92%), collagen per wet weight (51%) and GAG per wet weight (52%) [235]. A somewhat similar study of scaffold-free porcine chondrocyte constructs demonstrated beneficial responses to direct compression stimulation at strain amplitudes of 5-20% [229]. Following this loading, 200-300% increases in construct stiffness and a 250% increase in Young's modulus was measured [229]. Since the knee meniscus is also loaded in cyclic direct compression *in vivo* [43, 239] these results are promising for tissue engineering of the meniscus using scaffold-free self-assembly.

It is important to note that while this method is scaffold-free, it is not devoid of the utilization of biomaterials. Agarose hydrogel plays an important role in the self-assembly process as a hydrophobic negative mold used to prevent cell-substrate adhesion during tissue coalescence. Interestingly, it has also been shown that the compliance and surface roughness of this mold can alter the biomechanical properties (strength, stiffness) and even biochemical content (cellularity, collagen I/II, GAG) of self-assembled constructs [240]. It is possible that still other hydrogels and biomaterials may function to significantly modulate the quality of tissue formed from scaffold-free self-assembly.

7. Biochemical Stimuli in Meniscus Tissue Engineering

A large variety of biochemical stimuli have been applied in meniscus tissue engineering investigations. Growth factors are the most prominent biochemical stimuli for tissue engineering the knee meniscus (Table 5). Overall, for meniscus cell proliferation, b-FGF in particular has been seen to elicit a strong response [241-244]. One group studied the ability of nine growth factors (EGF, b-FGF, TGF-α, PDGF-AB, a-FGF, TGF-β1, PDGF-AA, IGF-I, and NGF) to stimulate proliferation of meniscus cells in monolayer over 4 days [241]. Of these nine, b-FGF, PDGF-AB, EGF, and TGF-α encouraged proliferation, with b-FGF inducing the greatest effect [241]. These four growth factors also promoted increased collagen synthesis of meniscus cells [241]. Another study compared monolayer proliferation of meniscus cells from the different tissue regions (inner/middle/outer) [245]. An up to 3-fold increase in DNA synthesis was demonstrated when PDGF-AB, HGF, and BMP-2 were applied to these cultured cells, while IGF-1 had no such effect [245]. Interestingly, cells from different regions responded differently, with BMP-2 having a slightly stronger effect on cells from the middle zone, and HGF exerting a slightly stronger effect on cells from the inner zone [245]. The effects upon monolayer meniscus cell migration were also examined. PDGF-AB and HGF stimulated migration in cells from all three zones of the meniscus, while EGF, IGF-1, IL-1, and BMP-2 promoted cell migration only in specific zones of the meniscus (outer and inner, middle and inner, outer and middle, and only middle, respectively) [245].

Aside from proliferation and migration, another chief function of growth factors in meniscus tissue engineering is to stimulate matrix synthesis. The TGF-β family, regarded as one of the most important for cartilage tissue engineering [246-248], has repeatedly demonstrated the ability to heighten meniscus cell synthesis of matrix proteins [102, 103, 232]. Since the ECM largely confers the mechanical properties which underlie the primary functions of the knee meniscus, this is particularly salient. An early study demonstrated increased proteoglycan synthesis of meniscus cells in monolayer, explant, and scaffold culture when treated with TGF-β1 [103]. Increased cell proliferation was also observed, in only the monolayer cultures [103]. Additionally, in scaffold and monolayer studies comparing TGF-β1, IGF-1, b-FGF, and PDGF-AB, only TGF-β1 stimulated significant simultaneous production of both collagens and GAGs over controls [244, 249]. TGF-β1 has also been

seen to up-regulate the expression and secretion of lubricin, or superficial zone protein (SZP) [250]. This protein is thought to provide essential function to cartilage by aiding in lubrication. By contrast, the same study found that interleukin-1β decreased SZP protein content and gene expression [250]. Finally, TGF-β has also interestingly been shown to inhibit meniscus cell proliferation [241]. This highlights the proliferation/production interplay in which meniscus cells are preferentially driven to one function or the other.

One potentially important function of growth factors may be to modulate matrix contraction. Both fibroblasts [251] and articular chondrocytes [252] exert local contractile forces on their surrounding matrices. Contiguous tissue constructs may actually benefit from controlled contraction, because ECM compaction and alignment can lead to anisotropy and greater mechanical properties. In fact, inhibition of fibroblast-mediated contraction has been shown to disrupt development of tendon mechanical properties [253]. Too much contraction, however, can render constructs of incorrect geometry [237]. However, the use of controlled contraction as a biophysical means of modulating cartilage development is relatively scarce in the literature. Both TGF-β1 and PDGF have been documented as growth factors involved in encouraging matrix contraction by meniscus cells, fibroblasts, and articular chondrocytes [232, 254-256]. FGF-2 and IGF-1 can also induce articular chondrocyte-mediated contraction of collagen II/GAG gel scaffolds [257]. The continued exploration of this topic may lead to interesting advances in the field.

