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# Remaining hurdles for tissue-engineering the temporomandibular joint disc

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## Abstract

The temporomandibular joint (TMJ) disc, a fibrocartilaginous structure between the mandible and temporal bone, is implicated in temporomandibular disorders (TMDs). TMDs symptomatically affect approximately 25% of the population, of which 70% have internal derangement of the disc. Treatments lack efficiency, motivating novel therapies, including tissue-engineering toward TMJ disc regeneration. Recent developments in scaffold-based or scaffold-free approaches, cell sources, and biochemical and mechanical stimulation result in constructs exhibiting native tissue mechanics. Safety and efficacy of tissue-engineered implants show promising results in orthotopic animal studies. However, many hurdles need to be overcome in tissue-engineering approaches, and clinical and regulatory pathways. Future studies present an opportunity for clinicians and researchers to work together toward safe and effective clinical trials.

### Keywords

tissue-engineering; temporomandibular joint disc

## Motivation for Tissue-Engineering of the Temporomandibular Joint Disc

The temporomandibular joint (TMJ) is a **ginglymoarthrodial joint** (see Glossary), central to speaking and chewing functions [1]. The TMJ contains a disc between a condyle and the glenoid fossa-articular eminence region [2] (Figure 1). The TMJ disc is biconcave and fibrocartilaginous in nature [2]. As the TMJ articulates, the TMJ disc may distribute the stresses that develop within the joint [3] (Figure 1). Trauma [4] and age-related degeneration [5] can cause abnormal loading in the TMJ, leading to temporomandibular disorders (TMDs). TMDs are characterized by orofacial pain and/or limitation in jaw movement [6–8], and symptoms are present in approximately 25% of the population [9]. Perplexingly, TMDs affect females up to 8.0-fold more than males [9–12]. In addition, TMDs affect mostly younger patients between 20-50 years of age [12–14]. As the second most common musculoskeletal condition resulting in pain and disability, TMDs cost an estimated $4 billion

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per annum in the United States (https://www.nidcr.nih.gov/research/data-statistics/facial-pain).

A specific subset of TMDs involve discal pathologies such as **internal derangement (ID)**, disc thinning, and disc perforation. ID affects about 70% of TMD patients [15]. Severe cases of ID present disc thinning and eventual disc perforation (Figure 2) in approximately 5-15% of ID patients [5,16,17]. However, ID and disc perforation can occur independently; the independent cases of disc perforation can be due to age-related wear [5]. These discal pathologies are the most prevalent manifestation of TMDs [15]. **Osteoarthritis (OA)** is also commonly seen in conjunction with ID [16,18], but the relationship between ID and OA is not understood; it is not known whether one precedes the other or if both share common causative events [18]. However, it is thought that TMJ disc pathologies such as ID or disc perforation are the first steps in a series of degenerative changes (i.e., OA) seen throughout the adjacent articulating, soft tissue surfaces [19].

Management of disc-related TMDs varies with disease severity [20]. Non- and minimally-invasive strategies include physical therapy [21], occlusal splints or adjustments [22], pharmacologic agents [23], sodium hyaluronate and corticosteroid injections [24], arthrocentesis [25], and arthroscopy [16]. However, these treatments are only palliative. Only 5% of TMDs are candidates for surgical intervention [26]; surgeries for TMDs include discectomy with or without disc replacement [27] and partial or full joint reconstruction with autologous [28] or alloplastic materials [29]. Discectomy has shown promise for symptom reduction but has shown degenerative remodeling of the joint as a result [30,31]. Costochondral rib grafts are used to reconstruct the mandibular condyle [28], but no autologous grafts exist for the complete joint [14]. Alloplastic total joint prostheses have been indicated for severe ankylosis, failure of autologous grafts, failure of Proplast-Teflon implants, or severe OA [32]. Most TMD patients range between 20-50 years of age [12–14], but the typical lifetime of alloplastic total joint prostheses is 10-15 years [33], making revisions likely within a patient's lifetime [14]. The use of alloplastic total joint prostheses is reserved as an option of last resort for a small subset of patients, creating a gap in terms of treatment options between non-invasive or minimally invasive strategies and end-stage surgical techniques.

The treatments described above do not provide mid-stage intervention for patients. To fill this gap, novel treatment strategies to improve patient outcomes must be developed. Tissue-engineering aims to regenerate the pathological tissues in TMD with biological neotissues to restore long-term function. Here, we focus on TMJ disc pathologies due to their overarching prevalence in TMDs [15]. In particular, we discuss recent tissue-engineering efforts (Table 1) and remaining hurdles for TMJ disc tissue-engineering.

## Recent Tissue-Engineering Efforts

Tissue-engineering employs scaffolds, cells, and various signals such as biochemical and mechanical stimuli (Figure 3). As discussed in this section, advances in materials engineering have resulted in a variety of scaffolds [34–36], while scaffold-free approaches, such as the selfassembling process [37–39], have also emerged in TMJ disc tissue-

engineering. In terms of cell sources, primary chondrocytes, mesenchymal stem cells (MSCs), and cell expansion technologies are also reviewed below (Table 1). Signals such as biochemical and mechanical stimuli for mechanical improvement of the TMJ disc (Table 1) are also discussed. This section also examines small animal models that have been used for examining the performance of these implants [36,39–43].

## Novel Scaffold-based and Scaffold-free Approaches

The primary purpose of scaffolds is to provide a template for cells to form tissues. Scaffolds can be functionalized with biomolecules to direct cell behavior and manufactured with mechanical properties similar to the tissues they are intended to replace. Ideally, scaffold degradation rates would match the rate of tissue formation. Scaffolds recently used in tissue-engineering the TMJ disc include natural materials and synthetic materials (Table 1). Two particularly interesting developments include novel scaffold fabrication methods and the emergence of scaffold-free approaches.

