5.517. Tissue Engineering of the Temporomandibular Joint

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Glossary

Ankylosis Hypertrophic bone growth from the mandible and/or the fossa resulting in fusion of the joint.

Arthrocentesis A minimally invasive procedure where a needle and syringe are used to flush and drain fluid from the joint.

Arthroplasty A surgical procedure that involves reshaping of the articular surfaces of the joint.

Etiology The study of disease causation.

Fibrocartilage A tissue that contains properties of both hyaline cartilage and fibrous tissue such as tendon. This is typically characterized by the presence of both collagens I and II.

Glycosaminoglycan Long chains of repeating disaccharides, which typically contain a negative charge.

Internal derangement An abnormal position of the TMJ disc relative to the mandibular condyle and glenoid fossa.

Occlusal splint Removable acrylic molds that cover the upper and lower teeth. Typically used to protect the teeth from grinding or clenching.

Orthognathic surgery A surgical procedure involving the cutting and repositioning of bones in the mandible or maxilla.

Proteoglycan A molecule composed of protein core with at least one, but often many glycosaminoglycan side chains.

Stress relaxation Testing modality to measure the viscoelastic properties of a material. A constant strain is applied to the tissue and the decaying stress is measured over time.

Abbreviations

bFGF	Basic fibroblast growth factor
BMP-2	Bone morphogenic protein-2
C-ABC	Chondroitinase-ABC
CS	Chondroitin sulfate
DS	Dermatan sulfate
ECM	Extracellular matrix
ePTFE	Expanded polytetrafluoroethlyene
FDA	Food and Drug Administration
GAG	Glycosaminoglycan
HA	Hydroxyapatite
IGF-I	Insulin-like growth factor-I
IL-1	Interleukin-1
MMP	Matrix metalloproteinase
PCL	Polycaprolactone
PDGF	Platelet-derived growth factor
PEG	Poly(ethylene glycol)
PGA	Polyglycolic acid
PLA	Polylactic acid
PLLA	Poly-L-lactic acid
TGF-β1	Transforming growth factor-β1
TMD	Temporomandibular joint disorder
TMJ	Temporomandibular joint
UHMWPE	Ultra-high-molecular-weight polyethylene
VEGF	Vascular endothelial growth factor

5.517.1. Introduction

Tissue engineering of the temporomandibular joint (TMJ), or jaw joint, is still in its early development. While a large body of knowledge exists for the characterization and tissue engineering of other synovial joints, only recently has the TMJ received significant attention. Tissue engineering of the functional TMJ tissues is a promising technology for the treatment of TMJ disorders (TMDs), potentially improving the lives of millions of people. Because of the complex loading patterns that engineered tissues will experience in the TMJ, complete design parameters from native tissue are critical. Unfortunately, neither the normal nor the diseased states of the TMJ are fully understood at present, hindering tissue engineering efforts. Within the TMJ, the fibrocartilaginous disc has received the most attention thus far, but efforts are underway to engineer the mandibular condyle cartilage and bone as well. Implantation of engineered tissues may help alleviate pain, restore range of motion, and return the patient to normal jaw function. The potential impact of a biological TMJ replacement is even greater considering the significant lack of long-term treatment options for TMD patients. This chapter provides a survey of the literature related to the rapidly expanding field of TMJ tissue engineering.

5.517.2. Gross Anatomy and Physiology of the TMJ

The anatomy and physiology of the TMJ have been reviewed in detail in the literature.[1–4] This section provides a summary of the pertinent anatomy and physiology for engineers. The TMJ is composed of the condyle of the mandible articulating against the glenoid fossa and articular eminence of the temporal bone with an interposed disc (Figure 1). The mandibular condyle is the moving component of the articulation, while the fossa-eminence remains stationary relative to the cranium. Both of the articulating surfaces of the TMJ are covered by fibrocartilage, unlike the knee, where the articulating surfaces are covered by hyaline cartilage. Positioned between the condyle and fossa-eminence is a fibrocartilaginous disc that is attached to the periphery of the joint and is free to move over both the superior and inferior articulating surfaces. The TMJ disc serves to increase congruity between these surfaces, distribute load, and aid in joint lubrication.[5] The TMJ is surrounded by a capsule, which encloses the intra-articular environment and attaches to the disc near the condylar head.

Movement of the TMJ is unique because it includes both rotation relative to the transcranial axis and translation forward relative to the skull base. During normal mastication, the joint movement is mainly rotational, allowing vertical opening and closing of the mouth. It is only during wide mouth opening (to around 40 mm) that translation becomes a major component of the movement.[4] Under normal rotational movements, a complex pattern of compression and shear loading occurs between the anterior side of the condyle and posterior slope of the eminence.[6] A healthy TMJ disc and synovial fluid act to dissipate these loads across the joint. If the disc becomes displaced, the lack of force dissipation results in abnormal loading patterns and can cause degradation of the joint.

The attachments of the TMJ disc with the surrounding tissues are extremely important for the coordinated movements of the TMJ. Anteriorly, the disc attaches inferiorly to the anterior condyle and superiorly to the eminence by bending with the joint capsule. Posteriorly, it attaches to the bilaminar zone, which is in turn attached superiorly to the temporal bone and inferiorly to the posterior condyle. Laterally and medially, the disc attachments blend into the joint capsule near its attachment to the condylar head. This complex attachment pattern allows the condyle to rotate relative to the disc but still allows the disc and condyle to translate as a single unit during wide mouth opening.[1] Additionally, the disc and

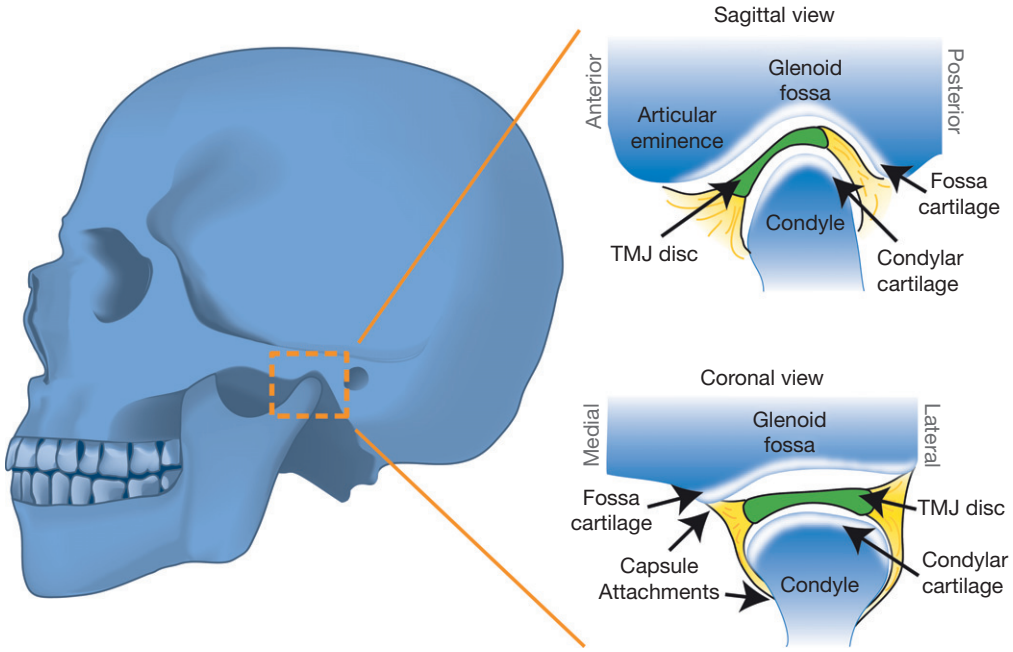

Figure 1 Location and anatomy of the temporomandibular joint (TMJ) in the sagittal and coronal planes. The TMJ is capable of both rotational and translational movement and is composed of three articulating structures: the mandibular condyle, TMJ disc, and the glenoid fossa. The mandibular condyle and glenoid fossa are both covered by fibrocartilage and the TMJ disc is positioned between these two structures.

its attachments separate the joint space into distinct inferior and superior regions. It has been proposed that the TMJ is mainly a translatory joint in the superior space and primarily a rotational joint in the inferior space.[7] This means that the loading patterns experienced by the two surfaces of the disc during normal motion are considerably different.