Phenotype maintenance or cell differentiation to fibrochondrocytes is another vital application of growth factors in meniscus tissue engineering. Relatively little work has been done in this area. However, it has been found that meniscus cell phenotype may be salvaged by exposure to FGF-2 during monolayer expansion [258]. Subsequent 3-D pellet culture of FGF-2 exposed meniscus cells revealed a 200-fold higher expression of collagen II and GAG than controls [258]. Fibrochondrogenic differentiation of human embryonic stem cells has also been performed [121]. CDMP-1 has also been explored in a PGA scaffold modality to enhance fibrochondrogenesis of dermal fibroblasts [259], and has been demonstrated to increase proteoglycan content and collagen II gene expression [260]. Lastly, exposure to TGF-β1 has also been suggested to push meniscus fibrochondrocytes towards a more chondrocytic phenotype [102]. Since meniscus fibrocartilage is a tissue with varying regions, either similar to or distinct from the hyaline articular cartilage produced by chondrocytes, this is a relevant result for prospective tissue engineers. These varying results demonstrate considerable potential in, and promise for, further investigations of fibrochondrogenic differentiation of cells.

Chondroitinase ABC (C-ABC) is another biochemical stimuli that has been employed in cartilage tissue engineering. This enzyme cleaves chondroitin and dermatan sulfate from proteoglycan chains while leaving collagen fibers unaffected [261, 262]. It has been suggested that a dynamic balance between the swelling pressure caused by proteoglycans and the restraining strength of the collagen network exists [263]. Subsequently, it has been hypothesized that enzymatic depletion of cartilage GAG content (which is afterwards recovered by cellular synthesis) may facilitate increased collagen network alignment and density, leading to heightened tissue tensile properties [232, 264-266]. Indeed, serum-free C-ABC treatment of tissue-engineered articular cartilage (in both self-assembled and agarose scaffold forms) has resulted in increased tensile properties versus untreated controls, as well as recovery of GAG content and compressive stiffness after 2 to 4 wks of culture post-treatment [264-266]. The repeated beneficial results of C-ABC on tissue-engineered articular cartilage motivate its use for tissue engineering meniscus fibrocartilage. Along these lines, self-assembled meniscus constructs (composed of meniscus cells and articular chondrocytes) treated with C-ABC have been seen to display approximate 2 to 3-fold increases in tensile modulus over untreated controls and GAG recovery after 3 wks of

culture post-treatment [232]. However, more studies using C-ABC for meniscus tissue engineering, especially in conjunction with other stimuli, are necessary.

Biochemical stimulus selection is not clear-cut, and the study of multiple agents (especially growth factors) in conjunction necessitates additional investigations. Culture conditions play a non-trivial role in modulating cell responses to biochemical stimulus administration, whether the treated tissue is arranged in monolayer, scaffold, explant, or self-assembled form. The presence of serum in the media of a study is also a critical variable [245, 267]. Futures studies may focus on unconventional growth factors, such as the serum-derived phospholipid agent lysophosphatidic acid (LPA). LPA is naturally present in mammalian sera at concentrations ranging from 1-5 μM [268], and has been studied extensively as an anti-apoptotic factor. Other potent agents may be derived from platelet-rich plasma, which has been shown to increase matrix deposition and proliferation of meniscus cells cultured in monolayer [269]. Finally, although fibrocartilage is a particularly important soft tissue, research into its generation with biochemical stimuli is nascent, and thus future work along these lines may produce significant medical advances. Much remains to be studied concerning biochemical stimuli used in tissue engineering the knee meniscus.

8. Mechanical Stimulation for Meniscus Tissue Engineering

Meniscus cells may respond positively to mechanical stimuli by enhancing the fibrocartilage ECM, or negatively by secreting matrix-degrading or inflammatory factors. The mechanical properties of the matrix can be enhanced through three general mechanisms: deposition, alignment, or compaction. To achieve these results, there are several possible methods of stimulating meniscus tissue. These include high and low shear, fluid perfusion, hydrostatic pressure, direct compression, and even ultrasound. However, most current efforts are centered on hydrostatic pressure and direct compression stimulation.

Explant and engineered tissue can demonstrate varied remodeling responses to hydrostatic pressure. For instance, leporine meniscal explants subjected to cyclic hydrostatic pressure at 1 MPa, 0.5 Hz, for 1 min on/14 min off over 4 hrs, have been shown to upregulate inflammatory factors and matrix degradation proteins [270]. In contrast, leporine meniscus cells seeded in PLLA constructs and stimulated with hydrostatic pressure at 10 MPa statically for 1 hr every 3 days exhibited beneficial responses [271]. In these constructs, collagen and GAG content, as well as compressive properties, were all significantly higher than control or dynamic hydrostatic pressure regimens run at 0.1 or 1 Hz [271]. Furthermore, when combined with TGF-β1, hydrostatic pressure stimulation of leporine meniscal cell-seeded PLLA constructs displayed additive increases in collagen and GAG deposition as well as a synergistic increase in compressive properties [272]. These results demonstrate the various responses meniscus cells may mount in response to hydrostatic pressure.

