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Considerations for Translation of Tissue Engineered Fibrocartilage From Bench to Bedside

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Fibrocartilage is found in the knee meniscus, the temporomandibular joint (TMJ) disk, the pubic symphysis, the annulus fibrosus of intervertebral disk, tendons, and ligaments. These tissues are notoriously difficult to repair due to their avascularity, and limited clinical repair and replacement options exist. Tissue engineering has been proposed as a route to repair and replace fibrocartilages. Using the knee meniscus and TMJ disk as examples, this review describes how fibrocartilages can be engineered toward translation to clinical use. Presented are fibrocartilage anatomy, function, epidemiology, pathology, and current clinical treatments because they inform design criteria for tissue engineered fibrocartilages. Methods for how native tissues are characterized histomorphologically, biochemically, and mechanically to set gold standards are described. Then provided is a review of fibrocartilage-specific tissue engineering strategies, including the selection of cell sources, scaffold or scaffold-free methods, and biochemical and mechanical stimuli. In closing, the Food and Drug Administration (FDA) paradigm is discussed to inform researchers of both the guidance that exists and the questions that remain to be answered with regard to bringing a tissue engineered fibrocartilage product to the clinic.
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Keywords: tissue engineering, fibrocartilage, translation, knee meniscus, temporomandibular joint disk

1 Introduction

Cartilage is a connective tissue that is classified by its biochemical properties into hyaline, elastic, and fibrous cartilage (also referred to as fibrocartilage). Of these, fibrocartilage is marked by the presence of type I collagen and traces of type II collagen. Glycosaminoglycans (GAGs) are present in fibrocartilage, albeit in lower amounts than in hyaline articular cartilage [1]. Areas in the body containing fibrocartilage include the knee meniscus [2], the temporomandibular joint (TMJ) disk [3], the pubic symphysis, the annulus fibrosus of the intervertebral disk, tendons, and ligaments. Fibrocartilage undergoes a range of stresses including tension, compression, and shear in different areas of the body. Much like hyaline articular cartilage, fibrocartilage has a naturally low regenerative capacity due to its avascularity [1]. Fibrocartilages are notoriously difficult to repair with limited clinical options. Tissue engineering may be a route to provide novel clinical treatments, but the pathway for these products can be ill-defined due to the low number of Food and Drug Administration (FDA)-approved cellular products. While FDA guidance documents exist for human cells, tissues, and cellular and tissue-based products (HCT/Ps) in general [4] and, specifically, for products intended to repair or replace hyaline articular cartilage [5], an equivalent document for fibrocartilage does not exist. Formation of clinically relevant, tissue engineered fibrocartilages would require satisfying a variety of design criteria and regulatory requirements. This review uses the knee meniscus and TMJ disk fibrocartilages as two examples to discuss how tissue engineered fibrocartilages may be translated from the bench to bedside.

In Secs. 2–4, anatomy and structure–function relationships of the knee meniscus and TMJ disk will be presented. Epidemiology of these tissues and the causal pathologies that lead to specific indications for current clinical treatments will be provided. Assays for characterization for histomorphological, biochemical, and mechanical properties of fibrocartilages will be explained. Together, anatomy, function, epidemiology, pathology, current clinical treatments, and characterization studies inform design criteria for tissue engineered fibrocartilages. In context to these design criteria, current tissue engineering methods for fibrocartilage, specifically the meniscus and TMJ disk, will be discussed via subsections on the selection of cell source, a scaffolding or scaffold-free approach, biochemical stimuli, and mechanical stimuli. In addition, evaluation of tissue engineered fibrocartilages and discussion of engineering a fibrocartilage spectrum will be provided. The final section of this paper will look toward the translation of tissue engineered fibrocartilage and how this type of product may be shepherded through the FDA paradigm. A focus will be considerations for preclinical animal models and clinical trials. Future directions will be recommended, motivation for FDA guidance will be discussed, and remaining questions or concerns will be presented.

2 Fibrocartilage Types, Epidemiology, Pathology, and Clinical Treatments

Fibrocartilage anatomy, function, epidemiology, and pathology all inform how tissue engineered fibrocartilage should be designed and made. Current clinical options and practices can inform how tissue engineered fibrocartilage may be deployed in the clinical setting and can, thus, inform design criteria as well. These are provided below.

2.1 The Knee Meniscus and Temporomandibular Joint disc. In 2005, more than 46 million adults incurred over $353 billion in direct healthcare costs related to different rheumatic

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conditions in the U.S. alone [6]. These conditions encompass those affecting fibrocartilages. Two fibrocartilages of high clinical relevance are the knee menisci and TMJ disk. Knee menisci are semicircular, wedge-shaped fibrocartilaginous tissues, located between the distal femur and the tibial plateau (Fig. 1) that protect articular cartilage via load distribution. The knee contains a medial and a lateral meniscus (Fig. 1). Under compressive load, the menisci's wedge shape causes tension to develop, which is resisted by circumferentially aligned collagen. A gradient of healing capabilities in the knee meniscus correlates with the degree of vascularity, with the capacity for healing decreasing as one moves closer to the innermost, avascular region (Fig. 1, white-white region).

The TMJ is a ginglymoarthrodial joint that contains a fibrocartilaginous disk situated between the mandibular condyle on the inferior side, and articular eminence and mandibular fossa on the superior side (Fig. 1). The TMJ disk is biconcave and consists of the anterior and posterior bands as well as the lateral, central, and medial zones that are collectively referred to as the intermediate zone (Fig. 1) [7]. The TMJ disk serves to increase congruity between the eminence and fossa, to distribute load, and to aid in joint lubrication [8]. The movement of the TMJ disk serves the rotational motion of the joint primarily in the rotational axis during normal mastication and the translational motion of the joint when the mouth is opened wide. During typical movements of the joint, loading patterns in the anterior portion of the mandibular condyle and posterior portion of the articular eminence lead to complex shear, compressive, and tensile forces on the fibrocartilaginous disk.

2.2 Epidemiology and Pathology. Meniscal lesions are the most common intra-articular knee injuries and most frequent cause of orthopedic surgical procedures in the U.S. [9]. This is reflected by the size of the meniscus repair market, which in 2008 was anticipated to increase at a compound annual growth rate of 10.6% to an estimated $318 million in 2015 [10]. Previously reported incidences of meniscal injury leading to meniscectomy

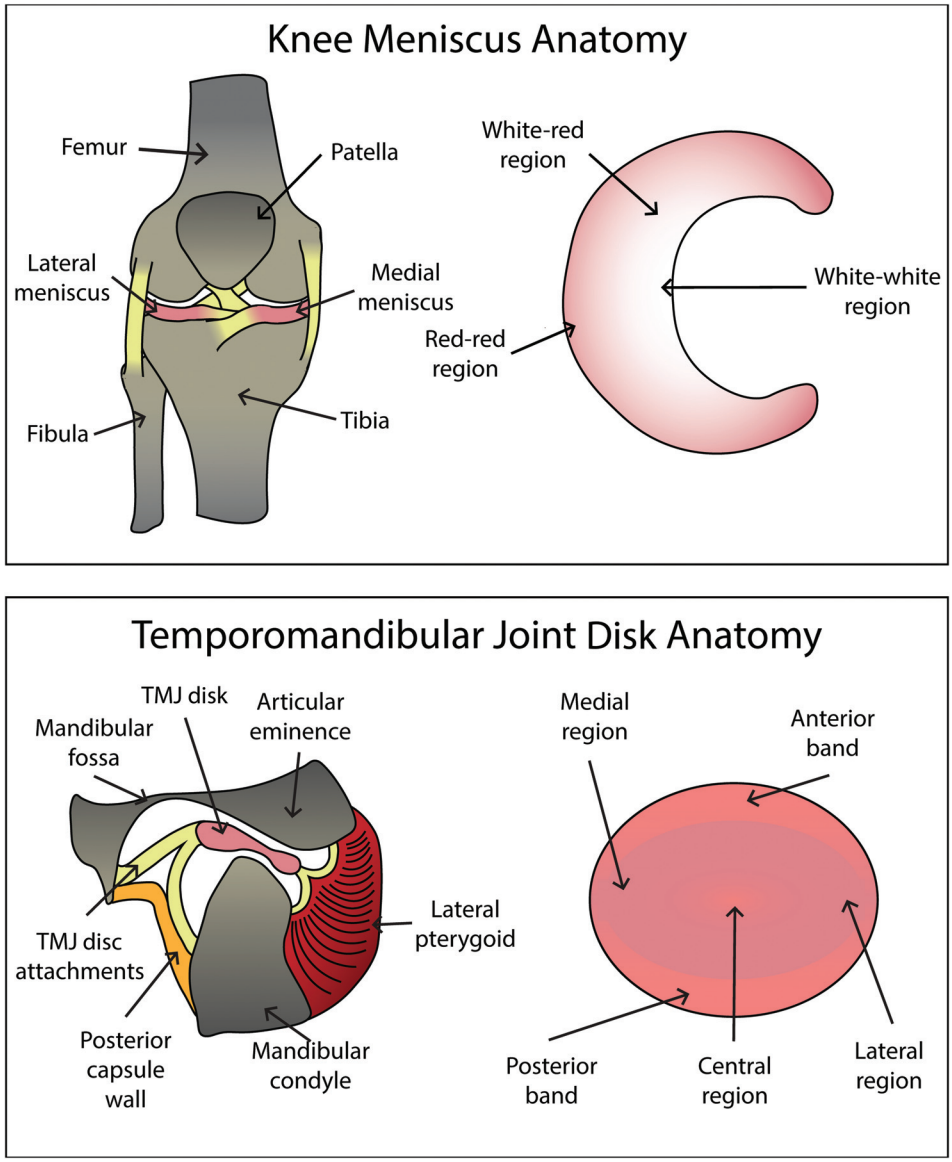

Fig. 1 Anatomy of the knee meniscus and TMJ disk. The anatomical structures of the knee are shown, with the menisci depicted between the femur and tibia. The transverse view is shown in the right panel, indicating the different vascular regions of each meniscus. The TMJ disk is shown from a sagittal view between the mandibular condyle and the articular eminence in an open jaw position. The disk from a transverse view is depicted in the right-hand panel.

were noted at 61 per 100,000 persons [11], but damage to the medial meniscus is significantly more prevalent than in the lateral meniscus (81% and 19%, respectively) [11–17]. Injury to the lateral meniscus, while less frequent, leads to the degeneration of knee function, lower Lysholm scale scores—a scale from 0 to 100 that measures patient-reported pain where 100 represents a better outcome with fewer symptoms or disability, and a higher rate of instability when treated via meniscectomy as compared to meniscectomy of the medial meniscus [16,17].