New fabrication methods allow for surface modifications of scaffolding materials. Layer-by-layer nanoassembly is one such fabrication method [34,44]. Titanium dioxide nanofilms are used to modify surfaces of scaffolds for tissue-engineering of bone [44] as well as cartilage [34]. These nanofilms are created by layer-by-layer nanoassembly, based on the principle of electrostatic charge, to coat various surfaces allowing for increased cell attachment, control of cell phenotype, and control of differentiation. In a study using titanium dioxide surface modification with seeded TMJ disc cells, cell proliferation and extracellular matrix (ECM) deposition increased with increasing thickness of nanofilms [34]. The matrix was reminiscent of a fibrous ECM, in contrast to a cartilaginous ECM. Type I collagen and decorin, approximately 0.34mg/mL and 0.31mg/mL, were present in higher amounts than type II collagen and aggrecan, approximately 0.14mg/mL and 0.28mg/mL, after 14 days of culture on 20 layers of titanium dioxide nanofilms [34]. Additional work needs to be performed to couple layer-by-layer nanoassembly with typical scaffold materials such as polycaprolactone (PCL) or polylactic acid (PLA).

Three-dimensional (3D) printing is a fabrication technique that achieves microprecise placement of scaffolding materials and functional biomolecules. 3D printing can create regional variation in scaffolds reminiscent of the native TMJ disc. For example, a dual-nozzle setup in a PCL-poly(lactic-co-glycolic acid) (PLGA) microsphere system allowed spatiotemporal delivery of transforming growth factor beta 3 (TGF-β3) and connective tissue growth factor (CTGF) [35,36]. The 100mg dosages of growth factor-embedded microspheres resulted in increased intermediate zone type II collagen and aggrecan deposition by approximately 2-fold compared to the 50mg dosage when analyzing immunofluorescence images of constructs seeded with bone marrow-derived MSCs [35]. However, growth factor-embedded microsphere application decreased compressive modulus in both dosages by at least 2-fold when compared to empty microspheres in both areas analyzed [35]. Similar trends were apparent in instantaneous and relaxation moduli indicating that mechanical properties did not necessarily trend with growth factor application and ECM content [35]. Compared to traditional scaffold-based approaches, 3D

printing offers the ability to create regional variation which can resemble native ECM content.

Scaffold-free approaches, such as the self-assembling process [37–39], have been developed to bypass issues related [45] to scaffold degradation products, e.g., acidity due to PLA degradation [46], fabrication byproducts, e.g., crosslinkers and plasticizers [46], and stressshielding of cells [47]. The self-assembling process recapitulates developmental aspects of cartilage formation to generate functional neotissues with characteristics resembling those of native tissues [45,48]. Specifically, it is the most prominent of these techniques for TMJ disc tissue-engineering because it has generated mechanically robust tissue [37]. Stimulation of self-assembled TMJ disc constructs by bioactive agents and mechanical compression resulted in values of approximately 3.5%, 2.75 MPa, and 2.25 MPa for collagen per wet weight, tensile **Young's modulus**, and ultimate tensile strength (UTS), respectively. Additional analysis of constructs created from cocultures of hyaline articular chondrocytes (ACs) and knee meniscus cells (MCs) found collagen fibril alignment reminiscent of native TMJ discs, exhibiting direction-dependent strains in finite element analysis. This was promising because it showed anisotropic tissue on par with the alignment of native tissue [38], which further substantiates scaffold-free tissue-engineering as an alternative to scaffold-based approaches.

While scaffold-free approaches do not necessarily have the flexibility of scaffold-based approaches, e.g., scaffold functionalization with biomolecules, these limitations can be overcome with exogenous stimulation, which can have various effects on scaffold-free constructs such as increased mechanical properties [49,50]. In addition, variation of the cell source can also have a large influence on the eventual properties of the resulting constructs.

## Cell Sources

Selection of a cell source is one of the most important considerations for TMJ disc tissue-engineering (Table 1). Options for primary cells range from native TMJ disc cells [34,51] to other cells from hyaline articular cartilage and the knee meniscus [38]. In addition, recent advances in cell expansion technologies [52–54] have allowed exploration of costal cartilage-derived cells [39]. MSCs are also heavily used [35,36,40,41,51,55–57].

Potential primary cell sources for TMJ disc tissue-engineering include TMJ disc cells, ACs, MCs, and costal chondrocytes (CCs). TMJ disc cells have been used in multiple studies [34,51], but the dearth of available, healthy tissue raises concerns for this source [58]. Thus, ACs and MCs have been considered [38]. Using AC-MC coculture with the self-assembling process resulted in a functional, anisotropic TMJ disc as discussed above [38]. With recent advances in cell expansion technologies that preserve chondrogenic phenotype [52–54], CCs might allow for either an autologous or allogeneic approach to replacing cartilages, as demonstrated previously in articular cartilage [59,60] and the TMJ disc [39]. Allogeneic CCs can be harvested from cadaveric tissue, while autologous tissue harvest procedures are conducted routinely for rhinoplasty and autologous TMJ reconstruction. Thus, existing surgical procedures may be sufficient for tissue regeneration purposes. The use of CCs can also remove or reduce donor site morbidity and virtually eliminate the potential of harvesting cells from OA tissue. When used in a hyaline articular cartilage model, CC

constructs have attained a functionality index (FI, described in Box 1) of 55% compared to the medial condyle cartilage properties [60]. These techniques and results offer promise of an alternative source of chondrocytes that can create mechanically stable constructs for other parts of the body such as the TMJ disc.

An array of MSCs from both adult and fetal tissues have been used, as previously reviewed [61]. MSCs from various tissues (Table 1) offer an autologous or allogeneic approach and can be isolated in large quantities, making these sources clinically relevant for construct formation. Perhaps the most interesting MSCs are those derived from the synovium because they were shown to synthesize cartilage oligomeric matrix protein, link protein, and glycosaminoglycans (GAGs), similar to ACs [62]. For example, synovium-derived MSCs on fibrin-chitosan scaffolds increased type I collagen expression approximately 2-fold *in vitro* and ECM deposition *in vivo* as evidenced by histological analysis when compared to pure chitosan scaffolds [40]. Progress using MSCs has resulted in morphological and biochemical biomimicry evaluated via histology, gene expression, and other biochemical assays [36,40,41,51], but future research should next focus on assaying functional properties of MSC-derived constructs via mechanical testing.

The choice of cell source remains a challenge within the field of TMJ disc tissue-engineering. Lack of standardization of mechanical testing modalities makes it difficult to compare sources head-to-head and to determine if one cell source is more suitable than another. Perhaps the most important characteristic to consider when choosing a cell source is mechanical stability of the resulting tissue-engineered construct due to the dynamic joint environment.