Direct compression stimulation of the meniscus has also been pursued, although insufficient loading or excessive loading can be detrimental. For example, static and dynamic loading regimens over 24 hrs using direct compression at 0.1 MPa and 0.08-0.16 MPa, respectively, have been shown to decrease mRNA levels of type I collagen, type II collagen, and decorin [273]. Furthermore, under the same regimens, mRNA for ECM-degrading matrix metalloproteinase-1 (MMP-1) and collagenase were both upregulated [273]. Similarly, dynamic compression of porcine meniscus explants (24 hrs, 0.1 MPa, 0.5 Hz) has been demonstrated to upregulate production of nitric oxide, a potent signaling molecule implicated in arthritis and meniscus degeneration [274]. Thus, the use of incorrect loading regimen parameters can lead to matrix and tissue degradation.

By contrast, certain other loading regimens have shown beneficial effects. Physiological loads on the meniscus *in vivo* may exceed 1000 N [39], and it is believed this compressive loading helps facilitate nutrient-waste exchange in the otherwise transport-limited and avascular meniscus. For example, it has been shown that tensile loading of meniscus cells can suppress the production of inflammatory factors [275]. Further, dynamic compression (2% oscillatory strain, 1 Hz, 1 min on/1 min off duty cycle, 4 hrs per day for 4 days) of meniscus explants has been reported to increase aggrecan expression compared to statically compressed samples [276]. A longer duration loading regimen (0.1 MPa, 0.5 Hz, for 24 hrs) has also been shown to increase protein (68%) and proteoglycan (58%) synthesis in meniscal explants [277]. Dynamic compression has also yielded other positive results in anatomically-shaped alginate scaffolds containing bovine meniscus cells [278]. The investigated loading regimen (7-15% strain, 1 Hz, 1 hr on/1 hr off duty cycle, 3 hrs per day, 3 days per wk for 6 wks) applied to meniscus constructs yielded increases in compressive equilibrium modulus and GAG content after 2 wks, and decreases in these values but increases in collagen content after 6 wks [278]. These results under different loading regimens highlight the varied biosynthetic responses that may occur following mechanical stimulus, and encourage the pursuit of additional research.

Of all of the factors being integrated to achieve successful engineering of the knee meniscus, mechanical stimulation is perhaps the one with the highest ambiguity and largest opportunity for improvement. Interestingly, while shear is generally thought of as detrimental to the chondrocyte phenotype, oscillatory fluid flow has been shown to upregulate calcium signaling and GAG production of meniscus cells in parallel plate flow chambers [279]. The use of shear and other forces to generate fibrocartilage may yield benefits for meniscus tissue engineering in the future. Due to the large amount of variable parameters (method, time of application, magnitude, duration, and frequency of stimulation) the optimal and most dramatic combination of effects for mechanical stimuli likely remains undiscovered.

9. Conclusions and Future Directions

This review has provided an account of current concepts in meniscus pathology and repair, as well as meniscus tissue engineering. Undoubtedly, the need for effective therapies based on tissue engineering approaches is exceedingly high. The driving factors for this are high incidences of meniscal lesions amongst several age groups in the general population and significant deficiencies associated with current repair techniques. Secondary to these factors are degenerative changes in articular cartilage, which lead to osteoarthritis and generate considerable socioeconomic costs for healthcare systems worldwide. Tissue engineering aims to ameliorate these problems by establishing new meniscus repair techniques that, for example, can yield constructs that restore mechanical function by integrating with or replacing the patient's tissue. Apart from its potential clinical benefit, a tissue-engineered meniscus can also be of great use in the study of developmental, regenerative, and degenerative processes in the knee.

Despite considerable diversity amongst current strategies for tissue engineering the meniscus, several important design principles are emerging. In general, these principles relate to a biomimetic approach to generating replacement meniscus tissue by recapitulating biological, structural, and functional features of the native meniscus. First, cells in replacement tissue must possess a similar phenotype to those found in native meniscus. This dictates the presence of a both fibroblast-like and chondrocyte-like cells. In cases of total meniscus replacement, this may also include vascularization in the outer periphery. Second, the biochemical content in the meniscus (i.e., collagen, GAGs) should closely mirror the regional variation displayed natively. This design principle will follow naturally from the

use of cells with appropriate phenotypes. Third, functional anisotropy must be manifest in an engineered meniscus. Functional anisotropy of mechanical properties may in turn be recapitulated from the presence of appropriate ECM content. Taken together, these principles of tissue engineering using biomimicry may guide tissue engineers to generating a fully functional meniscus.