Meniscal lesions are classified by their spatial alignment as vertical longitudinal (or longitudinal), radial, oblique, complex (or degenerative), and horizontal tears (Fig. 2). Complex tears are more likely to arise with increasing age, while other tears are more commonly attributed to traumatic injury. Oblique and vertical longitudinal tears represent 81% of meniscal tears [18,19]. Vertical longitudinal tears run parallel to the long axis of the meniscus and are perpendicular to the tibial plateau (Fig. 2). These tears divide the circumferentially aligned collagen fibers and are categorized as either complete or incomplete vertical longitudinal tears. The former is known as a bucket handle tear, which more commonly affects the medial meniscus. Bucket handle tears are often unstable and can cause mechanical symptoms or locking of the knee [18], and are more amenable to repair if found within a vascularized region of the meniscus [20].

Temporomandibular joint disorders (TMDs) encompass any issue with the jaw and the muscles that control it. TMDs are the second most common musculoskeletal condition resulting in pain and disability [21] and cost an estimated $4 billion per annum in healthcare in the U.S. alone. TMDs may cause pain in 20–25% of adults worldwide [22]. A gender paradox exists with TMDs because a 3.5-fold higher prevalence is seen in women than men [23,24]. This gender paradox has been well studied and has been hypothesized to occur due to hormone differences between genders [24]. TMD symptoms are wide-ranging, including clicking, restricted or deviating range of motions, and cranial and/or muscular pain [22].

Up to 70% of TMD patients suffer from internal derangement (ID) of the disk [25], where the TMJ disk is displaced from its normal anatomic position. Severe cases of ID are often presented with focal thinning of the disk, with eventual progression to larger areas of thinning or disk perforation (DP) (Fig. 2) [26]. Osteoarthritis (OA) often accompanies TMDs [27], but there is conflicting evidence of a clear causal relationship between ID and OA [28].

Epidemiological and economic data make the knee meniscus and TMJ disk highly significant fibrocartilages for tissue engineering. When one considers the mechanical behaviors of the knee meniscus and TMJ disk, and how these functions fail due to pathology, many similarities begin to emerge. For example, both fibrocartilages function under large magnitudes of mechanical stress; engineered implants must be ready to bear similar loads. While specific pathological features may differ for the knee meniscus and TMJ disk (tears for the meniscus and thinning or perforation for the TMJ disk), late-stage pathologies of both fibrocartilages are often treated by tissue removal without long-term options for replacement, leading to joint degeneration. The similarities lead to comparable design criteria for the tissue engineering of these fibrocartilages.

2.3 Current Clinical Treatments. Fibrocartilage treatments usually follow a path of two stages: nonsurgical methods followed by surgical intervention that range from minimally to highly invasive procedures. Nonsurgical methods may include physical therapy, analgesics for pain management, and behavioral modification, and are indicated for early disease stages. If no improvement in symptoms is shown, surgery may be indicated. Surgical options for fibrocartilage are limited and progress rapidly to final stage options, such as arthroplasty, beyond which, even fewer options exist [29]. Tissue engineered fibrocartilage could potentially bridge the gap between the early and end stages of fibrocartilage pathology.

Initial diagnoses of knee meniscus injuries begin with clinical examination using a variety of tests [18]. If a meniscal tear is identified, the tear's severity is categorized to determine treatment which includes repair via arthroscopy, partial or full meniscectomy, and allograft transplantation [18]. Therapeutic efficacy varies by indication in part due to anatomy. For example, tears found in the red-white region of the meniscus are more amenable to repair than the white-white region due to the higher levels of vascularity in that region [20]. If possible, meniscectomy should be reserved for cases refractory to repair because meniscal repair tends to yield better clinical outcomes than meniscectomy [30].

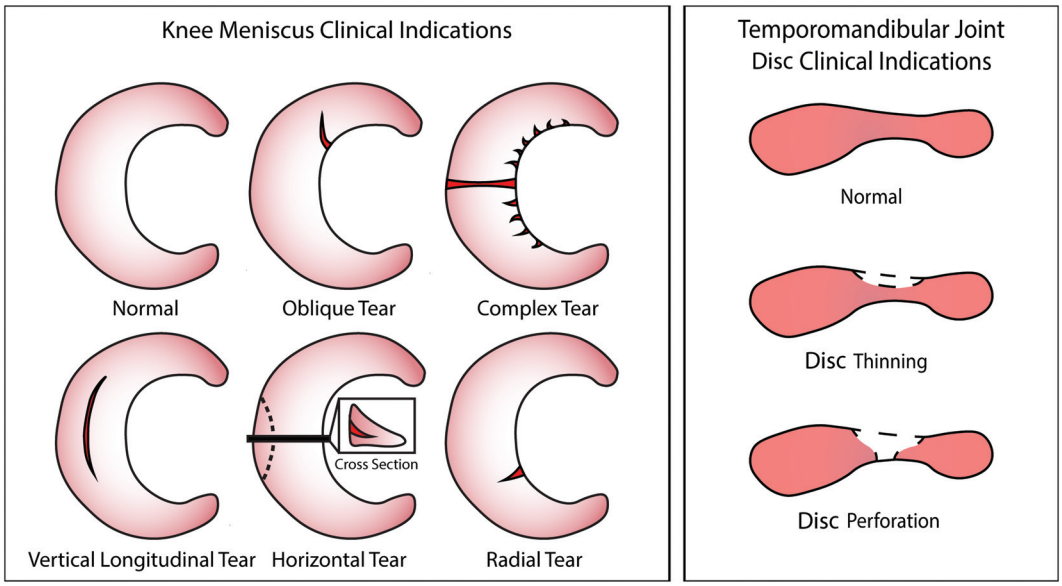

Fig. 2 Clinical indications of the knee meniscus and TMJ disk. Different clinical indications for the meniscus are shown including five different tears: oblique, complex, vertical longitudinal, horizontal, and radial tears. For the TMJ disk, disk thinning and DP are the clinical indications presented.

Meniscectomy removes parts of the knee meniscus or cleans up degenerative debris, leading to immediate pain relief, although this is not always observed. Meniscectomy virtually guarantees the emergence of OA [31]. While some meniscectomy patients report pain relief, a statistically significant increase in quality of life after meniscectomy over alternatives such as physical therapy has not been observed, illustrating the limitations of fibrocartilage removal without replacement [32–34].

Diagnosis of TMDs follows patients' report of pain in the TMJ, headaches behind or around the eyes, and pain spreading to the temple, neck, ears, and shoulders [27]. Patients will often undergo a physical exam and multiple imaging modalities, such as magnetic resonance imaging (MRI) and/or computed tomography [22]. Although many TMJ symptoms can resolve themselves [21,27], approximately 3–5% of TMD patients will require medical intervention in various forms.

Even in the most severe cases of TMDs, nonsurgical treatment is preferred [27]. Surgical options for TMDs are limited but include disk repositioning or discectomy with or without disk replacement [22,35]. Hemiarthroplasty is replacement of the articulating joint surface [36], most commonly the superior side in the TMJ with a vitallium alloy in the mandibular fossa-articular eminence region [37]. For certain indications such as ID, the disk can be repositioned in the correct anatomic position. Another option is discectomy, where the TMJ disk is removed. Postoperative follow-up in 3 years shows that discectomy increases mandibular motion [38] but is also associated with signs of degenerative changes including flattening of the articular surfaces and osteophytes [22,39]. Alloplastic disk replacements have been studied including Teflon-Proplast- [40] and silicone-based [22] implants. Biologic materials such as fat have also been explored [41], but all have required follow-up intervention. When a substantial portion of the joint is lost due to degeneration from trauma or significant degeneration in the articulating surfaces, total joint reconstruction may be indicated [22]. Costochondral grafts are used to replace the condyle in autologous TMJ reconstruction [42]. Alloplastic materials have been used in three FDA approved products [8,22] and often require secondary surgery due to the average patient age and resultant implant degradation [22].

As illustrated with the knee meniscus and TMJ disk, both nonsurgical and surgical options for fibrocartilage repair and replacement are lacking in long-term efficacy. Nonsurgical methods commonly treat symptoms and attempt to delay degeneration but are often unsuccessful in doing so. Surgical methods can cause degeneration in the joint space and commonly require additional surgical follow-ups. An important consideration for tissue engineers will be where and how engineered products might fit into