Like other diarthrodial joints, the TMJ is surrounded by a capsule, the inner surface of which is lined by synovium, a layer of cells that specialize in the production of synovial fluid. Synovial fluid serves two main functions within the TMJ. First, it acts as a lubricating fluid with a coefficient of friction of approximately 0.001.[8] Second, synovial fluid acts as a transmission medium for nutrients to the fibrocartilages within the joint and also serves to remove waste products. The volumes of synovial fluid in the inferior and superior joint spaces of the TMJ are about 0.5 and 1.0 ml, respectively.[2] As it nourishes all the tissues of the joint, the importance of a healthy synovium should not be overlooked by tissue engineers.

5.517.3. Characterization of TMJ Tissues

TMJ tissue engineering requires finely tuned design criteria in order for constructs to effectively handle the complex loading environment of the TMJ. These design criteria are determined through the characterization of the tissue in three major categories: cells contained in the tissue, its biochemical makeup, and its biomechanical properties. An understanding these components of TMJ tissues is critical for the development of mechanically functional engineered constructs, though the number of characterization studies of TMJ tissues remains relatively small in comparison to that of other synovial joints, such as the knee. Fortunately, recent characterization studies, particularly for the TMJ disc, have significantly increased our understanding of these complex tissues. Reviewing this information can greatly improve tissue engineering efforts by illuminating the TMJs structure–function relationships and providing gold standard specifications.

5.517.3.1. TMJ Disc

The TMJ disc is a biconcave fibrocartilaginous tissue that sits atop the mandibular condyle and articulates against the glenoid fossa of the temporal bone. It allows for smooth jaw movement during normal daily activities such as eating and talking. Because of its unique shape, the disc is commonly thought of consisting of three regions in the anteroposterior direction: the anterior band, intermediate zone, and posterior band (Figure 2). The intermediate zone also exhibits mediolateral variation and it is thus divided into the medial, central, and lateral regions. Finally, the two surfaces of the TMJ disc have varying properties, so the disc can also be classified into inferior and superior regions. The cellular, biochemical, and biomechanical properties that accompany this unique architecture provide the appropriate lubricating and cushioning functions for the joint. This section provides a summary of the salient TMJ disc properties for tissue engineers. Further information about the cellular, biochemical, and biomechanical properties of the disc has been reviewed in the literature.[7,9–12]

5.517.3.1.1. Cells

Similar to other fibrocartilages such as the knee meniscus, the TMJ disc contains a heterogeneous population of cells.

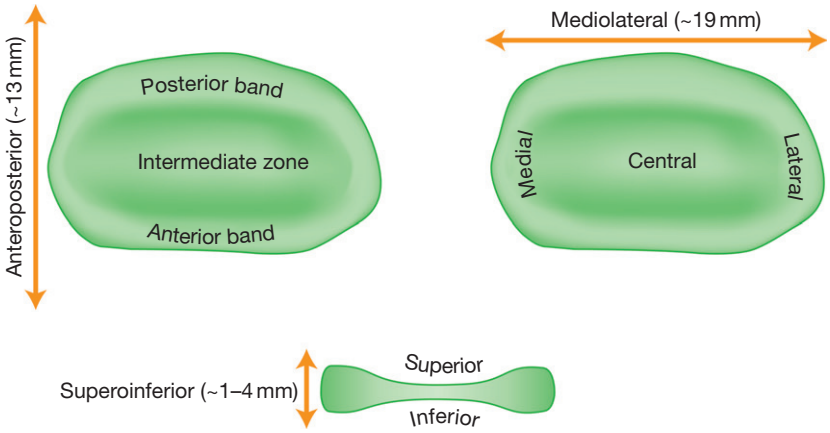

Figure 2 Regional variations and approximate dimensions of the temporomandibular joint (TMJ) disc. The TMJ disc is commonly classified into the posterior band, intermediate zone, and anterior band in the anteroposterior direction. In the mediolateral direction, the disc can be separated into the medial, central, and lateral regions. The disc exhibits a biconcave shape in the superoinferior direction, with each surface having distinct properties.

These cells possess characteristics of both fibroblasts and chondrocytes and are therefore termed fibrochondrocytes.[13] The overall cellularity of the disc is reported to be between 20 and 50 million cells per gram of tissue.[13,14] Although the TMJ disc contains multiple cell types, the overall population appears to be more fibroblastic than chondrocytic. Histological investigations have shown that approximately 70% of the disc cells are fibroblast-like, with the remaining 30% being chondrocyte-like.[15] The chondrocyte-like cells lack a pericellular matrix found around hyaline chondrocytes, and are mostly located in the intermediate zone.[15–17] Regional variations in cell number appear to vary with species. The anterior band was seen to contain the smallest number of cells in the porcine disc,[14,15] while the intermediate zone was found to have the fewest cells in primate discs.[17] Regardless of distribution, the heterogeneous fibrochondrocyte cell population has been seen in all species, and the difficulty involved in recreating this cellular environment should be appreciated by tissue engineers.

5.517.3.1.2. Collagen

The main extracellular matrix (ECM) component of the TMJ disc is collagen, which largely controls the functional properties of the tissue. Collagen makes up about 37% of the wet weight,[18] 50% of the wet volume,[19] or 69–85% of the dry weight.[14,20] Regional distribution of total collagen has been seen to vary depending on the animal model tested. The anterior and posterior bands were seen to contain most of the collagen in the rat disc,[17] while the intermediate zone was reported to contain more collagen in the porcine disc.[14] Although there are several types of collagen in the TMJ disc, collagen type I is by far the most prevalent.[13,17] Collagen type II, the primary component in hyaline cartilage, can be found in small amounts in the intermediate zone, surrounding chondrocyte-like cells.[13,17,21] Trace amounts of other fibrillar (type III) and nonfibrillar (types VI, IX, XII) collagens have also been found in the TMJ disc.[22–24] Collagen type I is by far the most prevalent component of the TMJ disc's ECM and will need to be recreated in a tissue replacement.

The orientation of collagen fibers in the TMJ disc is anisotropic, but there is a basic symmetry. Collagen fibers near the periphery of the disc align in a ring-like structure, while the collagen fibers in the intermediate zone run predominately in an anteroposterior direction.[21,25,26] In the center of the disc, transition regions are observed where the anteroposterior directed fibers of the intermediate zone meet the mediolateral directed fibers of the anterior and posterior bands.[26] It has been speculated that the outer ring of fibers serves to maintain the disc shape under both tensile and compressive loads.[27] The average collagen fiber diameter in the disc is $18 \pm 9\,\mu m$.[21] Finally, collagen fibers in the disc exhibit a wavy or crimped appearance throughout the full thickness of the tissue.[28] The unique ring-like collagen fiber orientation of the TMJ disc has important ramifications for the mechanical properties of the tissue, as described subsequently.

5.517.3.1.3. Glycosaminoglycans and proteoglycans

Together glycosaminoglycans (GAGs) and proteoglycans can contribute to the compressive and tensile properties of a tissue. GAGs are long repeating disaccharide chains with or without branching that possess at least one negatively charged side group. Proteoglycans are composed of a central protein core with one or many GAG side chains. There is little agreement in the literature about the total quantity and regional variation of GAGs in the TMJ disc. The total sulfated GAG content has been reported to be between 1 and 10% of the dry weight.[29,30] This is a large range, but most studies indicate that disc GAG content is below 5%.[14,20,21,29,31] Regionally, studies of the porcine TMJ disc have indicated that the posterior band has the least sulfated GAG.[14,21] Similar results have been seen in the bovine disc, where the bands were shown to have less GAG content than the intermediate zone.[31] The exact opposite distribution of GAGs has been seen in the primate disc, with the anterior and posterior bands having the highest content.[17] Regardless of conflicting results, it is clear that the GAG content of the disc is much lower than hyaline cartilage.[10]

The main proteoglycans in the TMJ disc are chondroitin sulfate (CS) and dermatan sulfate (DS). The GAG chains associated with these two proteoglycans make up 75–93% of the total GAG content of the disc.[20,21,31] Other proteoglycans including keratan sulfate and heparin sulfate have been found

in trace amounts.[20,21,29,31] As in the case of GAGs, there are conflicting reports about the regional proteoglycan distribution in the TMJ disc. In the rat disc, the highest concentrations of CS proteoglycans were found in the bands and the greatest concentration of DS proteoglycans was found in the intermediate zone.[32] The exact opposite trends in CS and DS proteoglycan distributions were seen in the porcine disc.[21] Unfortunately, the studies that have investigated regional distribution of proteoglycans and GAGs have all used different assays. As a result, it is difficult to determine whether the regional disparities seen are a result of interspecies variations or a byproduct of the different assays used.