To achieve these goals, several strategies have been proposed, with mixed results. Several cell sources have been studied for meniscus engineering, including autologous, allogeneic, xenogeneic, and stem cells. Yet, amongst these, no specific cell source has been established, although stem cells are particularly promising. Whilst the various categories of biomaterial scaffolds offer different advantages, no approach currently addresses all three fundamental requirements of a successful meniscus tissue replacement (satisfactory mechanics, bioactivity, and logistics). In this field, a scaffoldless self-assembly process utilizing a biomaterial mold is another potentially effective strategy for meniscus tissue engineering that largely fulfills some of the above criteria. However, and perhaps more importantly, it lacks complications associated with scaffolds such as integration, degradation, biocompatibility, and stress shielding. In addition to these approaches, the use of synergistic biochemical and biomechanical stimuli are fundamental to the creation of functional tissue. Thus, based on these advantages, the self-assembly process may provide sufficient flexibility to lead to functional tissue-engineered meniscus constructs.

Even though the existing studies in meniscus tissue engineering show promising results, research needs to progress further. At the basic level, more long-term scaffold and scaffoldless studies aimed at improving mechanical properties and matching them to tissue need to be undertaken. Especially vital is the avoidance of scaffold degradation before mechanically-competent tissue can be formed. In line with this, seeded cells must be encouraged to maintain their phenotype and synthetic capacity in a scaffold. Additionally, the reproducibility of engineered tissue needs to be established. Going a step further, experimental (animal) studies and well-designed prospective, randomized, and controlled clinical trials with long-term quantitative outcome measurement are key to *in vivo* evaluation of research in this field. The outlining of indications and contraindications, the selection of suitable patients for tissue repair with engineered meniscus, and the establishment of specialized surgical techniques are therefore necessary. Moreover, the development of non-invasive assessment procedures for generated tissue, both pre-implantation and post-implantation, is required. Directions for future research should also be guided by minimization of healthcare costs. These essential goals will be pivotal to the potential widespread clinical application of tissue-engineered meniscus. Although the challenge is vast, recent scientific advances suggest that a solution to this as-of-yet intractable problem may be emerging from the collaborative efforts of biomedical engineers, clinicians, and industry leaders.

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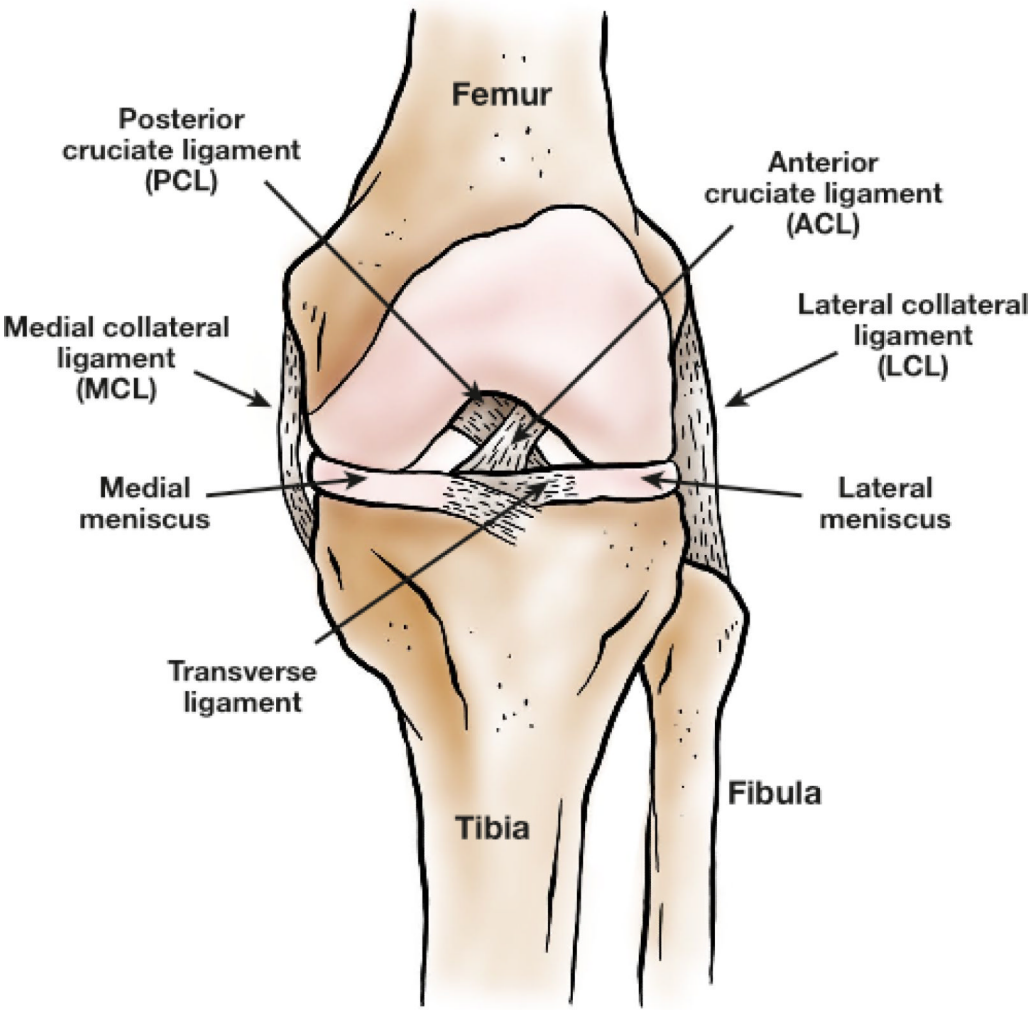

Figure 1. Anatomy of the knee joint: anterior view

The knee meniscus is situated between the femur and the tibia. Crossing the meniscus are various ligaments, which aid in stabilizing the knee joint.