## Improvement of Mechanical Properties of TMJ Disc Fibrocartilage

The TMJ disc functions in a dynamic environment of compression, tension, and shear [63,64]. Finite element analysis shows stresses in the TMJ disc during mouth opening to be greater than 7 MPa in compression, 4 MPa in tension, and 1 MPa in shear [65]. For comparison, the hip experiences approximately 7-10 MPa in compression and up to 18 MPa during stressful activities such as standing up [66,67]. Characterization of the native tissue should aim to define the gold-standard, design criteria for tissue-engineered TMJ disc constructs; the expectation is that replicating the native tissue's mechanical properties would allow for restoration of mechanical function. Thus, to engineer constructs with physiological levels of mechanical stresses in mind, various biochemical and mechanical stimuli, and also changes in scaffold processing (Figure 3) have been developed. For scaffold-free approaches, self-assembled constructs have approached native values in mechanical properties due to synergistic effects of biochemical and mechanical stimulation [38,39]

A majority of recent scaffold-based studies use only biochemical stimuli to improve construct mechanical properties (Table 1). Constructs stimulated with biochemical stimuli have been previously found to exhibit native tissue structure-function relationships. For example, insulin-like growth factor I and TGF-β applied to constructs created from TMJ disc cells increased collagen synthesis by greater than 400% at 3 weeks of culture, leading to higher aggregate moduli of 5 kPa [68]. However, constructs sometimes do not follow native tissue structure-function relationships [35] (e.g., increased matrix deposition leading to

increased mechanical properties). To overcome such deficiencies, mechanical stimulation may be considered. However, mechanical stimulation has not been employed in scaffold-based TMJ disc approaches, though it has been used in other fibrocartilages such as the knee meniscus. For example, hydrostatic pressure combined with TGF-β1 led to 4-fold higher collagen deposition and 3-fold higher GAG deposition, as compared to the unpressurized growth factor controls in MC-seeded PLA scaffolds [69]. Studies showing recapitulation of native tissue structure-function relationships should serve as models for future studies toward identifying additional stimuli. Biochemical stimuli must continue to be investigated, but, additionally, mechanical stimuli can be used to increase mechanical properties of engineered discs to withstand the dynamic *in vivo* environment.

Scaffold-free approaches have combined biochemical stimuli and mechanical stimuli to generate stiffer, stronger, anisotropic constructs, followed by examination of the resulting constructs in large animal models. Using a scaffold-free approach with AC-MC coculture, TGF-β1, chondroitinase ABC (C-ABC), and lysyl oxidase-like 2 (LOXL2) have been identified in the past as efficacious for fibrocartilage tissue-engineering, enhancing tensile Young's modulus and UTS by 245% and 186%, respectively [70]. In a self-assembled TMJ disc model using AC-MC coculture stimulated with only TGF-β1 and C-ABC, tensile Young's modulus, UTS, and collagen per wet weight increased by 2-fold or greater in the intermediate zone of the disc, as compared to controls [38]. Passive axial compression and these biochemical stimuli were combined and noted to exhibit synergism, showing 5.8-fold, 14.7-fold, and 13.8-fold increases in collagen per wet weight, tensile Young's modulus, and UTS, respectively, compared to unstimulated controls [38]. Moving to *in vivo* studies, TMJ discs engineered using all three stimuli (TGF-β1, C-ABC, and LOXL2) coupled with passive axial compression, yielded an FI (Box 1) of 42% of native properties with a passaged, allogeneic CC source [39]. By combining these three biochemical stimuli with mechanical stimulation, increased functional properties were achieved as compared to either alone. Thus, further synergistic effects of other biochemical and mechanical stimuli should be explored.

As reviewed elsewhere [49], strategies for other tissues, such as hyaline articular cartilage, can help inform further mechanical improvement of constructs. Similar designs and models can be used to engineer the fibrocartilage of the TMJ disc. For example, in a recent study on tension and its effects for articular cartilage engineering, continuous stimulation combined with a bioactive regimen increased the tensile properties by 5.8-fold over unstimulated controls in AC-derived, self-assembled constructs [71]. By improving mechanical stability using biochemical and mechanical stimuli, constructs continue to approach native tissue values. Attaining mechanical biomimicry is a crucial characteristic for constructs to perform satisfactorily when implanted into the orthotopic environment.

## Current Animal Models

Prior to human clinical trials, tissue-engineered implants are examined in relevant animal models to demonstrate initial safety and efficacy. Similar to TMJ disc tissue-engineering, development of animal models is based on design criteria. For the TMJ, similar anatomies, chewing patterns, and diets compared to humans, and ease of surgical access are included in

the design criteria. In addition, relative size of TMJ structures and animal cost may also determine which model to use. Animal models exist for various purposes such as observing the adverse reactions to an implant subcutaneously to examining surgically induced pathologies in orthotopic studies. Small animals such as mice and rats are economical, serve as pain models [72,73], and simulate OA and associated degenerative changes in the joint [74,75]. However, their small TMJ disc size limits studies to simple subcutaneous implantation as opposed to orthotopic studies in larger animals such as rabbits [43]. Moving toward orthotopic studies, rabbits allow for additional biochemical and histological analysis, and reliable mechanical testing [42], but present substantial differences from human size and loading conditions [43]. This motivates the use of large animal models that more closely resemble human anatomies and conditions [42].

Many preliminary studies involve subcutaneous implantation to examine possible adverse reactions and establish proof-of-concept. These studies, as reviewed [43], are commonly performed in mice or rats due to their low cost, without much consideration of anatomical or dietary similarities. For example, a fibrin-chitosan scaffold with synovium-derived rat MSCs was embedded into explanted TMJ discs with perforation defects and implanted into nude mice subcutaneously in a xenogeneic approach [40]. Histological analysis showed increased type I and II collagen deposition in the fibrin-chitosan scaffold, compared to the pure chitosan scaffold [40]. Although this study represents a disc perforation model, additional biochemical and mechanical analyses must be performed in larger animals to show reparative ability in the fully loaded orthotopic environment.