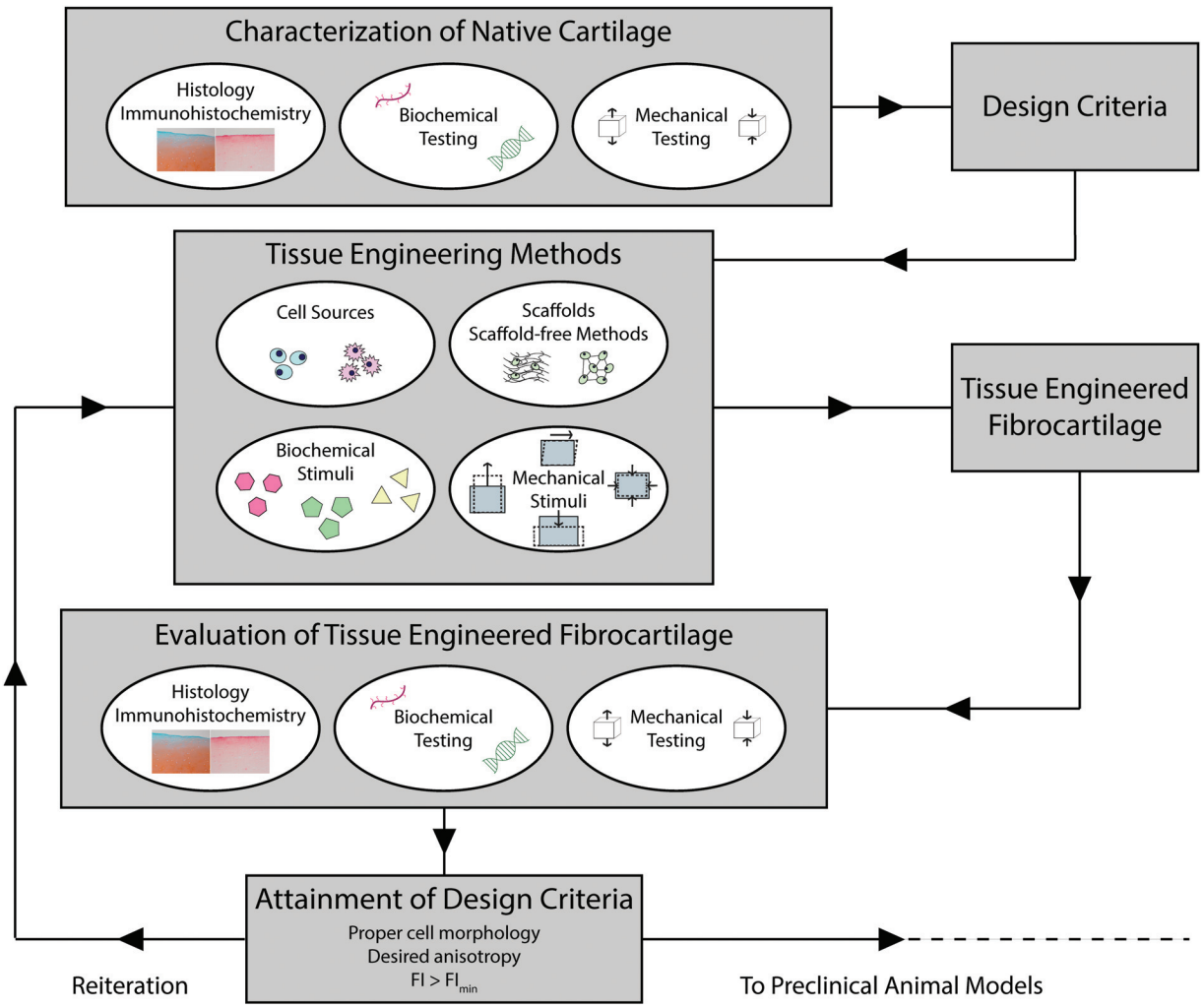

Fig. 3 Tissue engineering of fibrocartilage. Tissue engineering requires characterization of native cartilage from which design criteria can be specified. Tissue engineering parameters such as selection of a cell source, choice of scaffold or scaffold-free methodology, and use of biochemical or mechanical stimuli results in tissue engineered fibrocartilage which is subsequently tested for appropriate properties. If design criteria are met, the tissue engineered fibrocartilage and methodology used may move to preclinical animal models or the tissue engineering process might be reiterated to obtain improved tissue engineered fibrocartilage.

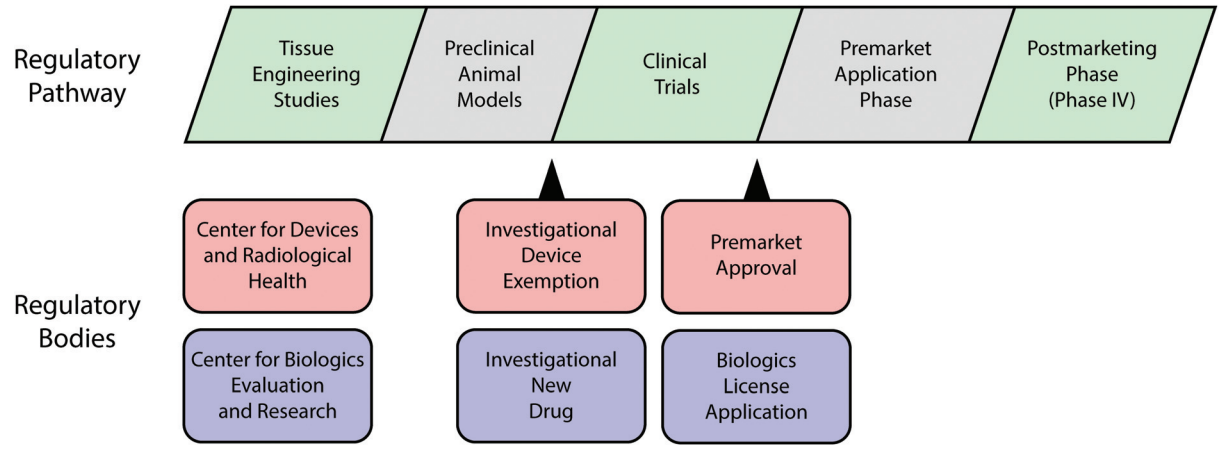

Fig. 4 The FDA paradigm. The FDA paradigm is outlined from tissue engineering studies to the postmarketing phase with appropriate milestones for CBER and CDRH depicted.

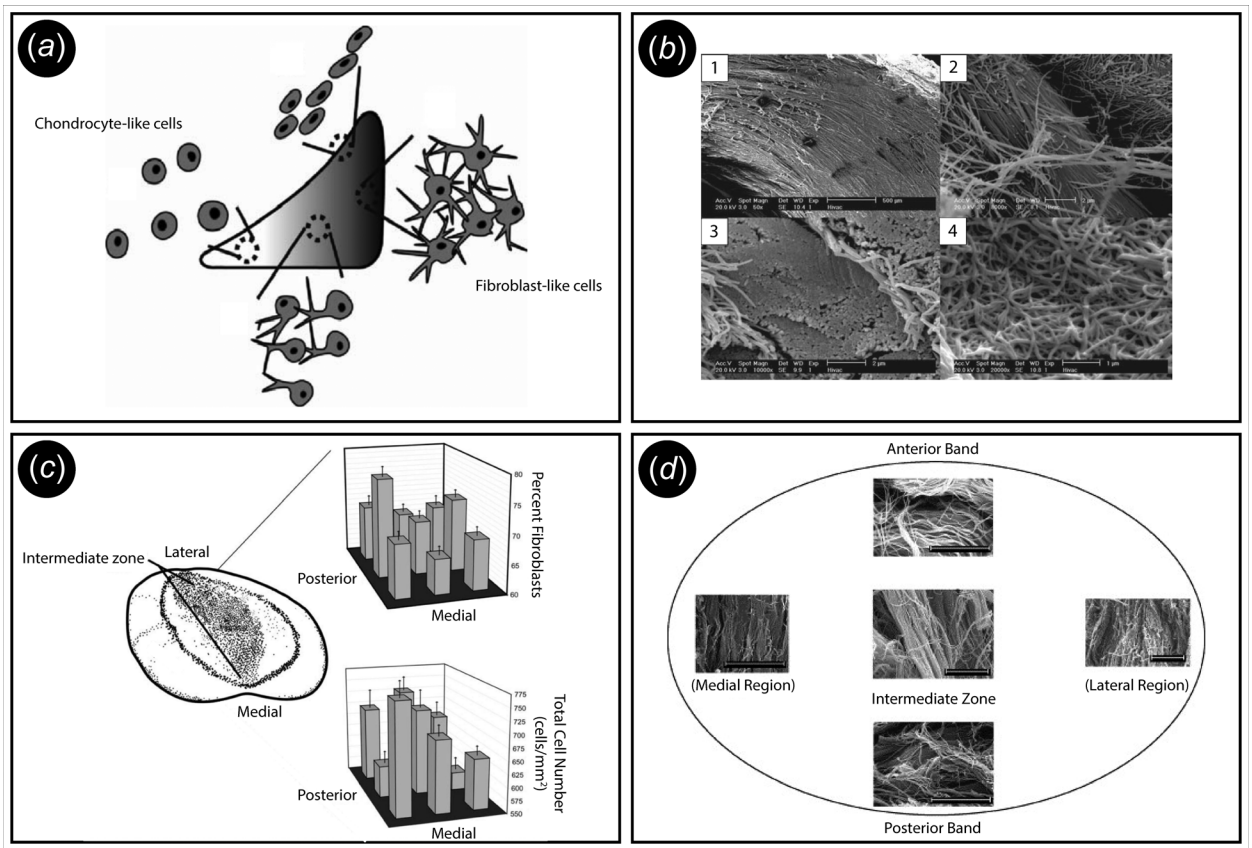

Fig. 5 Cell morphology and collagen alignment of the knee meniscus and TMJ disk. (*a*) A representation of the wedge-shape of the meniscus is depicted with the innermost region showing rounded, chondrocyte-like cells transitioning to spindle-shaped, fibroblast-like cells toward the outermost region. Figure reused with permission from Springer Nature: *Cellular and Molecular Bioengineering* [59]. (*b*) Scanning electron micrographs showing (1) the circumferential collagen alignment, (2) a close-up view depicting individual collagen fibers, (3) a cross section of a collagen bundle, and (4) the random collagen orientation on the outer surfaces of the meniscus. Figure reused with permission from SAGE Publications: *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine* [61]. (*c*) Ratio between fibroblasts and chondrocyte-like cells, and overall cellularity in the TMJ disk are reported, showing the posterior and anterior bands have a higher proportion of fibroblasts when compared to the intermediate zone. Figure reused with permission from Elsevier: *Journal of Oral and Maxillofacial Surgery* [47]. (*d*) Scanning electron micrographs of various regions of the TMJ disk showing primarily anteroposterior alignment in the intermediate zone, while the anterior and posterior bands show circumferential alignment. Scale bars are 10 μm except for the lateral region where the scale bar represents 200 μm. Figure reused with permission from Elsevier: *Matrix Biology* [56].

existing treatment modalities, such as serving as a bridge between early and late-stage surgical interventions.