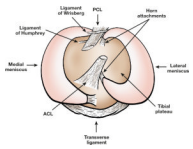

Figure 2. Anatomy of the meniscus: superior view of the tibial plateau

This view of the tibial plateau highlights the ligaments of Humphrey and Wrisberg, which attach the meniscus to the femur. The menisci are attached to each other via the transverse ligament. The horn attachments connect the tibial plateau to the meniscus.

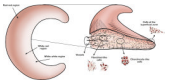

Figure 3. Regional variations in vascularization and cell populations of the meniscus

Left: Though fully vascularized at birth, the blood vessels in the meniscus recede during maturity. In adulthood, the red-red region contains the overwhelming majority of blood vessels. Right: Cells in the outer, vascularized section of the meniscus (red-red region) are spindle-shaped, display cell processes, and are more fibroblast-like in appearance, while cells in the middle section (white-red region) and inner section (white-white region) are more chondrocyte-like, though they are phenotypically distinct from chondrocytes. Cells in the superficial layer of the meniscus are small and round.

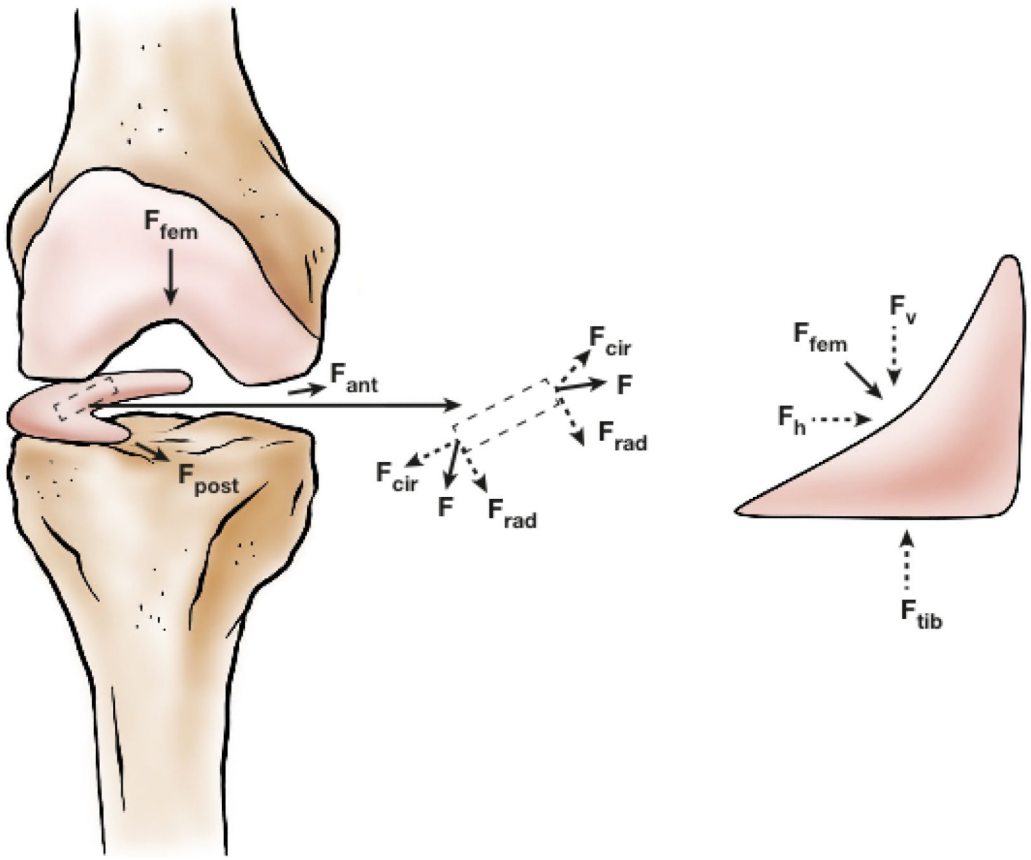

Figure 4. How force is transduced upon and throughout the knee meniscus

Free body diagram of the forces acting on the knee meniscus during loading (for simplicity, only the lateral meniscus is shown). During everyday activity, the menisci are compressed by the downward force of the femur. Since the meniscus is a wedge, the femoral force is enacted at an angle, and thus a vertical component exists which is countered by the upward force of the tibia. Additionally, a horizontal component of the femoral force exists, which is exerted radially outward on each meniscus. This horizontal force is in turn countered by the anchoring force of the attachments at the posterior and anterior horns of the meniscus. Additionally, as this compression occurs, circumferential stress is created along the meniscus. Therefore, the menisci function by converting compressive loads to circumferential tensile loads. At the same time, shear forces are developed between the collagen fibers within the meniscus while the meniscus is deformed radially.