5.517.3.1.4. Tissue mechanics

It is important to understand the mechanical properties of the TMJ, as engineered constructs will need to support the same loads imparted on the native tissue. The compressive properties of the disc have been studied fairly extensively, but the measured mechanical properties have varied widely between studies. Under unconfined compression, the porcine disc displays an instantaneous and a relaxed moduli of 500 and 30 kPa, respectively.[33,34] These results match well with those of other porcine and human disc studies.[35,36] The canine and bovine discs demonstrate a much larger compressive resistance, with instantaneous moduli of 31 and 15 MPa under unconfined compression and stress relaxation, respectively.[37,38] It is unclear whether these drastic differences are due to interspecies variations or the different testing modalities used. Regionally, the greatest instantaneous moduli have been seen in the anterior and medial portions of the disc from porcine and bovine samples.[33,34,38] Additionally, a large instantaneous modulus has been observed in the posterior band of the porcine disc, but this was not observed in bovine samples. The central portion of the disc is reported to have a modulus equal to or less than that of the anterior and posterior bands.[33,34,36,38] This is an interesting finding, as the center of the disc is generally reported to have more sulfated GAGs, which often correlates to compressive stiffness.[11] From these data, it appears that compressive properties are more closely related to total collagen content than GAG content, probably because of the exceedingly low GAG content in the TMJ disc.

Tensile testing of the TMJ disc has also resulted in large variations in reported mechanical properties. Reported tensile moduli for the TMJ disc range from 0.5 to 100 MPa.[37,39–41] This large range of recorded properties is related to dramatic variations in regional tensile properties, which are fairly consistent across studies. The tensile modulus of the porcine disc is higher in the anteroposterior direction than in the mediolateral direction.[40,41] Regionally in the mediolateral direction, the relaxation moduli of the posterior band, anterior band, and intermediate zone in the porcine disc are 23.4, 9.5, and 0.58 MPa, respectively.[41] Similar results have been seen in the canine disc.[39] The dramatic twofold decrease in tensile modulus between the posterior band and intermediate zone is interesting because it does not correspond to a dramatic difference in biochemical content. Instead, it is due to the fact that the collagen fibers in the intermediate zone are aligned in the anteroposterior direction, perpendicular to loading.[41] Tensile variation in the anteroposterior direction is not as substantial, but the central region is the stiffest followed by the medial, and then lateral sections.[37,41] It is clear that collagen alignment is important for the tensile properties of the tissue and should be considered in all engineering efforts.

5.517.3.2. Condylar and Fossa Cartilages

While characterization of the TMJ disc is not fully complete, it is far more comprehensive than the current characterization of condylar cartilage. This cartilage lines the articulating surface of the mandibular condyle and moves against the inferior surface of the TMJ disc. Like the disc, condylar cartilage is a fibrocartilaginous tissue with noted amounts of collagen types I and II. This tissue exhibits a zonal architecture and is commonly divided into four zones in a superior to inferior fashion: fibrous, proliferative, mature, and hypertrophic (**Figure 3**). The articulating fibrous zone is a fibrocartilaginous region that sits on top of a highly cellular proliferative zone. The mature and hypertrophic zones, which border the subchondral bone, are considered to be like hyaline cartilage. This section provides an overview of the salient properties of condylar and fossa cartilages. It should be noted, however, that scant information exists on fossa cartilage. More detailed reviews of structure and composition can be found in the literature.[12,42,43]

5.517.3.2.1. Cells

Similar to other fibrocartilages, the cells of condylar cartilage are a heterogeneous fibrochondrocyte population, but unlike the TMJ disc, true chondrocytes can be found in some regions.

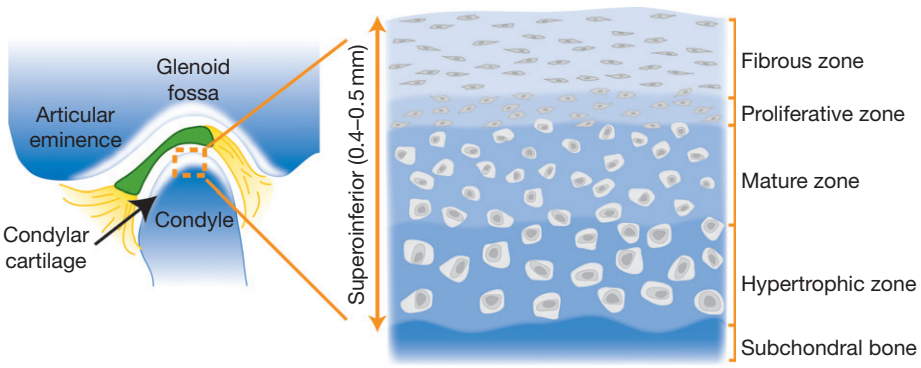

Figure 3 Zonal architecture and approximate dimensions of condylar cartilage. Condylar cartilage is commonly divided into four zones in the superoinferior direction: fibrous, proliferative, mature, and hypertrophic.

The articulating surface of mandibular cartilage contains flat fibroblast-like cells, which is indicative of this region being called the fibrous zone.[44,45] The proliferative zone is a highly cellular region that appears to play a role as a cell reservoir for the other zones. It produces cells for the overlying fibrous zone,[46,47] as well as chondrocyte precursors for the underlying mature zone.[48–50] Terminally differentiated chondrocytes are the main cell type in the mature and hypertrophic zones, although hypertrophic chondrocytes can be found near the junction with the subchondral bone.[51] The fact that the proliferative zone produces cells for all other zones should be noted by tissue engineers. If this layer can be purified, the cells it contains may be a potent cell source for engineering condylar cartilage.

5.517.3.2.2. Extracellular matrix

Although collagen appears to be the main constituent in condylar cartilage, there is little known about the exact quantity of collagen present. A value of 165 nmol of hydroxyproline/mg of dry weight has been reported,[52] as well as 2.2 $\mu g\,mg^{-1}$ of wet weight.[12] In contrast, the types of collagen present in condylar cartilage have been studied quite thoroughly using immunohistochemistry. Collagen type I can be found throughout the cartilage but is primarily located in the fibrous and proliferative zones.[16,53,54] Collagen type II is the primary collagen of the mature and hypertrophic zones,[16,53] although type X can also be found in these regions.[55] The fibrous zone contains collagen type III in addition to type I, which is representative of its fibrous nature.[54] More studies need to be completed on the quantitative distribution of collagens in condylar cartilage.

Collagen orientation in condylar cartilage has also been seen to have zonal heterogeneity. Microscopic investigations on the fibrous zone have indicated a transversely isotropic collagen fiber alignment, with sheets of fibers stacked on top of each other.[51,56,57] More recently, a macroscopic study of the fibrous zone showed anisotropic fiber orientation.[58] The proliferative zone is mostly cellular and contains few collagen fibrils.[59] The mature and hypertrophic zones exhibit randomly oriented collagen fiber bundles, indicating that there is an isotropic arrangement of collagen in these zones.[56,59] Overall, collagen organization results indicate the presence of a bilayered fiber structure with an anisotropic layer near the articular surface and an isotropic layer near the underlying bone.[43]

The total GAG content in mandibular cartilage is reported to be 6.4 $\mu g\,mg^{-1}$ wet weight in the rat,[54] or about 0.19 mg in the rabbit.[52] Keratin and chondroitin sulfates are the only GAGs that have been studied in mandibular cartilage, and there is contradictory evidence about their distribution. A primate study found these GAGs only in the mature and hypertrophic regions,[16] while they were found in the fibrous and proliferative zones of porcine and rat cartilage.[60,61] Regionally, cartilages from the anterior and posterosuperior portions of the condyle have been found to contain more CS than the superior region.[62] There is a zonal distribution of proteoglycans as well, with aggrecan located primarily on the mature and hypertrophic zones.[60,61] Decorin, on the other hand, is distributed fairly evenly throughout the cartilage.[63] Even though condylar cartilage contains chondrocytes, its GAG and proteoglycan content are significantly different from that of articular cartilage.

5.517.3.2.3. Tissue mechanics

Tensile testing of condylar cartilage has revealed a dramatic anisotropy in tensile stiffness, which matches the collagen fiber organization discussed earlier. The Young's moduli of condylar cartilage in the anteroposterior and mediolateral directions have been reported to be 9.0 and 6.6 MPa, respectively, when the cartilage is connected to subchondral bone.[64] When the cartilage is tested independent of bone, the moduli range from 8.0 to 11.0 MPa in the mediolateral direction, and 22 to 29 MPa in the anteroposterior direction.[58] This anisotropy has also been seen under dynamic shear testing, where the storage moduli of condylar cartilage were found to range from 1.50 to 2.03 MPa in the anteroposterior direction and 0.33 to 0.55 MPa in the mediolateral direction.[65,66] Overall, condylar cartilage is stiffer under tension and shear in the anteroposterior direction, which is also true for the TMJ disc.