2.4 Using Tissue Engineering for Fibrocartilage. The need for interventions that can delay or arrest joint degeneration motivates the development of tissue engineered fibrocartilages. In early to midstage pathologies, such as a partial vertical longitudinal tear in the knee meniscus or thinning of the TMJ disk, tissue engineered fibrocartilage implants may be used to bolster failing tissues to slow down or to arrest the degenerative process. Late-stage pathology where fibrocartilage removal by meniscectomy or discectomy is indicated may be combined with implantation of a tissue engineered fibrocartilage replacement. While there is hope for these strategies, there is currently a lack of tissue engineered fibrocartilage products on the market. Sections 3–5 outline the process of fibrocartilage tissue engineering (Fig. 3) and examine the necessary steps for translating a tissue engineered fibrocartilage product to clinical use (Fig. 4).

3 Characterization Studies of Fibrocartilages

Prior to carrying out tissue engineering studies, design criteria must be acquired. These are determined via characterization studies of the native fibrocartilage using histology, immunohistochemistry (IHC), biochemical testing, and mechanical testing (Fig. 3). Various animals commonly serve as models due to their anatomical, structural, and functional similarities to human tissues. Various reviews and comparative studies in the literature discuss different animal models and their similarities to human tissue for both the knee meniscus [43,44] and TMJ disk [45,46] and should be referenced to determine comparability. Test results establish the gold standards toward which tissue engineers aim for in terms of histomorphological, biochemical, and mechanical properties of the engineered tissue. This section will provide guidance for the aforementioned testing and will provide values for native knee meniscus and TMJ disk properties that are relevant to tissue engineering.

3.1 Histomorphological Properties. Histology and IHC allow for examination of a tissue's microscopic organization. In fibrocartilage, the distribution of different cell types [47–50], GAGs [48,50–54], and collagen [48,50,52–55] can be visualized using hematoxylin staining, Safranin O staining with a Fast Green counterstain, and Picrosirius Red staining, respectively. IHC uses antibodies for more specific visualization of the aforementioned items [53,56,57]. For example, multiple collagen types exist within fibrocartilages, and these can be discerned using IHC.

Histology, IHC, and microscopy techniques (e.g., polarized light, second harmonic generation) are used widely to elucidate fibrocartilage properties. For example, different cell types reside side-by-side in fibrocartilage, as seen in the meniscus where chondrocyte-like cells exist in its inner region and transition to a fibroblast-like phenotype in its outer region [58,59] (Fig. 5(*a*)). In the TMJ disk, the ratio of fibroblasts to chondrocyte-like cells varies by region as well, with the highest relative number of chondrocyte-like cells present in the intermediate zone [47] (Fig. 5(*c*)). GAGs were evenly distributed throughout young equine menisci, whereas samples from older horses showed distinct positive and negative staining locations [60]. IHC determined the presence of hyaluronic acid backbone, keratan sulfate, and chondroitin sulfate in the primate TMJ disk [57]. In addition, collagen fibers in an equine knee meniscus model were shown to be randomly organized in the distal and proximal surface layers [60,61] (Fig. 5(*b*)), while the innermost layer exhibited circumferentially aligned collagen fibers with parallel alignment in the red-red region [60]. Polarized light microscopy [62] and scanning electron microscopy [56] showed that collagen aligned primarily circumferentially of the human and porcine TMJ disks, with the intermediate zone showing alignment anteroposteriorly (Fig. 5(*d*)). Finally, IHC showed greater type I collagen staining than type II collagen staining throughout the porcine TMJ disk [56].

Overall, histology and IHC are an adequate starting point for confirming presence and distribution of cells, GAGs, and collagen within fibrocartilage. While useful for the visualization of tissue organization, histology and IHC are qualitative assays and should be supported by sufficient sample sizes and quantitative assays, such as biochemical and mechanical testing.

3.2 Biochemical Properties. Biochemical assays yield quantitative data that allow one to determine how similar properties of tissue engineered fibrocartilage are when compared with those of native tissue. DNA content can be quantified using, for example, PicoGreen [48,63]. Sulfated GAGs are often quantified using dimethyl methylene blue [48,54]. Collagen content can be measured by assaying for hydroxyproline [48,54,64]; a modified version of this assay which excludes use of perchloric acid to measure the collagen content has recently been published [64]. For quantification of specific types of collagen and GAG, enzyme-linked immunosorbent assay is used [53,56]. Pyridinoline content, a measure of collagen crosslinking, can also be quantified with high performance liquid chromatographic assays [48,54,65]. Much like histology and IHC, many of these biochemical assays can be performed to determine regional variation.

The knee meniscus extracellular matrix (ECM) is composed of water, fibrillar components, proteoglycans, and adhesion glycoproteins. Water, collagen, and GAGs account for the majority of components by mass and has been shown to be 72%, 22%, and 0.8%, respectively, in human menisci. The remainder of the tissue is made up of DNA (0.12%) and adhesion molecules. The distribution pattern of GAGs is as follows: 40% chondroitin 6-sulfate, 10–20% chondroitin 4-sulfate, 20–30% dermatan sulfate, and 15% keratan sulfate [66]. Collagen accounts for approximately 60–70% of the dry weight and includes types I–VI collagen [67]. Of these, type I collagen is by far the most predominant in the meniscus, accounting for more than 90% of total collagen [68]. The outer two-thirds of bovine menisci is composed primarily of type I collagen, whereas the inner one-third is 60% type II collagen and 40% type I collagen [69]. Pyridinoline collagen crosslinking has been shown to be highest in the inner region [70].

The biochemical composition of the TMJ disk is similar to the meniscus, being composed of primarily collagen and GAGs. Collagen is approximately 68.2% per dry weight in the porcine TMJ disk [71], while GAG content ranges from 0.273 to 0.936% per wet weight among species [51]. In a study on the structure–function relationship of the Yucatan minipig TMJ disk, the tissue showed regional variation in DNA content via PicoGreen assay ranging from 0.024% to 0.041% per wet weight [48]. In a study on the porcine TMJ disk using enzyme-linked immunosorbent assay to quantify GAGs, chondroitin sulfate was the most abundant GAG found, compromising 74% of the total GAG content [56]. For regional collagen variation, the intermediate zone had slightly more collagen per dry weight than the anterior and posterior bands of the disk, while in the mediolateral direction the central region contained significantly higher collagen than the lateral region [71]. In the Yucatan minipig TMJ disk, pyridinoline content was found to be significantly lower in the anterior and posterior bands than in the lateral and medial regions of the disk [48].

Biochemically, the knee meniscus and TMJ disk are similar due to their fibrocartilaginous nature. Both have similar ranges for collagen, GAG, and DNA content, and vary regionally as discussed previously. In addition, the meniscus and TMJ disk both are composed of primarily type I collagen in relation to other collagen types. Uniform biochemical characterization can be used for fibrocartilages and is a required quantitative step after performing histomorphological studies. Although biochemical assays may provide insight into structure, they should be supplemented by

mechanical testing to yield an understanding into fibrocartilage function.

3.3 Mechanical Properties. Inasmuch as fibrocartilages bear and distribute load, recapitulating the tissue's mechanical properties is a critical design criterion. Tension and compression tests are commonly used to derive target values. Uniaxial tensile testing provides tensile Young's modulus and ultimate tensile strength (UTS) [48,52,54,63,72]. For compression properties, creep indentation testing and incremental stress relaxation provide, among other properties, aggregate modulus [73–75], coefficient of viscosity [53,63,76], and instantaneous and relaxation moduli [48,51,54,62,63]. In addition to aggregate modulus, Poisson's ratio and permeability are also obtained from creep indentation testing [73,75,77]. These values can be derived from experimental data using different models based on linear elasticity, viscoelasticity including the standard linear solid model, poroelasticity, and mixture theories including the biphasic model. In-depth descriptions of these tests and their assumptions, performance, and mechanical models are available in the literature [1,78–82]. While no one testing modality is the gold standard for measuring mechanical properties, tissue structure–function relationships dictate which testing modality might be most informative when measuring characteristic properties of a native tissue. For example, the knee meniscus functions under compression, but its geometry causes tensile forces to develop within the tissue, and, thus, the tensile properties of a tissue engineered meniscus may be more indicative of whether it will be effective in replacing

Table 1 Selected list of key publications for fibrocartilage tissue engineering

Authors	Cell source	Scaffold or scaffold-free approach	Biochemical stimuli	Mechanical stimuli	Tissue engineered
Kasemkijwattana et al. [127]	Leporine MCs	Monolayer	Epidermal growth factor, IGF-1, bFGF, PDGF, TGF-β1, transforming growth factor alpha, acidic fibroblast growth factor, and nerve growth factor	None	Knee meniscus
Springer et al. [87]	Human and Porcine TMJ disk cells and articular eminence cells	Polyamide, polytetrafluoroethylene, and PGA scaffold	None	None	TMJ disk
Detamore and Athanasiou [97]	Porcine TMJ disk cells	PGA scaffold	IGF-1	Fluid-induced shear	TMJ disk
Eifler et al. [136]	Leporine MCs	Monolayer	None	Oscillatory fluid flow-induced shear	Knee meniscus
Bean et al. [95]	Porcine TMJ disk cells	PGA scaffold	Ascorbic acid	None	TMJ disk
Almarza and Athanasiou [98]	Porcine TMJ disk cells	Monolayer and PGA scaffolds	None	Hydrostatic pressure	TMJ disk
Aufderheide and Athanasiou [109]	Bovine ACs and MCs	Self-assembly	None	None	Knee meniscus
Johns et al. [89]	CCs, dermal fibroblasts, TMJ disk cells	Self-assembly	None	None	TMJ disk
Gunja et al. [125]	Leporine MCs	PLA scaffold	TGF-β1	Hydrostatic pressure	Knee meniscus
Huey and Athanasiou [53]	Bovine ACs and MCs	Self-assembly	TGF-β1, C-ABC	Tension and compression	Knee meniscus
Huey and Athanasiou et al. [76]	Bovine ACs and MCs	Scaffold–self-assembly	TGF-β1, C-ABC	None	Knee meniscus
Kalpakci et al. [106]	Bovine Acs and MCs	Self-assembly	TGF-β1, IGF-1	None	TMJ disk
Baker et al. [134]	Bovine MSCs	PCL scaffold	TGF-β3	Cyclic tension	Fibrocartilage
Hagandora et al. [100]	Caprine CCs	Poly (glycerol-sebacate) scaffold	None	None	TMJ disk
Hadidi and Athanasiou et al. [63]	Bovine ACs and MCs	Self-assembly	LPA	None	Knee meniscus
MacBarb et al. [124]	Bovine ACs and MCs	Self-assembly	C-ABC, TGF-β1	None	Fibrocartilage
Ahtiainen et al. [118]	Leporine adipose-derived MSCs	PLA scaffold	TGF-β1	None	TMJ disk
Moriguchi et al. [114]	Porcine synovium-derived MSCs	Cell sheet engineering	BMP-2	None	Knee meniscus
Makris et al. [54]	Bovine ACs and MCs	Self-assembly	TGF-β1, C-ABC, LOXL2	None	Fibrocartilage
Higashioka et al. [110]	Bovine ACs and MCs	Self-assembly	None	None	Knee meniscus
MacBarb et al. [135]	Bovine ACs and MCs	Self-assembly	None	Passive axial compression	TMJ disk
Murphy et al. [103]	Porcine CCs	Self-assembly	C-ABC, TGF-β1, LOXL2	None	TMJ disk
Murphy et al. [112]	Porcine CCs	Self-assembly	TGF-β1, bFGF, PDGF	None	Fibrocartilage
Legemate et al. [117]	Human bone marrow-derived MSCs	PCL scaffold	CTGF, TGF-β3	None	TMJ disk
Warren et al. [121]	None	PCL scaffold	None	None	Knee meniscus
Wang et al. [90]	Rabbit TMJ disk cells and synovium-derived MSCs	PLGA scaffold	TGF-β3	None	TMJ disk