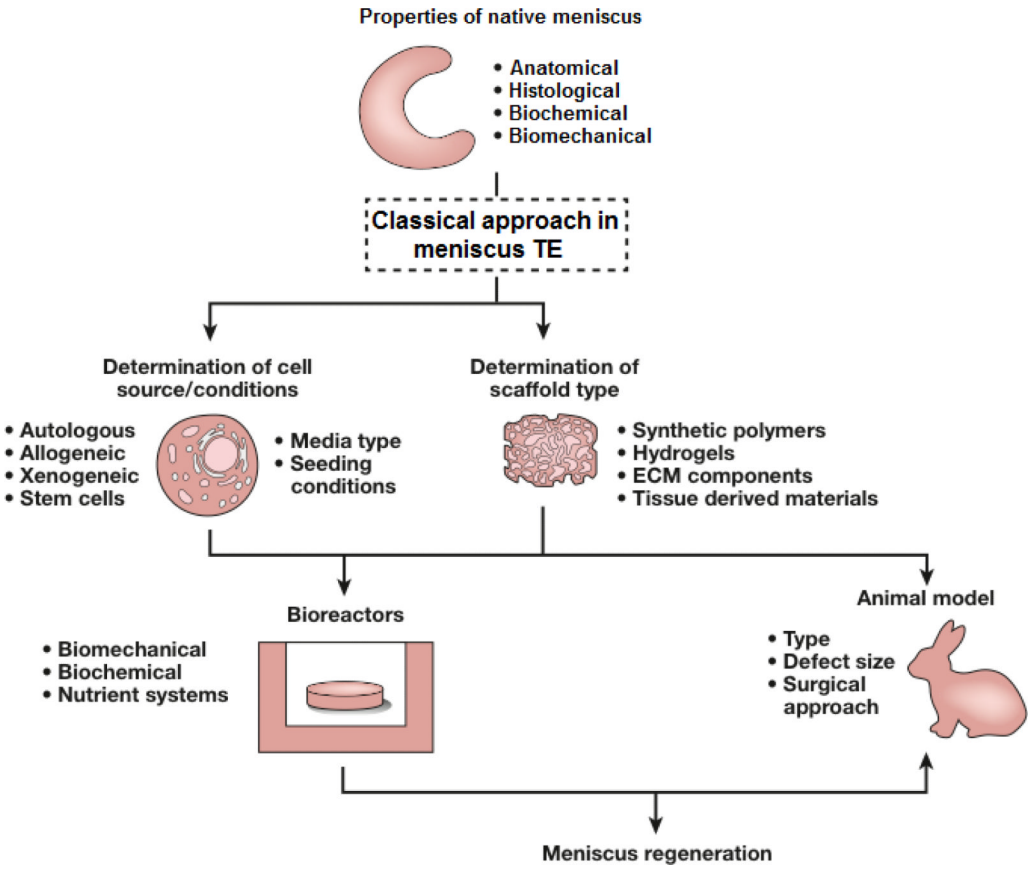

Figure 5. The classical, scaffold-based approach for meniscus tissue engineering

Generation of a functional meniscus requires several key considerations. Characterization of the meniscus is essential for establishing design parameters. Following this, judicious choice of cell type(s), scaffold material(s), and exogenous stimuli must be made. Implantation *in vivo* may be substituted for, or performed subsequent to, bioreactor culture. Using these tools, tissue engineering aims to regenerate or replace the meniscus.