Compressive testing of condylar cartilage has been conducted using numerous methodologies, but unfortunately there is no consensus about the anteroposterior variation in compressive properties. Studies using nanoindentation and dynamic compression have found that cartilage from the anterior region of the condyle was stiffer than that from the posterior region.[67,68] In contrast, studies using creep indentation and unconfined compression reported that the posterior cartilage was the stiffest.[69,70] With regard to mediolateral variation, multiple studies have agreed that cartilage from the medial region of the condyle is the stiffest.[67,70] In general, the aggregate modulus of condylar cartilage has been reported to be in the range of 45–75 kPa.[70] Unfortunately, there is no consensus about the regional variation in compressive properties of condylar cartilage, and more data are required to provide exact design requirements for tissue engineering.

Although little characterization of the fossa cartilage has occurred, the compressive properties have been tested using creep indentation. The average aggregate modulus of fossa cartilage was reported to be around 36 kPa, with cartilage from the posterior fossa being the stiffest and anterior fossa cartilage being the most compliant.[36] Overall, the fossa cartilage was found to be 57% thinner and 50% stiffer than the TMJ disc. Although this study provides a start for fossa characterization, a significant amount of further research is needed.

5.517.3.3. Mandibular Condyle and Temporal Fossa

The mandibular condyle, covered by a thin layer of fibrocartilage, is the major moving structure in the TMJ. It articulates against the glenoid fossa, also called mandibular fossa, which is a part of the upper temporal bone.[12] Looking at the structural organization of bones, they are typically composed of two microarchitectures: woven and lamellar bones, which are organized into dense cortical bone (compact bone) and porous cancellous bone (spongy or trabecular bone), as reviewed.[71] For example, underneath the condylar cartilage, a compact bone plate covers cancellous bone in the mandibular condyle.[42] From the viewpoint of chemical composition, bone is a well-organized composite matrix that is composed of a protein-based soft hydrogel template (e.g., collagens, noncollagenous proteins, and water) and hard inorganic components (such as hydroxyapatite, HA), as reviewed.[72] Specifically, a large amount of nanocrystalline HA, typically 20–80 nm long

and 2–5-nm thick, is found in the bone matrix.[71] Additionally, 90% of the organic phase in bone is made of type I collagen, which contributes to the elastic properties of bone. Other noncollagenous proteins, including various adhesive proteins (such as laminin, fibronectin, and vitronectin), bone-inductive proteins (such as osteopontin, osteonectin, and osteocalcin), growth factors, and cytokines, are found in the bone matrix to mediate cell–bone functions.[71,73] Unlike cartilage, bone has strong self-repairing potential. Various bone cells, including osteoblasts (bone-forming cells), osteoclasts (bone resorbing cells), and osteocytes (mature osteoblasts), are actively involved in normal bone functions, including ECM mineralization and new bone synthesis.

Clearly, the mandibular condyle's unique structure and composition must support a variety of mechanical loads during daily activities. To date, there have been several studies investigating the mechanical properties (such as stiffness and strength) of the cortical or cancellous bones in the TMJ.[74–78] For instance, it was reported that the cancellous bone of human mandibular condyle has anisotropic mechanical properties: the compressive elastic modulus and ultimate stress of axial specimens in mandibular condylar bones were 431 and 4.5 MPa when compared to 127 and 1.6 MPa of respective transverse specimens.[74] Cortical bone throughout the human mandibular condyle has been shown to posses significantly more stiffness than cancellous bone, exhibiting elastic moduli of roughly 12.2–26.6 GPa varying with different axes and locations.[76] It was also observed that the cortical plate on the lateral side was much thicker than the medial side. These anisotropic properties should be kept in mind when trying to engineer condylar bone. Unlike mandibular bone, little information exists about the bone of the glenoid fossa, and therefore, further characterization is needed.

5.517.4. Pathology of the TMJ

TMDs include a wide variety of conditions for which the etiology is not fully understood. Signs and symptoms of TMDs include limited mouth opening, deviation of the jaw during opening, dislocation, clicking, locking, and muscle pain during jaw movements.[79] Epidemiological studies report that about a quarter of the population has symptoms of TMD,[80] but after reviewing patient records, it appears that only 3–4% of the population seek treatment.[81] Three common pathologies of the TMJ which end up requiring clinical treatment are internal derangement, degenerative joint disease, and ankylosis. The first two mainly affect the soft tissues of the joint and the third affects the bony structures.

Internal derangement of the TMJ is defined as an abnormal relationship of the articular disc to the mandibular condyle and articular eminence.[2] It is believed to be the result of multiple pathological processes, including softening of the tissues, perforation of the disc, alterations in synovial fluid lubrication, and overactive musculature.[4] Disc displacement typically occurs on the anterior medial side of the condyle. The result of TMJ disc derangement depends largely on the extent and duration of the displacement. Long-term internal derangement typically results in altered loading patterns in the joint, reduced mobility, and increasing degradation of the soft tissues.[4] These long-term degenerative effects are thought to be caused by a direct mechanical injury and/or a hypoxia–reperfusion injury.[82] A progression of five stages of internal derangement has been described involving increasing joint degradation over time.[83] Most TMD patients with intermediate stage internal derangement progress into the later stages. An understanding of the internal derangement is particularly important, because a prior study has indicated that 70% of patients with TMD have disc displacement.[84]

Degenerative joint disease involves a catabolic loss of articular tissue and is a common form of degeneration in synovial joints. The main form of degenerative joint disease in the TMJ is osteoarthritis caused by excessive loading, but rheumatoid arthritis caused by autoimmune responses can also occur. Osteoarthritis of the TMJ is characterized by degradation and abrasion of the articular cartilage surfaces, which are accompanied by secondary inflammation.[85] The exact etiology of osteoarthritis in the TMJ is unknown, but it likely involves trauma to the joint, excessive loading, immobility, and increasing age.[86] During TMJ osteoarthritis, expression of matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) in the joint is elevated, resulting in increased inflammation and degradation of the soft tissues.[87] The result is abnormal remodeling and breakdown of the TMJ cartilages. TMJ degenerative joint disease can result from internal defragment, or it can develop independently.

The next TMJ pathology differs from the preceding conditions, because it involves a disorder of bone metabolism, rather than of soft tissue. TMJ ankylosis involves hypertrophic bone formation in the condyle or temporal bones. This excessive bone growth reshapes the articulating surfaces, and if given time to grow, can completely bridge the joint space. This results in immobilization of the mandible and a complete lack of joint function. Trauma to the joint cavity in young children, or repetitive trauma from surgeries is connected to the development of ankylosis.[88] Removal of the hypertrophic bone is typically not a long-term solution for ankylosis, as the bone will commonly regenerate.

Engineers must appreciate the complex pathologies of the TMJ. Without understanding the factors responsible for destruction of the native joint, it will not be possible to produce a permanent biological replacement. For example, if the joint degradation is initially caused by a displaced disc, not only must the disc be repositioned or replaced, but the catabolic and inflammatory environment of the joint must also be addressed. Because of the joint disease, the synovial fluid will be saturated with inflammatory factors that, if left unchecked, will likely lead to destruction of the newly implanted disc. An understanding of TMJ pathology will significantly increase the likelihood of success for future tissue-engineered TMJ replacements.

5.517.5. Current Therapies

As TMJ pathology progresses, an increase in symptoms often causes patients to seek clinical care. The most common reason for TMD patients to request medical care is pain.[5] Current clinical treatment options can be divided into four categories: noninvasive, minimally invasive, invasive, and alloplastic

replacement. As detailed elsewhere,[89] the goals for treatment of TMD patients should include (1) decreased joint pain, swelling, and reflex masticatory pain; (2) increased joint function; (3) prevention of further damage; and (4) prevention of disability and disease-related morbidity. This section provides an overview of the common clinical treatments for TMD. More detailed information can be found in the literature.[4,89]

The first stage of clinical treatments for TMD is noninvasive and generally includes occlusal splints and physical, myofunctional, and behavioral therapy as well as medications. Occlusal splints provide physical separation of the teeth. The goal of occlusal splints is to eliminate occlusal factors which trigger parafunctional habits and masticatory muscle hyperactivity, thereby reducing the involuntary overloading of the joint. Mandibular repositioning splints are used in order to achieve a repositioning of a dislocated TMJ disc. Because of the multifactorial nature of TMDs and the numerous types and concepts for occlusal splints, there are mixed reports on the effectiveness of this treatment.[90] Physical therapy, including active and passive joint movement and myofunctional therapy, is another important component of noninvasive therapy which has been seen to reduce pain in TMD patients.[91] Occlusal splints and physical therapy are commonly combined with behavioral therapy such as biofeedback and techniques for stress management, which has emerged as an extremely important component of TMD therapy. Nonsteroidal anti-inflammatory agents, such as ibuprofen, are the most common medications given for TMJ pain.