Note: Fibrocartilage tissue engineering studies were selected for their impact on the field. Authors, cell source, scaffold or scaffold-free approach, biochemical stimuli, mechanical stimuli, and type of engineered tissue are listed for these studies.

diseased tissue. Similarly, an analogous argument can be made for the TMJ disk; though the disk functions primarily under compression, the end result is principally tensile strain fields in the ECM. Values derived from mechanical testing of the meniscus and TMJ disk are provided below.

Since both the knee meniscus and the TMJ disk exhibit anisotropy, the mechanical properties depend on testing direction. The knee meniscus exhibits more robust tensile mechanical properties in the circumferential orientation rather than the radial due to the generally circumferentially aligned collagen fibers; this holds true throughout the depth of the tissue for the tissue's Young's modulus [72]. The Young's modulus is approximately 100–300 MPa in the circumferential direction and tenfold lower in the radial direction [2]. The meniscus has been shown to have an aggregate modulus of 100–150 kPa [75]. Incremental stress relaxation testing of porcine knee menisci in synovial fluid has yielded instantaneous and relaxation moduli for 20% strain of 2.37–6.75 MPa and 0.07–0.15 MPa, respectively, [83]. Values of mechanical properties can vary from species to species, as well as different testing modalities [77,84].

The mechanical properties of the TMJ disk display anisotropic, regional, and interspecies variations. Research on the Yucatan minipig TMJ disk revealed that UTS and tensile Young's modulus of the central region was highest in the anteroposterior direction, while the posterior band was stiffest and strongest in the mediolateral direction, when determined by uniaxial tensile testing [48]. Creep indentation testing shows that the medial region of the TMJ disk had the largest aggregate modulus at 28.9 ± 12.3 kPa and was found to be significantly higher than the anterior, posterior, central, and lateral regions [73]. Instantaneous and relaxation moduli for 20% strain in the Yucatan minipig TMJ disk were found to be 216–1540 kPa and 20.5–57.5 kPa, respectively, dependent on region [48]. Uniaxial tensile testing, creep indentation testing, and incremental stress relaxation all provide valuable design criteria.

As tissues that undergo constant mechanical loading, the gold standard for fibrocartilage functionality should accordingly be mechanical testing. Appropriate characterization of not only mechanical properties, but histomorphological and biochemical properties, defines the design criteria to be used in tissue engineering studies. By defining native tissue values, tissue engineers know what criteria they need to strive for and mimic within tissue engineered fibrocartilages.

4 Tissue Engineering of Fibrocartilage

The tools developed to address the design criteria for tissue engineering fall into the general category of cells, scaffolds, and signals. For fibrocartilage, of particular interest are the issues of finding an appropriate cell source, choosing a scaffold or scaffold-free approach, and identifying both biochemical and mechanical stimuli as depicted in Fig. 3. A selection of the most impactful studies outlined in Sec. 4 is summarized in Table 1. Sections 4.1–4.4 will include information on each of the aforementioned components with a focus on approaches shown efficacious when applied with a scaffold-free, self-assembling process of tissue formation.

4.1 Cell Sources. Cell sources used in tissue engineering of fibrocartilage vary from tissue-specific, terminally differentiated cells to various stem cell types. In terms of tissue-specific cells for tissue engineering of the knee meniscus, meniscus cells (MCs) and hyaline articular chondrocytes (ACs) [53,63,76,85] have been explored. For engineering the TMJ disk, TMJ disk cells [86–98], articular eminence cells [87], mandibular condyle cells [99], costal chondrocytes (CCs) [89,100–104], ACs [54,102,105–107], MCs [54,106,107], and dermal fibroblasts [89] have been explored. Mesenchymal stem cells (MSCs) are the most heavily examined stem cell population for tissue engineering of both fibrocartilages. Factors to take into account for all cells are an autologous versus allogeneic approach, coculture of cells, and various cell expansion technologies. For stem cells, additional considerations include their theoretically infinite ability to expand and suboptimal differentiation efficiency.

Autologous tissue-specific, terminally differentiated cells directly from native tissue, such as TMJ disk cells or MCs, offer the lowest risk of rejection, but sourcing can be a difficulty due to insufficient healthy tissue. Other cell sources that can potentially be derived in an autologous fashion for tissue engineered fibrocartilages include cells from hyaline articular cartilage [54,102,105–107], costal cartilage [89,100–104], tendon, and ligament [108]. Autologous sources require two surgical procedures on the same patient: one for harvest of the donor tissue and another for implantation of engineered tissue. An allogeneic approach, which employs cells from a nonself donor, mitigates the issue of multiple surgeries for the patient and donor site morbidity but is limited by a possible immune response and rejection. Traditionally, articular cartilage has been considered to be an immunoprivileged tissue; immune response against cells within cartilage is rare due to the dense ECM [1]. A recent minipig study showed minimal to no T cells, B cells, and macrophages within allogeneic, tissue engineered fibrocartilage implants in the TMJ disk [104], providing evidence that fibrocartilage, like hyaline articular cartilage, may also be immunoprivileged.

Cocultures of cells have been explored to recreate the various fibrocartilages that naturally contain different cell types and ECM composition. For example, a one-to-one coculture ratio of ACs and MCs [53,63,76], in comparison with other ratios, has been shown to be optimal in reconstituting the native meniscal cross section as well in providing adequate strength and stiffness [109]. Menisci that exhibit a more hyaline articular cartilage-like inner region and a more fibrous outer region have been engineered by seeding 100% ACs in the inner region and a one-to-one mix of ACs to MCs in the outer region. This regionally variant meniscus exhibited significantly higher compressive properties as well as GAG per dry weight in the inner region, while the outer region exhibited significantly higher circumferential tensile modulus and collagen per dry weight [110]. These compositional and functional properties mimic the biochemical and mechanical differences seen in native meniscus regions (Fig. 5(*b*)). For tissue engineering the TMJ disk, AC and MC cocultures [54,106,107], and CC and dermal fibroblast cocultures [89] have been examined. In AC and MC coculture, it was found that the presence of ACs is required to maintain a cylindrical shape by reducing contraction [106]. CC and dermal fibroblast coculture was inferior to CCs alone in terms of GAG content, total collagen, and type I collagen [89]. Coculture of multiple cell sources remains a viable option for creating more biomimetic tissue engineered fibrocartilages. Clinically, this may be more difficult to achieve using an autologous approach due to donor site morbidity and increasing number of surgeries as previously discussed, but an allogeneic approach might be appropriate if coculture were used.

Advances in cell expansion technologies that preserve cell phenotype, in combination with an allogeneic approach, have the potential to mitigate the concerns that repeat surgeries, donor site morbidity, and cell sourcing pose. For example, a combination of transforming growth factor beta 1 (TGF-β1), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) increases the postexpansion chondrogenic potential of CCs by increasing GAG content, altering the ratios of collagen types, and improving compressive properties engineered using treated cells [111]. After expansion, the phenotype of CCs can be preserved by culturing them in three-dimensional (3D) aggregates [112]. During this aggregate redifferentiation process, application of TGF-β1, growth differentiation factor 5 (GDF-5), and bone morphogenetic protein 2 (BMP-2) also improves biochemical and mechanical properties of neocartilage using treated cells [113]. This process allows defined expansion of cells and preservation of phenotype by aggregate culture, and is extremely promising for allogeneic approaches, increasing the impact one donor can have.

Stem cells offer a solution to sourcing issues by having a theoretically infinite capability to expand. Synovial MSCs have been explored for the repair of the meniscus in scaffold-free culture methods [114] as well as via injection [115,116]. TMJ disk engineering has used both MSCs from bone marrow [117] and adipose tissue [118]. The current limitation of stem cells for tissue engineered fibrocartilage formation lies in their suboptimal differentiation protocols, which often lack efficiency (i.e., only a low percentage of cells attain the target phenotype) and may result in "chondrocyte-like" cells [119] that may not form mechanically robust tissue engineered fibrocartilage. Additional concerns with stem cell use include tumorigenic potential and possible xenogeneic culture components. While stem cells for tissue engineered fibrocartilages have been used in research, their infinite expansion potential has yet to be realized clinically due to lack of efficiency.