Recent studies employed the rabbit for orthotopic evaluation of tissue-engineered TMJ discs [36,41]. For example, 3D printed PCL-PLGA microsphere scaffolds seeded with allogeneic, synovium-derived MSCs were implanted into the disc and noted histologically to degrade by 6 weeks [36]. Cells retained their chondrocyte-like phenotype *in vivo* [36]. Scoring of the condylar surfaces with an **OA score** resulted in values of approximately 3.9 and 2.4 for the scaffolds without and with growth factors, respectively, where a lower score represents a better outcome [36]. While these studies [36,41] demonstrate feasibility for implantation of tissue-engineered TMJ discs via histological analysis, mechanical testing is of paramount importance to show the integrity of tissue-engineered constructs.

Strides in animal studies are promising to the research community as they point to a feasible translation pathway for tissue-engineered constructs. The use of ectopic small animal and larger orthotopic models (e.g., the mouse and rabbit models) is a crucial first step in proof-of-concept work for the field. However, it will ultimately be regenerative studies in orthotopic animal models in species such as the minipig that will be most impactful for translation of tissue-engineered TMJ discs toward human clinical studies.

## The Path to Translation

Translational hurdles that remain (see Outstanding Questions) include tuning of construct mechanical properties toward biomimicry (Figure 3) as well as scale-up of area and thickness of implants (Figure 4, Key Figure). A recent minipig study, showing safe and efficacious implantation of TMJ constructs [39], establishes this orthotopic large animal

model as a cogent element in the translational pathway (Figure 4). Clinical and regulatory hurdles are also significant for translation of TMJ disc constructs (Figure 4).

## Application of Proper Tissue-Engineering Parameters for Tuning of TMJ Disc Constructs to the TMJ Mechanical Environment

Constructs must be tuned to the mechanical environment of the TMJ disc because they will be subject to compressive, tensile, and shear forces [63,64]. Theoretically, the required mechanical properties will depend on surgical technique, model, and animal. For example, it was shown that an FI (Box 1) of 42% was shown to be sufficient when implanted via the intralaminar fenestration surgical technique (Figure 5) in a focal thinning model in the Yucatan minipig [39]. When moving toward perforation or larger defects, this implant might be insufficient. On the opposite end, some constructs might be too stiff or strong compared to native values, as observed in some scaffold-based approaches [35], causing stress concentrations and possible degeneration on the articulating surfaces. Also, a mismatch in the rates of scaffold degradation versus tissue formation can lead to failure. Therefore, it is important to consider tuning mechanical properties by application of proper stimulation regimens, whether using a scaffold-based or scaffold-free tissue-engineering approach (Figure 3).

## Tailoring of Tissue-Engineered TMJ Discs to Human Discal Pathologies and Size

As the translational direction points to additional large animal orthotopic studies before human clinical trials commence, defect models must increase in size. As such, constructs must also scale-up (Figure 4). In the recent minipig study [39], a one-sided 3mm defect, mimicking disc thinning, was used. Future studies need to scale-up to a larger defect area to mimic increased disc thinning, in addition to two-sided defects to mimic disc perforation. To scale-up constructs to larger thicknesses, one might consider using larger scaffolds. But as scaffolds and constructs trend upward in thickness, it should be kept in mind that diffusion limitations increase. Decreased diffusion can result in shell-like neotissues with necrotic centers, that display inadequate mechanics. However, scaffold-free approaches might prove advantageous for creation of larger constructs to mimic disc thinning. Self-assembled articular cartilage constructs made of passaged ACs up to 25 mm dia. have been made by combining cytochalasin D, TGF-β1, C-ABC, and LOXL2, under a compressive load and in mechanical confinement [76]. This approach may allow for examining TMJ disc healing in larger defects that mimic clinically observed disc thinning. As such, a significant portion of future TMJ disc studies should investigate the scale-up of defects and constructs for relevance to human TMJ anatomy.

## Novel and Cogent Translational Studies

Orthotopic large animal models need to be performed to examine the safety and efficacy of tissue-engineered constructs prior to translation. Toward selection, possible species for performing regenerative studies include sheep [77], goats [78], dogs [79], farm pigs [80], and minipigs [81]. The farm pig and minipig are two suitable models that have been recently used for regenerative studies due to their similarities to humans in chewing patterns, diet, and anatomy [3,81–85].

In a recent study demonstrating safety and efficacy of a self-assembled, allogeneic, tissue-engineered implant for disc repair, a novel TMJ disc thinning model was created in the Yucatan minipig [39]. Because the implants were created from a CC source, implantation into the TMJ disc represented non-homologous use. Implants approaching native tissue values were stimulated by a regimen of biochemical and mechanical stimulation. To affix implants securely, the intralaminar fenestration surgical technique was developed (Figure 5) [39]. Although this was an allogeneic, non-homologous use which has potential to elicit an immune response, implant safety was shown by minimal to no immune response to the constructs, as assayed by histological staining for CD3, CD20, and CD68 for T cells, B cells, and macrophages. However, it was specified that additional work needs to further elucidate the immunological response [39], such as macrophage activation due to tissue-engineered implants [86–88] (Figure 4). In terms of efficacy, results showed that the tensile Young's modulus, integration at the repair-to-native tissue interface, and percent of defect closure were 3.4-fold, 3.2-fold, and 4.4-fold higher, respectively, compared to empty defect controls [39]. OA scores of the condylar surface treated with implants were 3.0-fold less than the empty defect controls [39], yielding a better clinical outcome overall. Together, these results demonstrate the feasibility of allogeneic TMJ disc tissue-engineered constructs in the orthotopic environment and pave the way for additional orthotopic large animal studies and future human clinical trials (Figure 4).

## Overcoming Additional Clinical and Regulatory Hurdles

In stark contrast to diarthrodial joints such as the knee, there is limited knowledge surrounding the TMJ, especially when it comes to developing new processes and products for repair or replacement of the TMJ disc. Compared to the TMJ, a greater variety of products, treatments, and studies exist for the knee. To illustrate these differences, one can consider indications and contraindications in the TMJ versus the knee. For example, in the knee, there are clear guidelines as to what constitutes small, large, partial thickness, and full thickness defects with concomitant treatment algorithms [89]. In contrast, it is not clear when a tissue-engineered treatment would be indicated in the TMJ. Currently, in the knee, tissue-engineered products are contraindicated for the OA milieu [90]. This has not been confirmed for the TMJ, though the expectation is that the constructs under OA conditions might succumb to the same fate as the native tissue [91]. Development of treatment guidelines and additional studies specific to the TMJ should continue, toward bringing TMJ-related knowledge to levels of other diarthrodial joints.