Following or in conjunction with noninvasive treatments, minimally invasive therapies include injections, arthrocentesis, and arthroscopy. Corticosteroid injections are used occasionally for severe inflammation, but repeated injections can lead to cartilage destruction.[92] Intra-articular injections of hyaluronic acid to increase lubricity of the joint have been suggested,[93] but have not yet been approved for clinical practice. During arthrocentesis, a needle is used to flush and drain the joint space, with the goal of removing inflammatory mediators and enhancing lubrication. Arthroscopy of the TMJ is mainly used for the diagnosis of early stage arthritis,[94] although some manipulation can be done such as removing fibrotic tissue.

Even though a majority of TMD patients can be managed with minimally invasive treatment, there is a subset of patients (~20%) who will require surgical intervention.[95] TMJ arthroplasty is a relatively common surgical procedure that requires the replacement of the disc with, for example, an autogenic material. Typically, the local temporalis muscle flap is used for disc replacement,[96] although a tissue-engineered disc would be of great utility in this situation. Hemiarthroplasty, reshaping the fossa or replacing it with an alloplastic implant, became popular in the 1960s, but is rarely used today because of concerns about degradation of the remaining joint tissues.[4] Although orthognathic surgery is an option for TMD patients, it is not frequently used, as the outcomes are poor for patients with preexisting TMJ degradation.[97] Instead, orthognathic surgery is now used in conjunction with total joint replacement to enhance facial symmetry.

When advanced degenerative disease is present in the TMJ, currently, the only clinical option is an alloplastic total joint replacement. Replacing all or part of the TMJ with an alloplastic material will always come under intense scrutiny on the basis of the poor history of these procedures. In the late 1980s, a Teflon–Proplast implant was approved for TMJ disc replacement by the Food and Drug Administration (FDA). These implants ended up fragmenting under normal loading conditions, leading to a large foreign body giant cell inflammatory response and causing immense resorption of the condyle and fossa.[98] In spite of this failure, alloplastic total joint replacements for the TMJ have been researched intensely for the last 20 years (**Chapter 6.621, Biomaterials and Their Application in Craniomaxillofacial Surgery**). Currently, there are three total joint replacement systems approved by the FDA, manufactured by Christensen, Biomet, and TMJ Concepts. TMJ total joint replacements generally consist of a chromium–cobalt–molybdenum condylar head articulating against an ultra-high-molecular-weight polyethylene (UHMWPE) fossa. The total alloplastic TMJ reconstruction is considered an appropriate treatment of advanced-stage degenerative TMJ disease, although the lifetime of the device and the long-term implications for the surrounding tissues have not been known yet.[89] Tissue engineering may provide a functional and permanent biological replacement for the TMJ, eliminating the need for alloplastic regeneration.

5.517.6. Tissue Engineering

While many tissues in the body when injured have an innate capacity to self-repair, there are some tissues that have little to no self-repairing capacity. The tissues of the TMJ fall into the latter category. Additionally, because of the complex interplay of tissues within the joint, a deficiency in one area can detrimentally affect the surrounding tissues, causing pathology of the joint as a whole. Widespread injury of the TMJ, combined with its limited reparative capacity, necessitates some form of clinical intervention in order to maintain normal function and eliminate pain for the patient. Presently, clinical therapies fall short of addressing the full spectrum of issues and are only semipermanent. Tissue engineering may address these deficits by providing permanent, biomimetic, replacement tissue systems for the TMJ. To achieve this, scientists use the tissue engineering paradigm (**Figure 4**). In this paradigm, native tissue is first characterized to create design parameters for tissue engineering (**Chapter 5.519, Biomaterials Selection for Dental Pulp Regeneration**; **Chapter 5.524, Biomaterials for Replacement and Repair of the Meniscus and Annulus Fibrosus**; and **Chapter 5.535, Cartilage Regeneration in Reconstructive Surgery**). These design parameters are then used to inform the selection of an appropriate cell source, bioactive factors, biomechanical stimulation, and/or scaffold for the creation of an implantable biomimetic tissue. In this section, current tissue engineering efforts for the disc, condylar cartilage, and condyle will be reviewed.

5.517.6.1. TMJ Disc

Characterization data for the TMJ disc have determined certain specifications that should be considered when tissue engineering a suitable replacement. It is known that this tissue houses a distinct cell population and has unique geometry and mechanical properties, brought on by the anisotropic behavior of its biochemical components. Tissue engineering of the disc,

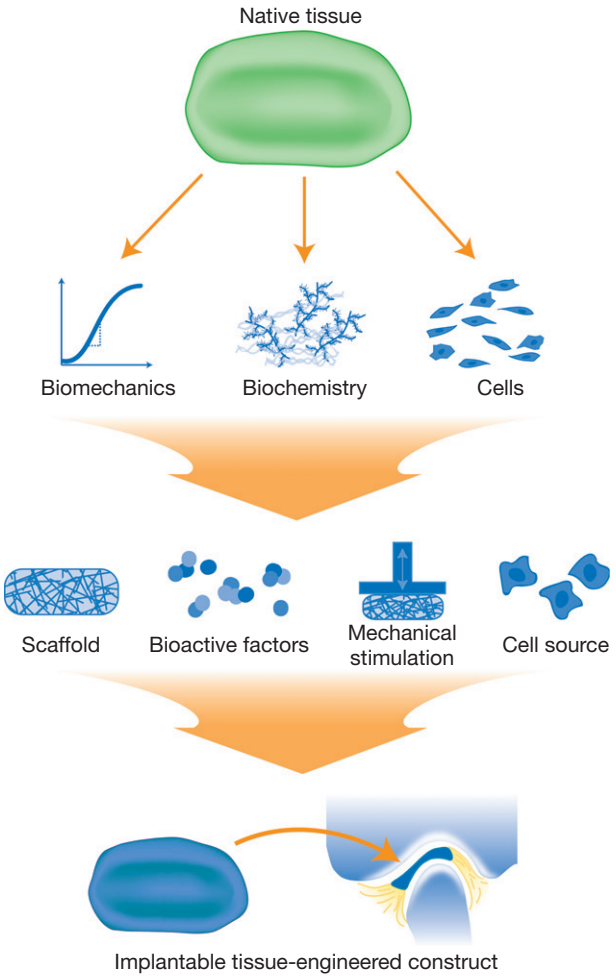

Figure 4 Tissue engineering paradigm for engineering temporomandibular joint (TMJ) tissues. The tissue engineering process is initiated by characterizing the biomechanical, biochemical, and cellular properties of the native tissue to create design parameters for tissue engineering. Next, cells are combined with scaffolds, bioactive agents, and mechanical stimuli to produce a tissue-engineered TMJ tissue that can be implanted *in vivo*.

therefore, must recapitulate these characteristics of the disc in order to preserve its function within the joint. Unlike other tissues of the TMJ, a considerable number of studies have investigated engineering of the disc. Although the first report of TMJ disc tissue engineering appeared in 1994, a majority of the tissue engineering efforts have been conducted recently. This section reviews important advances in cell and scaffold selection, as well as the role of bioactive factors and mechanical stimulation used in the field of TMJ disc tissue engineering.

5.517.6.1.1. Cell sources

Selection of a cell source is likely the most important aspect of any tissue engineering strategy (**Chapter 5.507, Tissue Engineering and Selection of Cells**). These cells are responsible for ECM production and maintenance, resulting in a functional replacement tissue. The most commonly used cells for engineering the TMJ disc have been primary disc cells.[99–109] Although primary TMJ disc cells have been studied extensively, there are two main problems with this cell source: (1) lack of donor cells and (2) donor site morbidity. It is possible to passage cells in monolayer to increase cell numbers, but unfortunately, TMJ disc cells dedifferentiate rapidly in culture and their phenotype is difficult to recover.[110–112] Because of concerns about donor site morbidity, costal chondrocytes have recently been investigated as an alternative cell source for TMJ disc engineering.[113–116] This research was prompted by the fact that oral surgeons already use costal rib grafts for the replacement of the mandibular condyle, and donor site morbidity is minimal.