To summarize, an autologous approach may be the ultimate goal because the cells are patient-specific, but not the most practical because the scarcity of healthy tissue remains an issue in these already diseased patients. An allogeneic approach may be the most translatable, especially with the advent of cell expansion technologies and evidence that suggests fibrocartilage as immunoprivileged. Allogeneic cells solve the issue of donor site morbidity and repeated surgeries from autologous approaches. Using stem cells may present the solution to the cell sourcing issue, but their translatability is not yet realized due to efficiency and possible tumorigenic potential. The selection of a cell source is among the most important choices a tissue engineer can make and should be well-informed by how a tissue engineered fibrocartilage will be translated.

4.2 Scaffold and Scaffold-Free Methods. For 3D cell culture of tissue engineered fibrocartilage, both scaffold and scaffold-free methods exist. Scaffolds can be used to direct cell behavior by engineering specific biochemical and mechanical cues into the biomaterial. In addition, scaffolds also allow immediate cell attachment and provide support to the cells. Tissues can also be engineered without scaffolds. Scaffold-free tissue engineering is particularly useful when one wants to avoid scaffold degradation products and stress shielding cells. With scaffold-free methods, degradation products and residual byproducts from fabrication and their associated toxicity to the cells do not need to be considered. Stress-shielding of cells via scaffolds is another consideration that is removed in scaffold-free approaches. While scaffolds retain the ability to directly alter cell behavior and support cells, for fibrocartilage tissue engineering, soluble and mechanical signals have both shown efficacy in directing cell performance in the absence of scaffolds.

A variety of scaffolding materials have been explored for tissue engineered fibrocartilages including alginate [86], polycaprolactone (PCL) [117], poly(glycolic acid) [86–88,93–98,105], decellularized matrix [120], polyamide [87], polytetrafluoroethylene [87], poly(glycerol sebacate) [100], type I collagen [91,99], poly(-lactic acid) (PLA) [88,105,118], and poly(lactic-co-glycolic acid) (PLGA) [90,117]. Considerations for scaffold formulations include degradation rates and products, and fabrication methods and resulting residual byproducts. Also, a recently added consideration may be compatibility with 3D printing because the technology is conducive toward producing tissue engineered fibrocartilages that are anisotropic and regionally variant, characteristics important in the function of native fibrocartilages. For example, anisotropic collagen alignment has been produced in 3D printed menisci [121]. Similarly, a regionally variant TMJ disk has been produced using 3D printing with PCL and spatiotemporal delivery of PLGA microspheres with connective tissue growth factor (CTGF) and transforming growth factor, beta 3 (TGF-β3) encapsulated [117]. The wide range of scaffolds available for knee meniscus and TMJ disk tissue engineering has been reviewed elsewhere [2,45,122].

Self-organization and the self-assembling process are techniques that generate 3D structures in a scaffold-free manner, but they are distinctly different. Self-organization is defined as any technique that produces biomimetic tissues with use of external forces or energy whereas the self-assembling process is defined as a spontaneous organization of cells that mimics native tissue structures without external forces or energy. Self-assembly occurs via the minimization of free energy through cell-cell interactions. Examples of self-organization include cell sheet engineering and bioprinting of cells. Self-assembly is used across multiple tissue types, including fibrocartilage. Self-assembly addresses considerations of scaffold-based methods by the creation of robust tissue engineered fibrocartilages that can immediately bear load and do not shield the cells from various stresses present in the joint environment [123].

4.3 Biochemical Stimuli. Biochemical stimuli are used to target cells and ECM molecules to improve mechanical properties. This can occur, for example, via increased production of ECM, improved collagen fiber alignment, or increased collagen crosslinking. For the production of scaffold-free, tissue engineered fibrocartilage, prior studies have applied a variety of growth factors including TGF-β1, small molecules such as ascorbic acid and phospholipid lysophosphatidic acid (LPA), and matrix modifying enzymes chondroitinase ABC (C-ABC) and lysyl oxidase-like 2 (LOXL2) separately and in combination.

Growth factors have been extensively studied for tissue engineered fibrocartilages. TGF-β1 [54,76,124,125], TGF-β3 [88,90,117], CTGF [117], PDGF [92,126,127], bFGF [92–94,127], insulin-like growth factor 1 (IGF-1) [88,92–94,106,126,128], and epidermal growth factor [126,127] are examples of growth factors that have shown various levels of efficacy in enhancing tissue engineered fibrocartilage formation. For example, TGF-β1 has been shown by microarray analysis to promote AC synthesis of ECM [129] and has shown similar effects in fibrocartilage studies [54,76,124,125]. Small molecules such as LPA and ascorbic acid have been studied as well. LPA increased values of tensile Young's modulus from 247 ± 89 kPa in control groups to 503 ± 159 kPa in stimulated groups, along with collagen fiber density and organization in meniscal tissue engineered fibrocartilage [63]. Ascorbic acid is a vital component to cell culture media and was found to be optimal at 25 μg/mL for cell concentration, collagen deposition, and aggregate modulus values in a TMJ disk model [95]. Enzymes such as the GAG-depleting enzyme C-ABC and the collagen crosslinking enzyme LOXL2 have been previously shown to have a positive effect on mechanical properties. Specifically in articular cartilage, C-ABC has been shown to increase tensile properties exhibiting an increase of 121% and 80% compared to untreated controls in UTS and Young's modulus, and allow for more type II collagen deposition as a result of GAG depletion [130]. For the native knee meniscus, LOXL2 has been shown to increase tensile properties approximately 1.9-fold during explant culture [131]. More thorough and extensive reviews of various biochemical stimuli and their effects on tissue engineered fibrocartilage are available in the literature [2,132,133].

Various growth factors and enzymes have also been used in combinations to create synergistic effects between increased ECM and more mature ECM. For example, increases in radial tensile moduli by fivefold over untreated controls of meniscal tissue engineered fibrocartilage were observed over untreated controls when a combination of TGF-β1 and C-ABC was applied [76]. A TGF-β1 and C-ABC combination can be used to tissue engineer other fibrocartilages as well because it has been observed to increase both tensile Young's modulus and UTS over unstimulated controls, reaching the lower range of native values [124]. Combining TGF-β1, C-ABC, and LOXL2 treatments during the culture of tissue engineered fibrocartilage led to further significant improvement of tensile Young's modulus and UTS by 245% and 186%, respectively, [54]. This combination has also been used to enhance mechanical properties and integration of TMJ disk tissue

engineered fibrocartilages, resulting in values of tensile Young's modulus of over 6 MPa and compressive instantaneous modulus of over 1200 kPa after 8 weeks in culture [103]. The biochemical stimuli that have been used and their varying efficacy might warrant additional research into novel, synergistic combinations of stimuli.

4.4 Mechanical Stimuli. Mechanical forces exerted naturally on native fibrocartilage are critical in tissue development and homeostasis. Native fibrocartilages experience tension, compression, hydrostatic pressure, and shear, and each of these forces has been applied to tissue engineered fibrocartilage as well. Prior tissue engineering studies involving mechanical loading either alone or combined with biochemical stimuli have resulted in significant increases of mechanical properties and also anisotropy.

Tension and compression are two commonly applied mechanical stimuli for tissue engineered fibrocartilage. While typically applied as separate stimuli, in fibrocartilage they often work together. For example, in the meniscus when a compressive load is applied, tensile strains develop due to the meniscus' wedge shape [2]. Meniscal tissue engineered fibrocartilage comprised of a nanofibrous matrix seeded with MSCs was subjected to dynamic tensile loading, leading to an increase in tensile modulus by 16% [134]. Independently of tension, passive axial compression of 0.1 N in a TMJ disk model has been shown to increase collagen and GAG content significantly as well as increase relaxation and tensile Young's modulus by 96% and 255%, respectively, over controls [135]. Combining TGF-β1 and C-ABC treatments with direct tension-compression loading during culture significantly increased instantaneous modulus (threefold), relaxation modulus (twofold), and tensile Young's modulus in the radial (sixfold) and circumferential (fourfold) directions of self-assembled meniscal fibrocartilage. The direct compression-tension bioreactor for menisci was fabricated such that the platens matched the curved surface and elliptical shape of the meniscal tissue engineered fibrocartilage, ensuring simultaneous compression and tension stimulation [53].

Although less often examined, hydrostatic pressure and shear also have been used to tissue engineer fibrocartilage. When subjected to a hydrostatic pressure loading regimen, PLA scaffolds seeded with MCs exhibited increases in ECM production exhibiting threefold higher GAG deposition and fourfold higher collagen deposition [125]. In a study on TMJ disk cells on PLA scaffolds, hydrostatic pressure was applied at 10 MPa either intermittently at 1 Hz or continuously for 4 h a day. Type I collagen was highest in the continuous stimulation group compared to the nonloaded and intermittent stimulation groups [98]. Fluid shear, while typically regarded as being a detrimental mechanical stimulus for the maintenance of a chondrocyte-like phenotype, may merit exploration for tissue engineered fibrocartilages. Exposing MCs to oscillatory fluid flow in parallel plate flow chambers has been shown to upregulate calcium signaling and GAG production [136]. Use of a rotating bioreactor in TMJ disk cell culture led to earlier and greater contraction compared to the control. This resulted in a denser ECM and cell composition; however, total ECM content and compressive stiffness were not significantly different [97]. Overall, there is currently not enough evidence to conclude whether fluid-induced shear is beneficial for tissue engineered fibrocartilages.

Using mechanical stimuli on tissue engineered fibrocartilages is an effective way to increase ECM production and organization, which subsequently results in more robust mechanical properties. This in conjunction with a biochemical stimulus regimen may also lead to synergistic effects, further enhancing tissue engineered fibrocartilage functionality. While there are limited studies using mechanical stimuli on tissue engineered fibrocartilage, many of the stimuli discussed here have been extensively studied for hyaline articular neocartilage in other reviews [137]. Further examination of mechanical stimulus regimens for tissue engineered fibrocartilage is warranted because specific application times and load amounts can have either beneficial or detrimental effects.