One must also consider fixation and associated surgical approaches. The intralaminar fenestration surgical technique (Figure 5) was successful in treating early-stage disc thinning, but in the minipig [39]. However, in 5% of TMD cases requiring surgery [26], it is not yet obvious how one may be able to attach a partial or whole, tissue-engineered disc (Figure 4). Surgeons and researchers must continue to collaborate to develop surgical approaches for implantation of tissue-engineered implants, as they are of utmost importance to the success of the tissue-engineered treatment.

With regard to clearing the regulatory hurdle, the TMJ's proximity to the brain (Figure 4) may necessitate more stringent safety requirements than products for other joints such as the

knee. These requirements may include analysis of the synovial fluid in the TMJ, but also the neighboring cerebrospinal fluid. Notoriously, mechanical failure and resulting degradation of the Proplast-Teflon disc implants resulted in exposure of the brain cavity [92–94]. Additionally, current large animal work has yet to investigate fully immunological implications related to TMJ disc implants (Figure 4) or how immunomodulation may be used in a proinflammatory environment [95]. In terms of regulation, the FDA has not previously guided a tissue-engineered TMJ disc product [96], thus raising the question of establishing TMJ-specific safety and efficacy guidance documents. Future research in the field needs to establish the safety of tissue-engineered TMJ discs by elucidating the immune response. Additionally, researchers need to communicate with regulatory bodies, such as the FDA, to obtain guidance on how tissue-engineered TMJ disc products need to be demonstrated as safe and efficacious.

## Concluding Remarks

While recent advances propel TMJ disc tissue-engineering forward, many hurdles still exist. To summarize, the pressing challenges include improvement of mechanical properties of constructs, scale-up of implant dimensions, determination of indications for tissue-engineered discs, development of surgical techniques, analysis of the immunological response, and regulation by the FDA (see Outstanding Questions). Tissue-engineering and basic science investigations for TMDs will continue to drive the field. The field should focus toward addressing questions in the clinical and regulatory spaces. Specifically, studies should pay attention to developing novel surgical techniques and associated fixation methods toward human clinical trials. For each new tissue-engineering approach, regulatory requirements need to be satisfied by demonstration of TMJ-specific safety and efficacy in large animal models. As regulatory bodies turn their attention toward clinical trials, these data will be the primary preclinical assessment of implants. Considering the momentum toward significant preclinical studies, it is an exciting time to be in the field of TMJ disc tissue-engineering. After the early success shown in the orthotopic study performed in the Yucatan minipig [39] and the identification of clinical and regulatory hurdles discussed here, there is new impetus to develop tissue-engineering solutions to begin addressing the various intractable TMJ trauma and degenerative ailments. The possibility of translating tissue-engineered TMJ discs is increasingly being realized.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Glossary

**Ginglymoarthrodial joint**
a joint functioning in both rotation and translation

**Internal derangement (ID)**
misalignment or displacement of the TMJ disc from a normal anatomic position

**Mastication**
the mechanical grinding of food into smaller pieces by teeth

**Osteoarthritis (OA)**
a slowly progressing joint disease characterized by degenerative changes in the cartilage and subchondral bone; presents through wear of the cartilage or underlying bone and presence of osteophytes; commonly affects large diarthrodial joints such as the knee, but also joints such as the TMJ

**OA score**
a semi-quantitative measure of the severity of osteoarthritis based on histomorphological analysis of cartilage, underlying bone, and degenerative marks such as osteophytes; a higher number indicates increased degeneration; standardized by various groups including the Osteoarthritis Research Society International (OARSI) or the International Cartilage Regeneration and Joint Preservation Society (ICRS)

**Ruminants**
an even-toed, hoofed mammal (e.g., bovine, ovine) that chews regurgitated food from its first stomach

**Young's modulus**
a material property defining the stiffness of a material when deformed by uniaxial tension or compression; measured as the ratio of stress (force per unit area) to strain (change in length divided by original length)

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## Highlights

- Current treatments for TMJ disorders lack long-term efficacy and are palliative, motivating tissue-engineering for repair or replacement of the injured or ailing tissues in the TMJ, such as the disc.
- Scaffold-based or scaffold-free approaches, cell sources, biochemical stimuli, and mechanical stimuli are all elements of the tissue-engineering process that need to be considered to tailor TMJ disc construct properties.
- Large animals can serve as models of human TMD; orthotopic implantation in large animal models is a necessary translational step.
- The first successful orthotopic study of the TMJ disc in a large animal model has primed the field for translation of tissue-engineered constructs; however, there are still numerous hurdles prior to human clinical trials.

## Outstanding Questions

- How do researchers achieve tuning of tissue-engineered constructs to the mechanical environment of the TMJ disc?
- Can researchers scale-up constructs, in area and thickness, to be relevant to human discal pathologies and size?
- For what cases will tissue-engineered products be indicated (or contraindicated)?
- Can novel surgical procedures be developed for accessing the TMJ, and fixing and implanting tissue-engineered TMJ disc constructs orthotopically?
- What is the local and systemic responses to tissue-engineered TMJ discs *in vivo*?
- How would tissue-engineered constructs for the TMJ disc be regulated by the FDA?