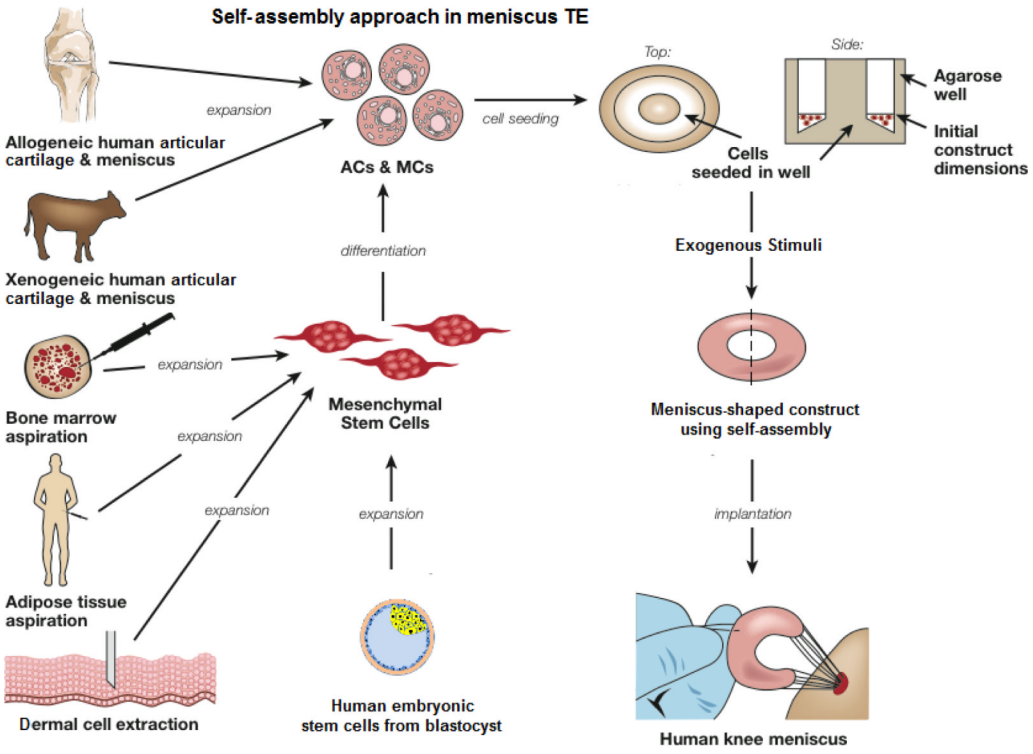

Figure 6. The strategy of tissue self-assembly for meniscus tissue engineering

This approach utilizes a hydrogel mold to form completely biologic tissue constructs. Selection of cells is of paramount importance. Following isolation from allogeneic or xenogeneic sources, articular chondrocytes (ACs) and meniscus cells (MCs) are expanded to achieve the high numbers needed for robust tissue engineering. Mesenchymal stem cells, but also potentially embryonic stem cells, are a promising alternative cell source for subsequent differentiation into meniscus cells. Cells are then seeded in high density in a non-adherent biomaterial mold, secreting ECM that coalesces into a continuous tissue over several days. Exogenous stimuli are added during culture to increase the synthetic activity and functional properties of neotissue, which is eventually implanted *in vivo*.

Table 1

Compressive properties of the knee meniscus.

Study	Species	Location	Aggregate modulus (±SD; MPa)	Permeability (±SD; 10^{-15} m^4 N^{-1} s^{-1})
Sweigart et al. [37]	Human	Medial superior:		
		Anterior	0.15 ± 0.03	1.84 ± 0.64
		Central	0.10 ± 0.03	1.54 ± 0.71
		Posterior	0.11 ± 0.02	2.74 ± 2.49
		Medial Inferior:		
		Anterior	0.16 ± 0.05	1.71 ± 0.48
		Central	0.11 ± 0.04	1.54 ± 0.49
		Posterior	0.09 ± 0.03	1.32 ± 0.61
	Bovine	Medial superior:		
		Anterior	0.21 ± 0.06	6.22 ± 2.55
		Central	0.14 ± 0.05	5.73 ± 6.19
		Posterior	0.11 ± 0.04	4.73 ± 2.56
		Medial Inferior:		
		Anterior	0.16 ± 0.06	5.79 ± 4.31
		Central	0.11 ± 0.03	5.65 ± 4.13
		Posterior	0.13 ± 0.06	5.40 ± 5.36

Table 2

Tensile properties of the knee meniscus.

Study	Animal type	Direction	Location	Stiffness (±SD; MPa)
Fithian et al. [38]	Human	Circumferential	Lateral meniscus:	
			Anterior	159.1 ± 47.4
			Central	228.8 ± 51.4
			Posterior	294.1 ± 90.4
			Medial meniscus:	
			Anterior	159.6 ± 26.2
			Central	228.8 ± 51.4
			Posterior	294.1 ± 90.4
Tissakht et al. [280]	Human	Circumferential	Lateral meniscus:	
			Anterior	124.58 ± 39.51
			Central	91.37 ± 23.04
			Posterior	143.73 ± 38.91
			Medial meniscus:	
			Anterior	106.21 ± 77.95
			Central	77.95 ± 25.09
			Posterior	82.36 ± 22.23
		Radial	Lateral meniscus:	
			Anterior	48.47 ± 25.67
			Central	45.86 ± 24.20
			Posterior	29.85 ± 12.77
			Medial meniscus:	
			Anterior	48.31 ± 24.35
			Central	46.20 ± 27.56
			Posterior	32.55 ± 11.27
Lechner et al. [281]	Human	Circumferential	Medial meniscus:	
			Anterior	141.2 ± 56.7
			Central	116.4 ± 47.5
			Posterior	108.4 ± 42.9

Table 3

Current research findings on meniscus repair techniques (alternatives to meniscectomy) for the avascular zone.