To completely eliminate concerns about primary cell sources, progenitor cells will likely need to be used for future TMJ tissue engineering efforts. Recently, a series of self-renewing and highly potent human stem cells, such as multipotent mesenchymal stem cells (MSCs), umbilical cord matrix stem cells, and pluripotent embryonic stem cells (ESCs), have emerged and have shown promise for TMJ tissue regeneration. These stem cells, as shown in **Figure 5**, have a large proliferation capacity enabling them to expand without losing their phenotype. Even after expansion, they are able to differentiate into cartilage, bone, and tendon/ligament. Both adult and embryonic stem cells have been shown to be capable of differentiating into fibrochondrocytes that can be used for TMJ disc engineering.[117–120] Additionally, progenitor cells from the skin have been shown capable of differentiating down a chondrogenic lineage in response to ECM molecules.[121,122] Future studies will need to further investigate the differentiation of progenitor cells and their application in TMJ tissue engineering.

5.517.6.1.2. Scaffolds

Scaffolds are an important consideration in tissue engineering as they provide the constructs' initial mechanical integrity and allow for cell attachment. The first TMJ disc tissue engineering study used a porous collagen scaffold and produced constructs with appreciable size and ECM.[99] Similar success was seen with porous polyglycolic acid (PGA) and polylactic acid (PLA) scaffolds. Both materials were shown to support cell attachment and matrix production for up to 12 weeks.[123] Another early study compared PGA, polyamide filaments, expanded polytetrafluoroethylene (ePTFE), and bone blocks for disc engineering.[100] While all scaffolding materials supported cell attachment, there was poor ECM production in all groups. The majority of more recent TMJ disc engineering efforts have used PGA nonwoven mesh scaffolds.[101–103,105–107] While PGA scaffolds do support cell attachment and biosynthesis, PGA fibers degrade too rapidly, producing constructs of very small size. As a result, Allen and Athanasiou[109] compared the use of PGA to that of poly-L-lactic acid (PLLA) nonwoven meshes. PLLA scaffolds produced constructs with enhanced dimensions and mechanical integrity compared to PGA.[109] Additionally, encapsulation of TMJ disc cells in alginate hydrogels has been investigated, but cell viability and ECM production were quite low after 4 weeks.[101] Overall, significantly better results have been observed when culturing TMJ disc cells on natural and synthetic mesh scaffolds than encapsulating the cells in hydrogels.

Although scaffolds are typically an integral part of tissue engineering, it is also possible to produce scaffold-less constructs. Recent efforts to engineer the TMJ disc using costal chondrocytes have produced large functional constructs using a scaffold-less 'self-assembly' technique.[113–116] In this

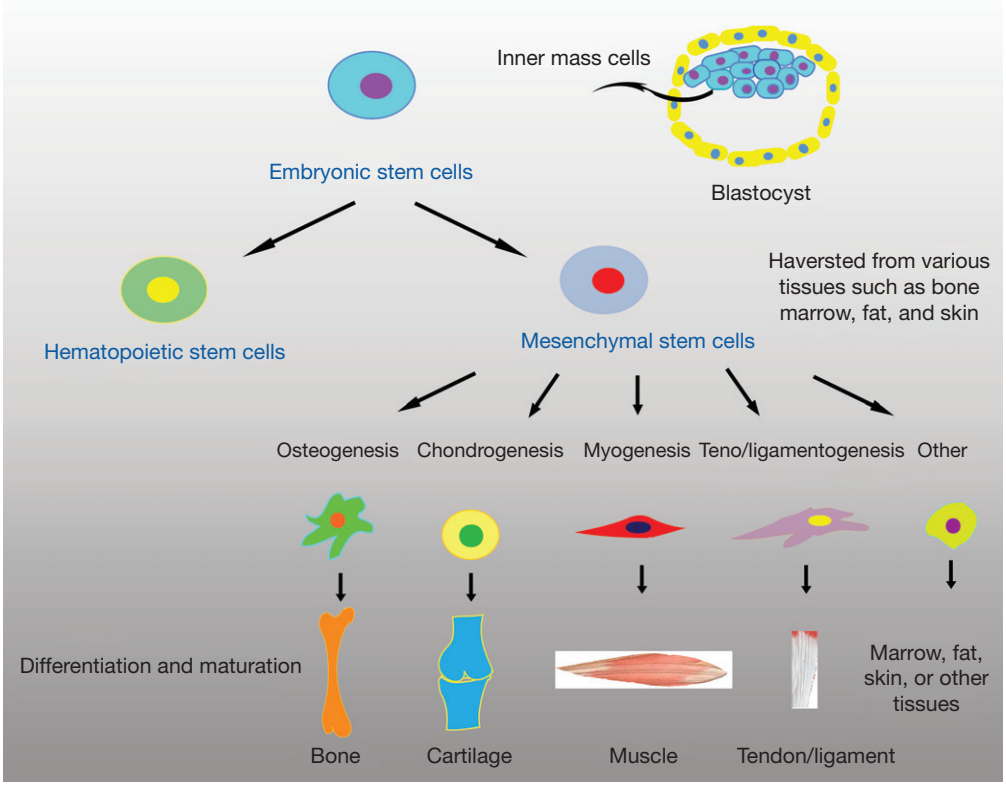

Figure 5 The hierarchal structure of human embryonic and mesenchymal stem cells. Embryonic stem cells are derived from the inner cell mass of the blastocyst and can differentiate down any of the three germ lineages. Mesenchymal stem cells are multipotent and can differentiate into any mesenchymal tissue, including cartilage and bone.

procedure, cells are seeded at very high density into a nonadherent well, which forces the cells to bind to one another.[124] The cells then secrete their own ECM scaffolding over time. Ultimately, both scaffold-less and scaffold-based approaches have seen beneficial results for tissue engineering the TMJ disc, and both techniques should be further investigated.

5.517.6.1.3. Bioactive agents

Growth factors are commonly used in tissue engineering because of their ability to enhance cellular proliferation and/or biosynthesis (**Chapter 5.522, Bone Tissue Engineering: Growth Factors and Cytokines**). So far, five different growth factors have been investigated for TMJ disc tissue engineering: platelet-derived growth factor (PDGF); basic fibroblast growth factor (bFGF); transforming growth factor-β1 (TGF-β1); transforming growth factor-β3 (TGF-β3); and insulin-like growth factor-I (IGF-I). In monolayer culture, TGF-β1, IGF-I, and bFGF have all been shown to increase TMJ disc cell proliferation and biosynthesis.[13,125] It was noted that high concentration of growth factors favored cell proliferation, while low concentrations of growth factors favored biosynthesis.[125] In three-dimensional culture, the effects of growth factors in TMJ disc tissue engineering have been investigated with both PGA and PLLA mesh scaffolds. On PGA scaffolds, both IGF-I and TGF-β1 were shown an increase in the collagen synthesis of porcine TMJ disc cells.[103] In contrast, with PLLA constructs, only TGF-β1 showed a significant increase in biochemical and biomechanical properties.[109] This differential effect may be related to the fact that PGA degrades much faster than PLLA. Growth factors have also been used to enhance TMJ disc tissue engineering using costal chondrocytes. IGF-I enhanced the cellular and biochemical properties of scaffold-less costal chondrocyte constructs.[114]

Although growth factors have received the most attention, other bioactive agents can have a significant impact on TMJ disc tissue engineering as well. TMJ disc cells cultured in media with 25 $\mu g\,ml^{-1}$ of ascorbic acid produced constructs with higher collagen content than cells cultured under concentrations of 0 or 50 $\mu g\,ml^{-1}$.[107] Molecules such as ascorbic acid are important to ECM protein synthesis and should be considered when choosing a media for tissue engineering. Recent evidence from articular cartilage engineering suggests that using catabolic agents can improve construct properties. Natoli *et al.*[126] recently demonstrated that applying chondroitinase-ABC (C-ABC, a GAG removing enzyme) during the midpoint of culture can improve the tensile properties of engineered cartilage. The GAGs that were depleted by C-ABC return by the end of culture and there is no loss in compressive properties.[126] Nontraditional bioactive agents such as this deserve future investigation for TMJ disc tissue engineering.