**Box 1:**

### The functionality index compares constructs properties to native tissue values.

Values for biochemical content, such as overall collagen (Col) and glycosaminoglycan (GAG) content, accompany values for various mechanical properties such as ultimate tensile strength (UTS), Young's modulus ($E^T$), compressive relaxation modulus ($E^r$), and compressive instantaneous modulus ($E^i$). Ranging from 0% to 100%, a value of 100% represents perfect recapitulation of native values. Subscripts serve to designate native (N) or tissue-engineered (TE) values.

$$FI(TE \mid N) = \frac{1}{6}\left[\left(1-\left|\frac{GAG_N - GAG_{TE}}{GAG_N}\right|\right)+\left(1-\left|\frac{Col_N - Col_{TE}}{Col_N}\right|\right)+\left(1-\left|\frac{E^i_N - E^i_{TE}}{E^i_N}\right|\right)+\left(1-\left|\frac{E^r_N - E^r_{TE}}{E^r_N}\right|\right)+\left(1-\left|\frac{E^T_N - E^T_{TE}}{E^T_N}\right|\right)+\left(1-\left|\frac{UTS_N - UTS_{TE}}{UTS_N}\right|\right)\right] * 100\,\%$$

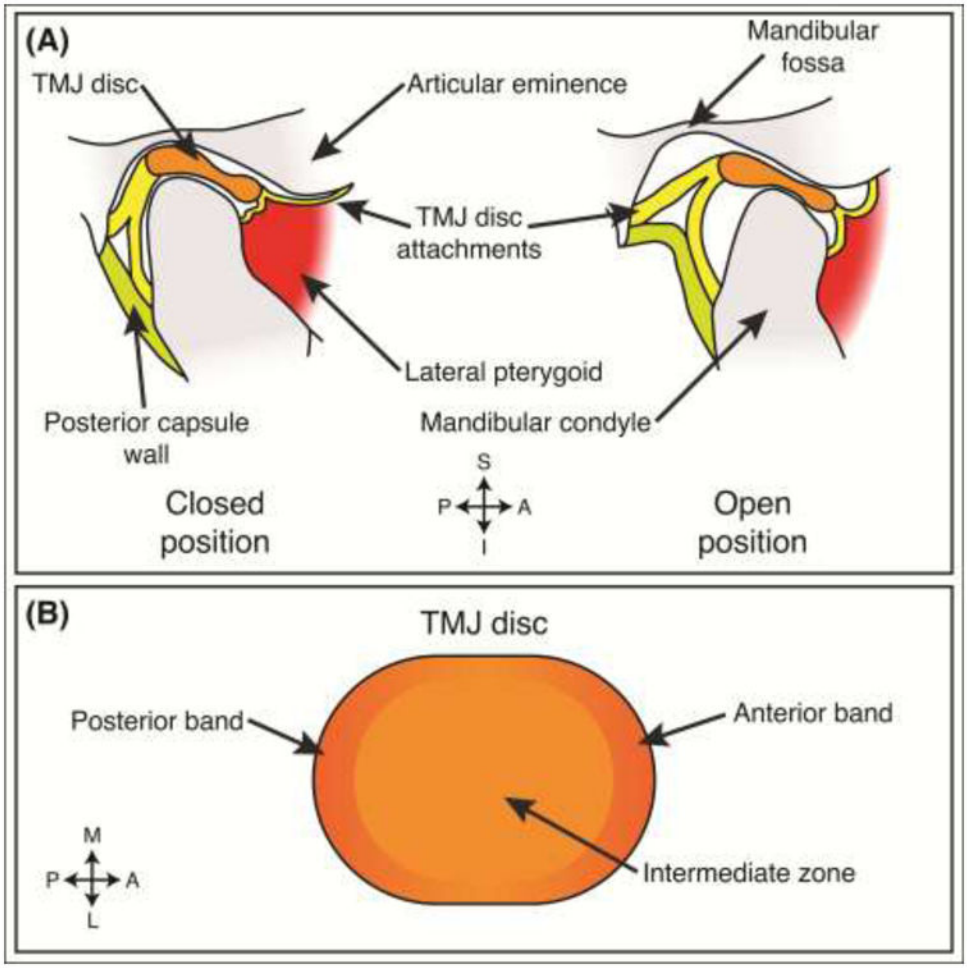


**Figure 1: TMJ disc anatomy.**

(A) Depending on the open or closed position of the joint, the TMJ disc is situated between the mandibular condyle and the articular eminence-mandibular fossa region. In this sagittal view, the disc is held in place by disc attachments, present at all angles (e.g., lateral, medial, posterior, anterior), surrounding the disc. The joint is separated into two joint capsules delineated by the TMJ disc. (B) The disc is regionally composed of two bands in the anterior and posterior portions of the disc. The middle portion of the disc is referred to as the intermediate zone. S – superior, I – inferior, A – anterior, P – posterior, M – medial, L – lateral.