4.5 Toward Tissue Engineering the Fibrocartilage Spectrum. Due to the spectrum of fibrocartilage structures in the body, each tissue engineering strategy will be slightly different. The outlined studies here provide insight into current tissue engineering methodology for the knee meniscus and the TMJ disk, but the approach to the pubic symphysis or annulus fibrosus of the intervertebral disk might require different methods. However, the concepts discussed in the prior sections can be used generally to approach tissue engineered fibrocartilages in a uniform manner. One way to tailor the tissue engineering approach used is application of multiple types of stimuli, varying the cell source, or using a different scaffolding or scaffold-free approach. Taking these considerations into account is critical when designing and carrying out tissue engineering studies. By properly considering these factors, a translational approach can be created and quickly shifted from basic research to preclinical animal models. This can eventually result in transition to clinical trials and a tangible product that can be put through the FDA paradigm (Fig. 4).

4.6 Evaluation of Tissue Engineered Fibrocartilage. Histomorphological, biochemical, and mechanical testing of tissue engineered fibrocartilage yields properties that can be compared with those of native tissue to determine whether the tissue engineering design criteria have been met. All evaluation methods outlined in Sec. 3 can be applied to tissue engineered fibrocartilage (Fig. 3). The quantitative values derived from these assays can be statistically compared to each other to determine whether one tissue engineering modality is more efficacious than another. Quantitative values can also be normalized to native tissue values in the form of a functionality index (FI), Eq. (1). The FI accounts for biochemical and mechanical properties found in native tissue and normalizes tissue engineered values to those of native tissue. The FI provides a quantitative value that reflects the overall quality of tissue engineered constructs that can be compared to each other. For example, the TMJ disk FI accounts for GAG, total collagen, instantaneous modulus values, relaxation modulus values, tensile Young's modulus values, and UTS values. The FI in Eq. (1) weighs each of the metrics equally [104,138]. The FI varies between 0% and 100%, where 100% is the value of native fibrocartilage.

$$\mathrm{FI}(\mathrm{TE}|N) = \frac{1}{6}\left[\begin{array}{c}\left(1-\left|\frac{\mathrm{GAG}_N-\mathrm{GAG}_{\mathrm{TE}}}{\mathrm{GAG}_N}\right|\right)+\left(1-\left|\frac{\mathrm{Col}_N-\mathrm{Col}_{\mathrm{TE}}}{\mathrm{Col}_N}\right|\right)+\left(1-\left|\frac{E_N^{20i}-E_{\mathrm{TE}}^{20i}}{E_{\mathrm{N}}^{20i}}\right|\right)\\ +\left(1-\left|\frac{E_N^{20r}-E_{\mathrm{TE}}^{20r}}{E_N^{20r}}\right|\right)+\left(1-\left|\frac{E_N^{T}-E_{\mathrm{TE}}^{T}}{E_N^{T}}\right|\right)+\left(1-\left|\frac{\mathrm{UTS}_N-\mathrm{UTS}_{\mathrm{TE}}}{\mathrm{UTS}_N}\right|\right)\end{array}\right]*100\% \quad (1)$$

Similarly, a knee meniscus FI might include similar components with the addition of radial tensile modulus to account for the tissue's anisotropy.

It is important to note that a perfect FI of 100% is not necessarily needed for proper functioning of tissue engineered fibrocartilage in vivo. For example, an FI of 42% was adequate for a TMJ disk thinning model in the Yucatan minipig, where the implanted disk exhibited mechanical robustness in situ, adaptively remodeled, and improved integration stiffness [104]. For specific models of fibrocartilage injury, appropriate FI values need to be established for the translation of tissue engineered fibrocartilages that researchers can aim for.

It is important to note that the tissue engineering approach must meet established design criteria (Fig. 3). As discussed, this can be measured by an index such as an FI, but other characteristics such as cell morphology and tissue anisotropy need to be evaluated qualitatively or using other measurements. If the tissue engineering approach does not meet design criteria in any of these categories, the process can be reiterated, and the approach can be modified to meet the target design criteria (Fig. 3). Upon meeting design criteria for the tissue engineering phase, researchers still need to demonstrate safety and efficacy in preclinical animal models and approved by the FDA before a tissue engineered fibrocartilage can be marketed as a therapy.

5 Toward Translation of Tissue Engineering

Tissue engineered fibrocartilage safety and efficacy must first be reviewed and cleared by the FDA before it can be marketed for clinical use. After tissue engineering studies, tissue engineered fibrocartilages should be demonstrated as safe and effective in animal models before examining the products' effects in humans. This section will present the FDA paradigm (Fig. 4), diving into preclinical animal models and clinical trials, and discussing considerations for both. Because there is lack of approved tissue engineered fibrocartilage products existing for repair or replacement, this section uses existing articular cartilage guidance as a way to infer how tissue engineered fibrocartilage products might be regulated. This section closes with a discussion on areas where additional guidance from the FDA is desired, for example, through the creation of a fibrocartilage guidance document analogous to that which exists for articular cartilage.

5.1 The Food and Drug Administration Paradigm. Tissue engineered fibrocartilage products will be regulated as HCT/Ps, a category of products containing or consisting of human cells or tissues intended for implantation, transplantation, infusion, or transfer into humans [4]. Much like tissue engineered products for hyaline articular cartilage [5], tissue engineered fibrocartilage products will be regulated through two centers of the FDA: the Center for Biologics Evaluation and Research (CBER) and/or the Center for Devices and Radiological Health (CDRH). CBER and CDRH co-authored the FDA guidance document for products intended to repair or replace hyaline articular cartilage [5], and this document can give insight into how tissue engineered fibrocartilage products might be regulated given similarities between the two tissue types.

If an HCT/P is minimally manipulated, intended for homologous use, and uncombined with another object, then it is only subject to regulation under Section 361 of the Public Health Service (PHS) Act and title 21 of the Code of Federal Regulations Section 1271.3(d)(1). These HCT/Ps are referred to as 361 products and do not require premarket approval. Examples of 361 products include bone (including demineralized bone), ligaments, tendons, and cartilage, which may have been sourced from cadaveric tissues. In terms of specific fibrocartilage products, cadaveric fibrocartilaginous tissue to be used as an allograft such as the knee meniscus and TMJ disk would fall under the category of 361 products. Otherwise, HCT/Ps are regulated as drugs, and/or biological products under Section 351 of the PHS Act and/or the Federal Food, Drug, and Cosmetic (FD&C) Act and are referred to as 351 products. Examples as provided by the FDA include cultured cartilage cells, cultured nerve cells, and gene therapy products. For fibrocartilage, expanded TMJ disk cells or MCs might fall under this category as well as tissue engineered fibrocartilage cultured using the self-assembling process.

Under the CDRH, products are regulated as devices under the FD&C Act. Human collagen and preserved umbilical cord vein grafts are in this classification. Biomaterial scaffolds without combination of cells for fibrocartilage repair or replacement may fall into this category. In addition, certain HCT/Ps can be classified as combination products by the Office of Combination Products and assigned to CBER or CDRH for primary jurisdiction. One example is cultured cells on synthetic membranes or combined with collagen. This product has potential to be regulated as a device or biological product, but is currently under review and may be regulated by CBER under device or 351 product regulations [4]. Tissue engineered fibrocartilage with use of a scaffold and seeded chondrocytes may fit into this category. Due to the many ways and materials with which fibrocartilage can be engineered, the FDA's classification of tissue engineered fibrocartilage products can vary. Consultation with the FDA is recommended if there is confusion as to the categorization of a specific tissue engineered fibrocartilage product.

Following product classification, a sponsor seeking FDA approval may consult guidance documents and the regulation of other approved products to determine data that need to be collected and submitted to the FDA. Guidance documents specifically for tissue engineered fibrocartilage products have not been published, but a guidance document has been published for products intended for repair or replacement of hyaline articular cartilage, which shares many similarities with fibrocartilage. In addition, autologous cultured chondrocytes on a porcine collagen membrane is an approved cellular and gene therapy product whose pathway to regulatory approval may offer insights for tissue engineered fibrocartilage products. The guidance document for articular cartilage products contains nonbinding recommendations to the industry on preparation and submission of investigational device exemption (IDE) and/or an investigational new drug (IND) application. Recommendations for classification of products, preclinical data, biocompatibility testing, and clinical study protocols are described. For example, goats, sheep, and horses are listed as the most frequently used large animal models for testing biological response, durability, toxicology, dose response, lesion size and location, appropriate endpoints, and use of arthroscopic or MRI imaging evaluations for articular cartilage repair [5]. Fibrocartilage large animal models are similar to the ones employed for articular cartilage with the addition of the minipig, farm pig, and dog [43,46,48,139]. For clinical trials, design, controls, study populations, endpoints, implantation procedures, and patient follow-up are all discussed as well [5]. Examples of measures that may be used to assess endpoints for articular cartilage products are the Knee Injury and Osteoarthritis Outcome Score (KOOS), IKDC Subjective Knee Evaluation Form-2000, and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [5]. For fibrocartilage within the knee such as the meniscus, these scoring systems might be adaptable while the TMJ disk fibrocartilage might need new indices created. This motivates the creation of a standardized scoring system for fibrocartilages throughout the body.

Guidance documents as well as meetings with the FDA help to provide clarity on the process by which a product receives FDA approval, and this process is briefly depicted in Fig. 4. Tissue engineering studies yield a product candidate that is then tested in preclinical animal studies to generate data for submission of an IDE and/or IND application dependent on product classification. An IDE/IND is necessary for clinical trials. Clinical trials are conducted in phases, and considerations for clinical trials include defining and measuring endpoints, the surgical procedures used, and patient follow-up. Upon completion, data from the trials are

submitted via a premarket approval and/or a biologics license application (BLA) to the FDA. These applications will be under review for a time-period known as the premarket application phase where the FDA reviews the data for safety and efficacy of the product. FDA approval allows the product to be marketed. Product safety and efficacy continues to be monitored in the post-marketing phase, sometimes referred to phase IV clinical trials. For more information on the FDA paradigm and general translation of tissue engineering products, readers are directed to a recent review [140].