Author	Type of study	No. of cases	Mean age (years)	Follow-up time(s) (mo.)	Concurrent ACLT	Type of lesions	Technique	Cases Completely Healed (%)	Evaluation
Arnockzy et al. [189]	Experimental	12	-	6	-	-	Exogenous fibrin clot + suture	100	Histology
Henning et al. [138]	Clinical	153	23	41	Majority	Longitudinal, radial, flap, horizontal split, bucket handle, complex	Exogenous fibrin clot + suture	64	Arthrogram + follow-up arthroscopy
Arnockzy and Warren **[14]**	Experimental	15	-	2, 5	-	-	Vascular access channels + suture	100	Histology
Vangesnes et al.[282]	Experimental	30	-	1, 5	-	-	Neodymium laser + suture	0	Histology
Jitsuiki and Ikuta [283]	Experimental	14	-	1	-	-	Free synovium allograft	Almost 100	Histology
Zhang et al. [132]	Experimental	21	-	1, 2	-	-	Trephination + suture	69	Histology + biomechanical testing
Cisa et al. [284]	Experimental	44	-	12	-	-	Transfer of pedunculated synovial flap	75	Histology
Rubman et al. [52]	Clinical	91	28	18	Majority	-	Arthroscopy + suture	25 (38 partial healing)	Follow up arthroscopy
van Trommel et al. [137]	Clinical	5	20	4, 71	Minority	Radial split	Fibrin clot + suture	60 (4 mos), 100 (71 mos)	Follow up arthroscopy + MRI + clinical exam
Tienen et al. [285]	Experimental	24	-	3, 6	-	Longitudinal	Porous polymer implant in partial thickness access channel	100	Histology
Uchio et al. [131]	Clinical	48	24	21	Majority	-	Rasping without suturing	71 (21 incomplete healing)	Second look arthroscopy
Papachristou et al. [286]	Clinical	25	20	36	-	Longitudinal	Suture	40	Clinical examination
Pollo et al. [287]	Experimental	10	-	1, 3	-	-	Photoactive laser technique	100	Histology
Petersen et al. [288]	Experimental	18	-	0.5, 1	-	-	Suture coated VEGF/PDLLA	0	Histology

Author	Type of study	No. of cases	Mean age (years)	Follow-up time(s) (mo.)	Concurrent ACLT	Type of lesions	Technique	Cases Completely Healed (%)	Evaluation
Noyes et al. [289]	Clinical	71	16	18, 51	Majority	Longitudinal, horizontal, radial, complex	Suture	75	Follow up arthroscopy (18 mos) + clinical exam (51 mos)

ACLT: Anterior cruciate ligament transection.

Table 4

Leading biomaterials strategies for meniscus tissue engineering rated on three primary criteria. Categories were qualitatively rated from one to four stars in mechanics (mechanical properties, geometry, anisotropy, lubrication), bioactivity (cell phenotype, ECM synthesis, immunogenicity, potential for host tissue integration), and logistics (supply, material processability and sterilization, ease of surgical implantation).

Rated Criterion	Synthetic Scaffolds	Hydrogel Scaffolds	ECM Component Scaffolds (e.g., collagen, GAG)	Tissue-Derived Scaffolds (e.g., SIS, decellularized matrix)	Scaffold-Free (e.g., self-assembly)
Mechanics	***	*	***	**	***
Bioactivity	*	**	**	****	****
Logistics	****	****	***	*	**

Table 5

Influence of selected growth factors administered to meniscus cells.

Growth Factor	Effects	Culture Conditions
TGF-β1	↑↓ Proliferation	Monolayer [103, 241, 242]
	↑↑ Collagen Synthesis	Monolayer [249]; Scaffold [242, 244]; Explant [290]; Scaffoldless [232]
	↑↑ GAG/Proteoglycan	Monolayer [103, 249]; Scaffold [103, 244, 291]; Explant [103, 290]; Scaffoldless [232]
	↑ SZP Secretion	Monolayer [250]; Explants [250]
	↑ Contraction	Scaffold [255]
b-FGF	↑↑↑ Proliferation	Monolayer [241-243]; Scaffold [244]
	↑ Collagen Synthesis	Monolayer [241, 243]; Scaffold [244]
	↑ GAG/Proteoglycan	Monolayer [243, 249]; Explants [290]
PDGF-AB	↑↑ Proliferation	Monolayer [241, 245]; Scaffold [292]; Explant [292]
	↑ Collagen Synthesis	Monolayer [241]; Scaffold [242]
	↑ GAG/Proteoglycan	Monolayer [249]; Explant [290]
	↑ Contraction	Scaffold [254]
	↑↑ Migration	Monolayer [245]; Scaffold [292]; Explant [292]
IGF-I	↑ Proliferation	Monolayer [103, 242]; Scaffold [244]
	↑↓ Collagen Synthesis	Monolayer [249]; Scaffold [242]
	↑ GAG/Proteoglycan	Explant [290]
	↑ Migration	Monolayer [245]
EGF	↑ Proliferation	Monolayer [241]
	↑ Collagen Synthesis	Monolayer [241]
	↑ Migration	Monolayer [245]
HGF	↑↑ Proliferation	Monolayer [245]; Scaffold [292]; Explant [292]
	↑↑ Migration	Monolayer [245]; Scaffold [292]; Explant [292]