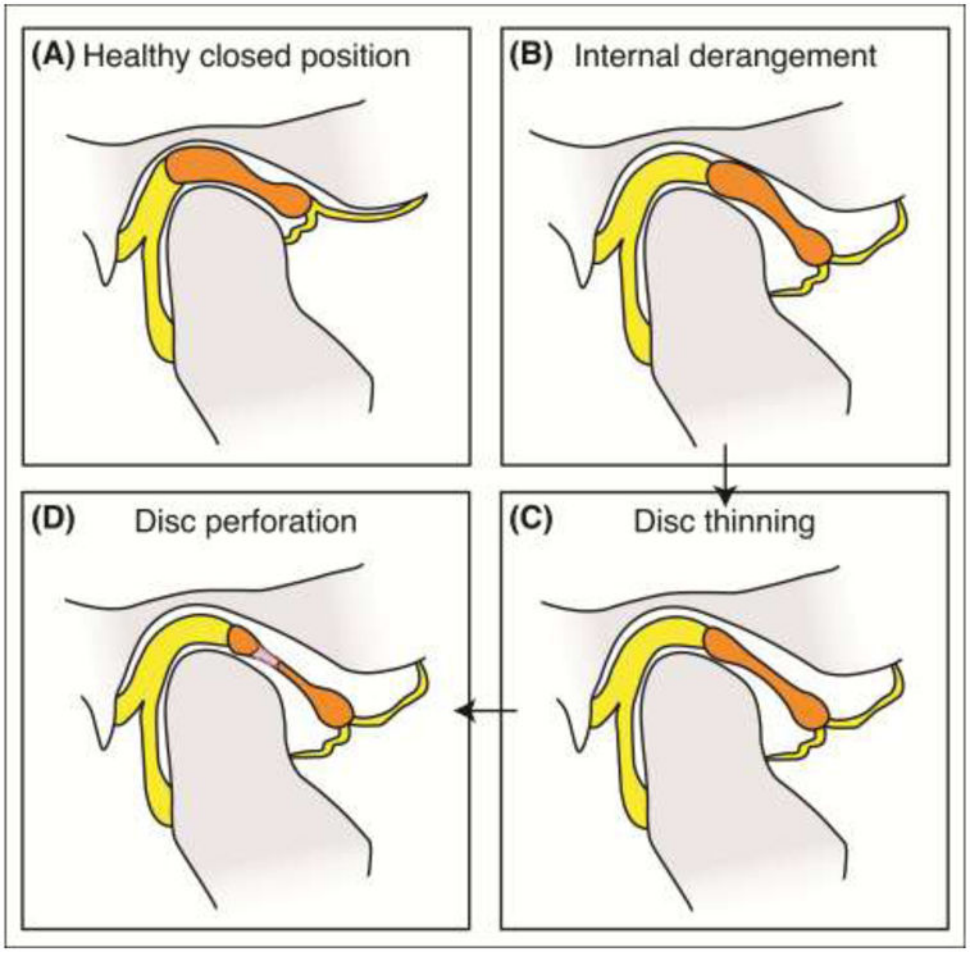


**Figure 2: Internal derangement of the TMJ disc.**

(A) A healthy closed jaw position is shown. (B) The most common type of internal derangement is shown, where the disc is displaced anteriorly. Progression of the joint in this configuration often causes (C) disc thinning and (D) eventual disc perforation.

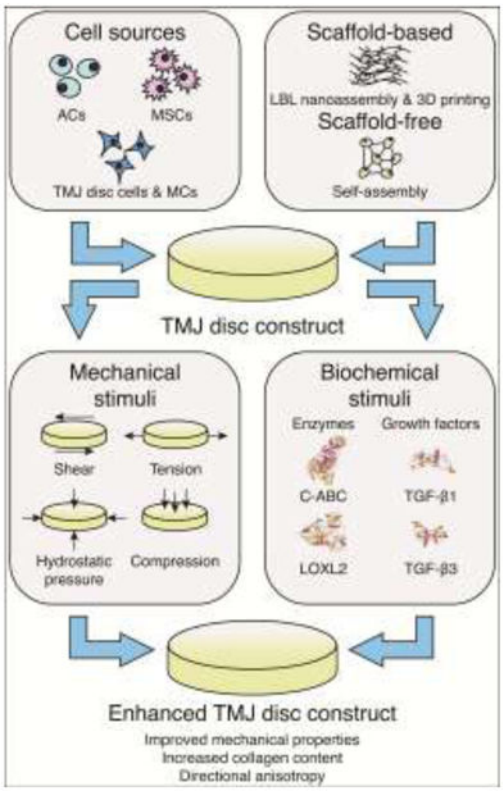


**Figure 3: Tissue-engineering paradigm of TMJ disc constructs.**

Combination of an appropriate cell source and scaffold-based or scaffold-free approaches can be used for fabrication of a TMJ disc construct (upper panels). Via the application of various biochemical and mechanical stimuli, an enhanced, biomimetic construct can be tissue-engineered (lower panels). ACs – hyaline articular chondrocytes, MSCs – mesenchymal stem cells, MCs – knee meniscus cells, LBL – layer-by-layer, 3D – three-dimensional, C-ABC – chondroitinase ABC, LOXL2 – lysyl oxidase-like 2, TGF-β – transforming growth factor beta.

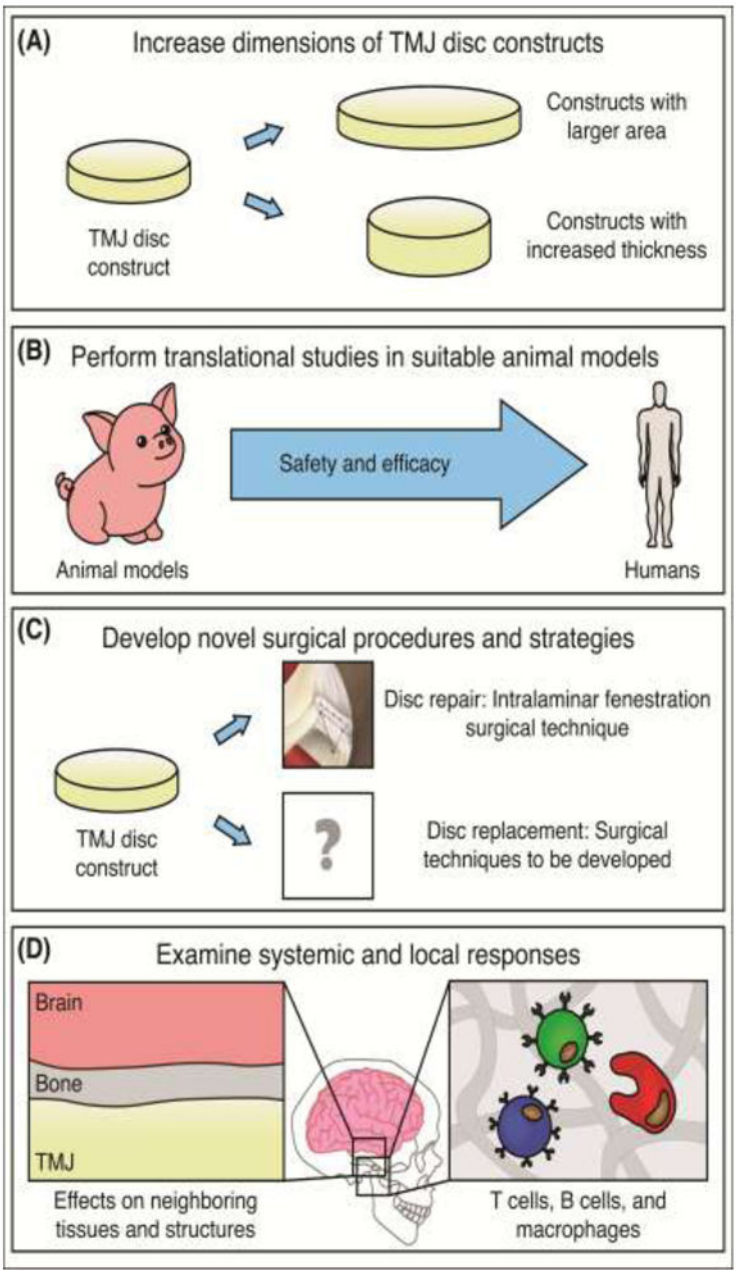


**Figure 4: Toward the path to translation.**

(A) Constructs should be tailored for human discal pathologies and size, potentially increasing in both area and thickness. (B) Prior to translation through regulatory bodies such as the FDA, animal studies must be performed in proper large animals, such as the minipig. (C) Novel surgical procedures for disc repair and disc replacement need to be developed as well. (D) Additional studies also need to be performed to examine local and systemic responses to tissue-engineered TMJ discs in the orthotopic environment. Upon overcoming these hurdles, the TMJ disc tissue-engineering field will be closer to human clinical trials.