5.517.6.1.4. Mechanical stimulation

The native TMJ disc experiences significant loading which encompasses compression, tension, and shear components (**Chapter 5.506, Effects of Mechanical Stress on Cells**).[127] As the disc is such a mechanically important tissue, it makes

sense that mechanical stimuli may be required to produce an optimal tissue engineering construct. The first study to investigate mechanical cues on TMJ tissue engineering used a rotating wall bioreactor to create a low-shear fluid environment. Constructs grown in the rotating wall bioreactor ended up being statistically the same as culture controls, and no benefit of the low-shear environment was observed.[104] Continuous hydrostatic pressure of 10 MPa has been shown to increase ECM synthesis of TMJ disc cells both in monolayer and in PGA scaffolds.[105] In contrast, intermittent hydrostatic pressure of 10 MPa applied at 1 Hz was seen to be detrimental to TMJ disc cell biosynthesis. In two-dimensional culture, TMJ disc cells exposed to dynamic tensile strain significantly reduced production of MMPs in response to the proinflammatory cytokine interleukin-1β (IL-1β).[128] While it is clear that mechanical cues have an impact on TMJ disc tissue engineering, more research is needed to determine whether other stimuli used in articular cartilage engineering, such as compression and shear, are beneficial.

5.517.6.2. Condylar Cartilage

Of the three TMJ tissues for which tissue engineering has been attempted, condylar cartilage has received the least amount of attention. The first report of three-dimensional condylar cartilage engineering did not appear in the literature until 2007. Similar to the disc, condylar cartilage has limited repair capabilities and is an excellent candidate for tissue engineering. To this point, disc and condylar cartilage engineering have been conducted independent of each other, although tissue engineering results from one tissue will likely benefit the other. As engineering of condylar cartilage is a new field, efforts thus far have focused on the issues of cell source and application of bioactive agents.

5.517.6.2.1. Cell sources

The majority of condylar cartilage tissue engineering efforts thus far have used primary cells from the tissue.[129–134] Currently, two methods have been used for harvesting primary condylar chondrocytes. The first uses collagenase to digest the cartilage,[129] and the second allows the cells to migrate out of tissue explants.[130] The collagenase method isolates cells from all four cartilage zones, whereas the tissue explant method isolates only cells from the fibrous zone. While condylar chondrocytes do grow in culture and respond to growth factors, they show relatively low ECM production *in vitro*.[42] Because of the low matrix production, chondrocytes from other parts of the body have been used for condylar cartilage engineering. Articular chondrocytes from the ankle have been compared to condylar chondrocytes during both monolayer and three-dimensional culture. In monolayer, condylar chondrocytes showed greater proliferation but ankle chondrocytes produced tremendously more matrix.[135] On PGA scaffolds, ankle chondrocytes again produced an order of magnitude more ECM than condylar chondrocytes.[136] Future studies using primary cells will likely use a chondrocyte source from somewhere other than the condyle.

Because of the standard concerns about primary cell sources (insufficient cell number and donor site morbidity), progenitor cells have begun to be investigated for condylar cartilage engineering. Recently, human umbilical cord mesenchymal stromal cells (hUCMSCs) have been identified as an attractive cell source for condylar engineering. hUCMSCs are multipotent stem cells that develop from the extraembryonic mesoderm of the umbilical cord. When hUCMSCs were compared to condylar chondrocytes for tissue engineering, hUCMSCs were seen to proliferate more rapidly and produce significantly more matrix.[134] External stimuli have been shown to increase the matrix production of hUCMSCs even further.[119,120] Capitalizing on the deficiencies of primary cells, multipotent stem cells appear to have a bright future in condylar cartilage engineering.

5.517.6.2.2. Scaffolds

While there are many potential scaffolds for condylar cartilage engineering, at this point, only two scaffolding materials have been used. The most common scaffold choice has been a PGA mesh.[119,120,134,136] The choice of PGA has likely been influenced by TMJ disc engineering where it had previously been used. As mentioned earlier, PGA supports good cell attachment and matrix production, but degrades too rapidly in culture. Other than PGA, one study has investigated encapsulation of condylar chondrocytes in alginate gel beads.[137] After 4 weeks, the cells maintained a chondrogenic phenotype based on immunostaining. As the field of condylar cartilage expands, it is clear that attention will need to be paid to scaffold choice.

5.517.6.2.3. Bioactive agents

To date, the use of bioactive agents in condylar cartilage engineering has focused on the application of growth factors to condylar chondrocytes and hUCMSCs. The growth factors investigated are similar to those used for TMJ disc engineering, including bFGF, IGF-I, TGF-β1, and epidermal growth factor (EGF). A high concentration of bFGF appears to have the greatest effect on condylar chondrocyte proliferation, although it may inhibit ECM biosynthesis of the cells.[131–133] IGF-I has been shown to be a potent promoter of both cell proliferation and matrix synthesis, particularly GAG production.[133,138,139] Condylar chondrocyte biosynthesis has also been shown to increase with TGF-β1 application, but it is unclear whether it exhibits positive effects on cell proliferation.[133] Cells from the fibrous zone of condylar cartilage have been show to be stimulated by EGF, but the remaining zones have not been investigated.[130] Finally, IGF-I has also been shown to enhance the fibrochondrogenesis and matrix synthesis of hUCMSCs.[119,135] These results provide the basis for further investigations into using external stimuli to enhance condylar cartilage engineering.

5.517.6.3. Mandibular Condyle

Unlike tissue engineering the TMJ disc which began in the early 1990s, condylar engineering did not appear in the literature until 2000. Fortunately, bone tissue engineering in general has been studied extensively. Although still lagging behind the TMJ disc, there has been more effort to engineer the mandibular condyle than the condylar cartilage. The current field of mandibular condylar tissue engineering combines a variety of cell types (i.e., osteoblasts, chondrocytes), mandibular-condyle-shaped scaffolds (i.e., polymers, ceramics), and bioactive factors (i.e., TGF-β, IGF-I, bFGF), to

restore the functionality of damaged tissues. A majority of the efforts thus far have been focused on creating a condyle-shaped scaffold as this is a nontrivial shape.

5.517.6.3.1. Cell sources

Mature osteoblasts and chondrocytes are the most commonly used cell types to regenerate new bone and cartilage tissues. Specifically, osteoblasts have been investigated as one of the main cell sources for subchondral bone repair.[140] They can actively interact with proteins and minerals to adhere, proliferate, and develop a mineralized ECM and differentiate into mature bone. Weng *et al.*[141] created a tissue-engineered mandibular condylar construct via a combination of osteoblasts, chondrocytes, and scaffolds. They seeded osteoblasts into a PGA/PLA scaffold and then painted chondrocytes onto the surface of the scaffold.[141] This study showed that the formation of a bone/cartilage composite *in vivo* is promising for future mandibular condylar reconstructions.

The lack of clinical translatability for primary cells has also been recognized in mandibular condyle engineering. A recent push has been made to investigate the use of stem cells in condylar engineering. Many studies have revealed promising results of bone marrow-derived MSCs for repairing condylar defects.[142–145] Alhadlaq and colleagues[146] once encapsulated the chondrogenically and osteogenically differentiated bone marrow MSCs into biphasic poly(ethylene glycol) (PEG)-based hydrogels in order to create a human-shaped mandibular condyle. After 8 weeks of *in vivo* implantation, it was demonstrated histologically that cartilaginous and osseous phenotypes were present in two stratified layers.[143] In summary, stem cells are emerging as a promising cell source for mandibular condyle tissue regeneration, although they require more investigations to fully explore their medical potentials.