5.2 Preclinical Animal Models. Currently, there are limited approved fibrocartilage HCT/Ps or clinical trials. Putting this in context of Fig. 4, the general state of fibrocartilage tissue engineering currently straddles the phases of tissue engineering studies (discussed in Sec. 4) and preclinical animal studies. Animal studies provide preclinical data that show how the product functions in vivo. Animal studies are used to assess biological responses, the durability of repair, toxicology, dose response, lesion size and location, appropriate endpoints mirroring those to be used in humans, and use of arthroscopic and/or MRI evaluations as has been previously outlined [5]. Aside from examining the host, testing modalities outlined in Sec. 3 should also be applied to the tissue engineered fibrocartilage implant both before and after implantation. Data on how the implant's biochemical, mechanical, and cellular properties change or remain the same will inform the success of the tissue engineering process and implant performance in vivo. Similar to using the FI to optimize tissue engineering procedures, the FI can be used for in vivo studies to determine, for example, implant properties that correlate with a durable repair response. It is worth noting that, unlike suggestions found in the hyaline articular cartilage product guidance document which only touches on compressive testing modalities [5], an appropriate FI for fibrocartilage should include both tensile and compressive properties due to the way fibrocartilage functions. Correlation of the implant's FI to host response might further inform eventual release criteria for the manufacturing of tissue engineered fibrocartilage products. An index such as the FI for general fibrocartilage tissue engineering would be informative to the field and allow comparison of various tissue engineering strategies for different fibrocartilages.

Ideally, preclinical studies in animals would test a version of the product that is identical to that which will be used in clinical studies. Investigating a product that contains human cells in animal models could require immunosuppressive agents to avoid rejection upon implantation, and this can be difficult if not impossible to implement in certain animal models. Recently, a review on experimental immunosuppression and immunomodulation has been published and may help provide strategies by which these can be applied to xenogeneic or allogeneic animal models [141]. Alternatively, one can test an analogous cellular product in terms of cellular characteristics and biological activity, derived from the animal species used in studies in an allogeneic strategy.

Preclinical data can be obtained from a combination of small and large animal studies. Small animal models, such as rodent and leporine models, allow for larger, more economical studies. However, for fibrocartilage injuries, surgical procedures in small animals may become difficult due to small joints that provide little space for operating. Translational applications in humans for tissue engineered fibrocartilage are best modeled in large animals that replicate human biomechanics as much as possible. As noted previously, goats, sheep, and horses are recommended for examining hyaline articular cartilage repair [5], but other species may suit fibrocartilage studies better. For example, menisci in pigs and sheep are most similar to humans' in terms of size and proportion [44], while ovine menisci are also similar to humans' in terms of composition and biomechanics [142]. For the TMJ disk, the Yucatan minipig has also been deemed a suitable comparative model to humans in terms of its structure–function relationships [48], and has seen success in a regeneration study by our group which used CCs to tissue engineer allogeneic TMJ disk fibrocartilage [104]. As such, the pig (including minipigs) and sheep may prove useful as large animal models for fibrocartilage studies, especially in those regarding the knee meniscus and TMJ disk.

For each animal model, details such as the specific surgical procedure for implanting the fibrocartilage product, how that surgical procedure may translate to human studies, how the study models particular indications, and specialized recovery or postoperative care must all be considered. For example, in a recent study where a focal thinning defect model was used, there was careful consideration of the minipig's postoperative diet [104]. After TMJ surgery, a diet consisting of mainly soft foods or liquids as opposed to hard foods is more amenable to repair. Thus, even if an animal model displays anatomical and functional similarities to humans, it does not automatically mean that the model should be chosen if surgical, husbandry, or other aspects listed previously cannot be adequately developed for the animal.

5.3 Clinical Trials. After obtaining preclinical data and approval of an IDE and/or IND, clinical trials can commence. Phase I and II trials commonly contain small patient cohorts compared to phase III trials. Phase I trials are meant to determine safety and dosage of the tissue engineered fibrocartilage product. Phase II trials determine product efficacy and possible side effects of fibrocartilage therapies. Phase III trials examine long-term safety and efficacy in larger patient cohorts.

While animal models may inform endpoints in humans, it is ultimately clinical trial data that will be used in final approval for market. Because explanting implanted tissue engineered fibrocartilages would impair function, it is oftentimes not possible to test human implant properties as done in preclinical animal models. Therefore, endpoints are often defined via subjective scales, such as pain and range of motion testing. Development of a standard fibrocartilage scoring system would be of great value to clinical trials of tissue engineered fibrocartilage products. Arthroscopic evaluation, histologic evaluation, serological assessments for inflammation, and imaging might also inform endpoints [5].

Considerations that ensure successful repair in animals should likewise be thought out in clinical trials. For example, surgical approaches such as technique and postoperative care must be standardized and inspected particularly in multicenter trials to minimize center-to-center variability. In addition, for the indication that a tissue engineered fibrocartilage product intends to treat, participants that undergo current gold standard treatment should also be enrolled to demonstrate the tissue engineered product's efficacy over standard of care. For example, for late-stage pathology of the TMJ disk such as perforation, either discectomy or total joint reconstruction is often indicated. These two clinical treatments will ultimately be two treatments that a tissue engineered TMJ disk may be compared to. Finally, follow-up of treatment with tissue engineered fibrocartilage will be required in these patient populations. It is common for the FDA to require safety and efficacy data over a number of years to compare short-term results of the tissue engineered fibrocartilage to current clinical treatments. The FDA will also use these data to evaluate claims of the product. For successful execution of clinical trials, these considerations should be taken into account to gain FDA approval for commercialization.

5.4 Future Directions. Tissue engineering approaches of fibrocartilage have improved markedly within the last decade, allowing for the fabrication of more mechanically robust tissue engineered fibrocartilages. However, as previously discussed, current clinical treatments that address indications such as meniscal tears and TMJ disk perforation require follow-up clinical procedures within a short time frame. In addition, there is a lack of tissue engineered fibrocartilage products on the market. This may be

due, in part, to a dearth of clarity on how tissue engineered fibrocartilage products can be translated.

Outlined here is the FDA paradigm as seen through current documentation and resources with numerous specific considerations for preclinical animal models and clinical trials of potential fibrocartilage products. The considerations discussed here are just an example of what must be taken into account when going through the FDA paradigm. Clarification of important considerations and guidelines must occur in order to allow translation of tissue engineered fibrocartilage products. As such, the field should gravitate toward studies that have translational implications and perhaps ask for the FDA to create a guidance document similar to the one that exists for articular cartilage products [5]. A guidance document would provide recommendations to researchers and streamline translational advances to tissue engineered fibrocartilage products used in the clinic.

There are a number of remaining questions and concerns surrounding the creation of such a guidance document. One concern is how such a document can be created when there are multiple types of fibrocartilaginous tissues in the body varying in function. As examined earlier, there are actually significant similarities between meniscus and TMJ disk pathologies and current clinical treatments that allow for similar tissue engineering approaches to be used for both. These tissues are just two fibrocartilage examples. Hence, discussion and exploration of other fibrocartilaginous tissues like the pubic symphysis and annulus fibrosus of the intervertebral disk are warranted. Along those same lines, critics might question the inclusion of numerous different pathologies, ranging from early to late-stage, within one document. One option might be to focus in on pathologies that are associated with degeneration of the tissue where tissue engineering might be able to bolster the early to midstage degeneration via repair or replace the tissue completely for late-stage pathologies. Finally, as discussed with the FDA paradigm, clinical endpoints must be measured. A major hurdle remaining is the development of standardized indices or measurement systems for fibrocartilage in general. Evaluating tissue engineered fibrocartilage by an FI was suggested for tissue engineering and preclinical studies but remains a question for measurement of clinical endpoints in phased human trials.

In summary, tissue engineering of fibrocartilage addresses the limitations of current clinical treatments. There has been limited translation of tissue engineered fibrocartilage products from the bench to the bedside. Throughout the FDA paradigm, there are many considerations to be included in the guidance document as discussed earlier. However, there are still several hurdles and remaining questions before the creation of a fibrocartilage guidance document analogous to that which exists for articular cartilage can come to fruition.

6 Conclusion

This review has highlighted tissue engineering of fibrocartilage, using the knee meniscus and TMJ disk as primary examples. Anatomy, function, epidemiology, pathologies, and current clinical treatments were reviewed to elucidate the need for tissue engineered solutions that are both biochemically and mechanically reminiscent of native tissue. Prior to tissue engineering fibrocartilage, design criteria must be attained via characterization of native tissue in the species of interest. Design parameters such as cell sourcing, scaffold versus scaffold-free methods, as well as biochemical and mechanical stimuli alone or in combination were discussed to create a fibrocartilage spectrum. Evaluation of the resultant tissue engineered fibrocartilages was also examined for comparison to previously characterized properties of native tissue.

Navigation of the FDA paradigm was discussed to motivate the translation of studies from laboratory bench to bedside in the clinic. We have recommended collaboration and open communication with the FDA to create a fibrocartilage guidance document analogous to that which exists for articular cartilage. Regulation of tissue engineered fibrocartilage and considerations for preclinical animal models and clinical trials were highlighted to encourage standardization among the field. Ultimately, this review looks to the future of tissue engineered fibrocartilage products, which are the culmination of decades-long research efforts. While there remains much to be accomplished, the field is now closer than ever to alleviating prominent fibrocartilage conditions.

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Nomenclature

Col_N = native collagen content
$\mathrm{Col}_{\mathrm{TE}}$ = tissue engineered collagen content
$E_N{}^T$ = native tensile Young's modulus
$E_N{}^{20i}$ = native instantaneous modulus
$E_N{}^{20r}$ = native relaxation modulus
$E_{\mathrm{TE}}{}^T$ = tissue engineered tensile Young's modulus
$E_{\mathrm{TE}}{}^{20i}$ = tissue engineered instantaneous modulus
$E_{\mathrm{TE}}{}^{20r}$ = tissue engineered relaxation modulus
FI (TE/N) = functionality index of tissue engineered fibrocartilage in relation to native tissue
GAG_N = native glycosaminoglycan content
$\mathrm{GAG}_{\mathrm{TE}}$ = tissue engineered glycosaminoglycan content
UTS_N = native ultimate tensile strength
$\mathrm{UTS}_{\mathrm{TE}}$ = tissue engineered ultimate tensile strength

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