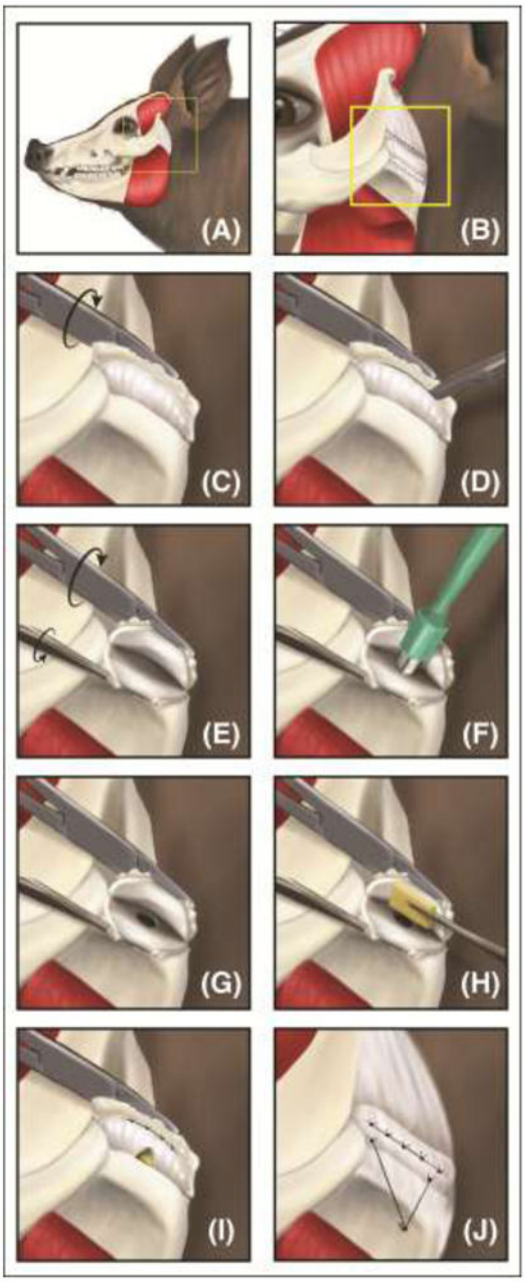


**Figure 5: The intralaminar fenestration surgical technique.**

(A-B) Through a preauricular incision, the TMJ was exposed. (C-E) Surgeons fileted the disc open with a scalpel, and (F-G) created a one-sided thinning defect via a biopsy punch. (H) A tissue-engineered disc was placed between the two laminae and (I) sutured back together. Sutures attached to the side of the disc instead of on the articulating surfaces allowed for continued loading of the TMJ disc while healing; this placement avoided possible stress concentrations and resulting degeneration. (J) The lateral attachment is recreated by use of an anchoring system. From Vapniarsky, *et al.*, 2018 [39]. Reprinted with permission from AAAS.

**Table 1:**

**Recent tissue-engineering studies of the TMJ disc published since 2013.**

Summary of the scaffold-based or scaffold-free approaches, cell sources, species, biochemical stimuli, mechanical stimuli, and implantation sites of the constructs are provided.

| Author, Year | Reference | Scaffold-based or Scaffold-free Approach | Cell Sources | Species of Cell Sources | Biochemical Stimuli | Mechanical Stimuli | Animal Model Tested (Implantation Site) |
|---|---|---|---|---|---|---|---|
| Vapniarsky, *et al.* (2018) | [39] | Self-assembling process | CCs expanded to passage 3 | Yucatan Minipig | TGF-β1, C-ABC, LOXL2 | Passive axial compression | Yucatan Minipig (Orthotopic) |
| Matsuka, *et al.* (2018) | [57] | Decellularized TMJ discs | Wharton's jelly-derived MSCs | Human | None | None | None |
| Bousnaki, *et al.* (2018) | [55] | Chitosan and alginate scaffolds | Dental pulp stem cells or human nucleus pulposus cells | Human | Unidentified * | None | None |
| Wang, *et al.* (2018) | [51] | Coculture cell sheet seeded on PLGA electrospun scaffolds | TMJ disc cells and synovium-derived MSCs | Rabbit | TGF-β3 | None | None |
| Ronald & Mills (2016) | [34] | Titanium dioxide nanofilms | TMJ disc cells | Cow | None | None | None |
| Tarafder, *et al.* (2016) | [36] | Polycaprolactone scaffolding with PLGA microspheres | Bone marrow-derived and synovium derived MSCs | Human/Rabbit | CTGF, TGF-β3 | None | Rabbit (Orthotopic) |
| Legemate, *et al.* (2016) | [35] | PCL scaffolding with PLGA microspheres | Bone marrow-derived MSCs | Human | CTGF, TGF-β3 | None | None |
| Juran, *et al.* (2015) | [56] | Decellularized TMJ discs with laser micropatterning | Wharton's jelly-derived MSCs | Pig | Epidermal growth factor, platelet-derived growth factor BB | None | None |
| Wu, *et al.* (2014) | [40] | Fibrin gel and chitosan scaffold | Synovium derived-MSCs | Rat | TGF-β3 | None | Nude Mice (Subcutaneous) |
| MacBarb, *et al.* (2013) | [38] | Self-assembling process | ACs and MCs | Cow | TGF-β1, C-ABC | Passive axial compression | None |
| Ahtiainen, *et al.* (2013) | [41] | Poly(lactic acid) scaffold | Subcutaneous adipose-derived MSCs | Rabbit | TGF-β1 | None | Rabbit (Orthotopic) |

* It is unclear what biochemical stimuli are in the chondrogenic medium used in the study by Bousnaki, *et al.* because it is a proprietary formulation.