5.517.6.3.2. Scaffolds

Scaffolds play an important role in mandibular condylar tissue engineering through providing structural and mechanical supports for cell growth and tissue formation. Because of the ease of fabrication, good biocompatibility, suitable mechanical properties, and controllable biodegradability, natural or synthetic polymers have been extensively used as bone, and osteochondral tissue engineering scaffolds. Popular polymers for tissue engineering the mandibular condyle have been PEG, polycaprolactone (PCL), PLA, PGA, and poly-lactic-*co*-glycolic acid (PLGA). Ueki *et al.*[147] implanted PLA/PGA/gelatin sponges (PGS) with or without recombinant human bone morphogenic protein-2 (BMP-2) into condylar defects of rabbit TMJs. After 4 weeks, it was observed that the PGS scaffolds with or without BMP-2 induced new bone and cartilage-like tissues in the TMJ.[147] In another study, a tissue-engineered bone construct with a mandibular condyle shape was obtained by combining osteogenically differentiated MSCs and PLGA scaffolds.[148]

Calcium phosphate ceramics such as HA and tricalcium phosphate (α- or β-crystalline TCP) share a similar crystal structure and chemical composition with natural bone. As a result, they have good osteoconductive and osteoinductive properties and have been considered as popular bone substitutes, filler materials, and bone tissue engineering scaffolds.[72] For mandibular condylar tissue engineering, calcium phosphates are usually fabricated with polymers into a composite. The Hollister group has generated various load bearing tissue-engineered scaffolds with appropriate bulk geometry and microarchitecture through image-based design and solid free-form fabrication methods from polymers (PLA, PGA, PLGA, PPF, etc.), ceramics (HA or TCP), or their composites (PLA/HA, PPF/TCP, and HA/TCP).[149–153] For instance, a biphasic PLA/HA composite scaffold was made and fibroblasts with BMP-7 and chondrocytes were separately seeded into the HA and PLA phases.[150] The results showed simultaneous growth of bone, cartilage, and a mineralized interface tissue in the tissue-engineered scaffold. Thus, this technology holds the potential for repairing osteochondral defects in the TMJ.

Natural tissues or organs have numerous nano features and cells directly interacting with nanostructured ECM. Therefore, biomimetic nanomaterials, which have basic structural units, grains, particles fibers, or other constituent components smaller than 100 nm in at least one dimension, have been investigated for TMJ implants, bone, and cartilage regenerations.[72,154] For example, by using chemical vapor deposition technology, a nanostructured diamond film with high hardness and enhanced toughness was deposited on articulating surfaces of TMJ implants and exhibited excellent biocompatibility and mechanical properties.[155] In addition, some *in vitro* studies showed that nanophase HA significantly enhanced osteoblast adhesion and inhibited undesirable fibroblast adhesion compared to conventional HA.[71] Venugopal and colleagues[156] demonstrated that osteoblast proliferation, alkaline phosphatase activity, and mineralization were significantly improved on the electrospun fibrous PCL/HA/gelatin nanocomposite when compared to PCL alone. It was also reported that the electrospun PCL nanofibrous scaffolds effectively induced chondrogenic differentiation of MSCs *in vitro*,[157] and bone formation *in vivo*.[158] Even though few results of nanostructured scaffolds for mandibular condylar tissue engineering are available, it is a promising research field because of its use of biomimetic surface topography, increased wettability, and better mechanical properties.

5.517.6.3.3. Bioactive agents

Even though condylar engineering is a new field, one recent study has investigated the effects of growth factors on tissue development. Srouji *et al.*[159] evaluated *in vivo* mandibular defect repair by hydrogel scaffolds with IGF-I and TGF-β1. After 6 weeks, significant bone formation was observed in the mandibular defects implanted with TGF-β1, IGF-I, and TGF-β1 + IGF-I incorporated hydrogels.[159] Although this study provides a preliminary insight, more research needs to be performed to determine the full potential of bioactive agents for condylar engineering.

5.517.7. Future Directions for TMJ Tissue Engineering

TMJ tissue engineering has progressed quite dramatically over the last 10 years. Now, there are investigators actively working on biological replacements for the disc as well as

the cartilage and bone of the condyle. The current literature provides a reference point for tissue engineering challenges such as cell source and scaffold selection, although the amount of prior work varies between tissues. There is still a significant amount of work that needs to be completed to produce functional replacements for TMJ tissues. Clear directions for the future of TMJ tissue engineering include progenitor cells, enhanced external stimulation, and engineering of the remaining TMJ tissues.

5.517.7.1. Progenitor Cells

Previous work using primary TMJ cells has allowed the characterization of these cells *in vitro*, but a clinically relevant tissue-engineered construct will likely not contain these cells. Problems with primary cells have been discussed earlier and include a lack of donor tissue and high donor site morbidity. A practical cell source for TMJ engineering should originate from healthy tissues which, when removed, should not result in significant morbidity.[12] The likely choice is progenitor cells, whether adult or embryonic. Direct comparison has shown that multipotent progenitor cells outperform TMJ cells.[134] Both MSCs and embryonic stem cells have shown the ability to differentiate down fibrocartilaginous and osteogenic lineages.[118,120,143,160] Although stem cells have been used for TMJ tissue engineering, different cell types have been used for each tissue in an investigator dependent manner. Additionally, the differentiation of these progenitor cells into TMJ-like cells is not fully understood. In the future, there should be coordinated efforts to determine the appropriate progenitor cells for all TMJ tissues, as a total biological joint replacement must be the ultimate goal.

5.517.7.2. Mechanical Stimuli

Even though a significant number of TMJ tissue engineering studies have been completed, only three have investigated the effects of external mechanical stimuli. As TMJ is a frequently loaded joint, it makes sense that mechanical stimulation would enhance TMJ engineering. Biomechanical stimuli have been used extensively in articular cartilage engineering with great success.[161] Stimuli that may be beneficial for TMJ tissue engineering include compression, tension, shear, and hydrostatic pressure. All of these mechanical loads are present in the TMJ.[12] Hydrostatic pressure[105] and tensile loading[128] have both shown promise for disc engineering and should now be carried forward toward tissue engineering of other TMJ tissues. Compression and shear have not yet been evaluated for TMJ engineering, but should certainly be incorporated into future studies.

5.517.7.3. Other TMJ Tissues

While current tissue engineering efforts have focused on engineering the disc and condyle, other tissues of the joint, including the fossa cartilage, disc attachments, and capsule, should also be considered. Each of these tissues plays an important role in the joint, and needs to be considered toward engineering a total biological TMJ replacement. Although the fossa-eminence is not well characterized, fossa cartilage and bone engineering are likely to benefit directly from condylar cartilage and bone engineering studies. The disc attachments and joint capsule on the other hand are distinct tissues that will require independent characterization and tissue engineering efforts.

5.517.7.3.1. Disc attachments

Although significant attention has been paid to characterization and engineering of the disc, very little focus has been placed on the disc attachments. These attachments connect the disc to the capsule and bony structures of the joint. The discal attachments are important for keeping the position of the disc in the joint relative to the condyle and fossa.[1] Maintaining disc position is critical for preserving normal loading patterns, and a breakdown in the discal attachments will result in joint degradation. Characterization of the native disc attachments will provide important information about how a tissue-engineered disc should be implanted in the joint. It is possible to anchor an engineered disc directly to the condylar head, but this will prevent movement of the disc relative to the condyle and alter the loading pattern in the joint. A more likely solution would be to engineer a disc with its attachments so that the attachments could be anchored to the condyle and sutured to the capsule. This would allow a natural movement of the disc within the joint. Future studies will need to investigate the properties and the tissue engineering potential of the disc attachments.

5.517.7.3.2. Joint capsule

Like the discal attachments, the capsule is a critically important but poorly understood component of the TMJ. Globally, the capsule provides a barrier which isolates the intra-articular joint spaces. Unlike the fibrocartilages of the joint, the TMJ capsule is innervated with various groups of nerve endings, including Pacinian corpuscles.[4] Inclusion of these nerve endings may be necessary for a physiologically normal joint replacement. Additionally, the capsule is lined with synovium, which produces the lubricating and nourishing synovial fluid for the joint. Lubrication is critically important for maintaining normal TMJ loading and must be incorporated into an engineered replacement. The exact difficulties involved in recreating the TMJ capsule will need to be investigated in the future. Ultimately, all of the TMJ tissues, including the fossa, disc attachments, and capsule, will need to be tissue engineered to produce a total biological replacement for the TMJ.

5.517.8. Conclusions

Although it has only recently received attention, the field of TMJ tissue engineering is growing rapidly. The pathology of the TMJ is complex, but it is important to address these diseases for the millions of people suffering from TMD. Even though characterization of native TMJ tissues has not been completed yet, the available literature has provided a rough set of design and validation criteria on which tissue engineering efforts can be based. Current tissue engineering efforts provide a basis for selecting a cell source, scaffold, and external stimuli, although technological advancements provide new options regularly. The rapid increase in TMJ disc characterization and engineering studies over the last 10 years provides

optimism for the remaining TMJ tissues that are not as well studied. It is clear that significant effort must be put forth before the ultimate goal of creating a functional biological replacement for the TMJ can be reached, but the future looks bright for this technology.

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http://www.arthritis.org/ – Arthritis Foundation.
http://www.tmjoints.org/ – Jaw Joints and Allied Musculo-Skeletal Disorders Foundation.
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