Is tissue engineering of the TMJ disc a feasible process?

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Abstract

Temporomandibular joint (TMJ) disorders are common and difficult to remedy. Tissue engineering is one alternative that seeks to improve TMJ surgical treatment options. Tissue engineering aims to replace diseased or injured tissue with biologically engineered constructs. These constructs should reproduce native function and limit an immune response. To achieve tissue engineering success, it is important to first understand the tissue's cellular, biochemical, and mechanical properties in order to create validation and design criteria. Reviewed herein are the known properties of the TMJ disc and initial attempts toward TMJ disc tissue engineering. Important aspects of tissue engineering are scaffold selection, cell source, biochemical factors, and mechanical stimuli.

Motivation

The temporomandibular joint (TMJ), or jaw joint, is used throughout normal everyday functions such as eating or talking. Thus, disease or injury of this joint greatly decreases a patient's quality of life. Common activities become difficult and painful for patients with a TMJ disorder (TMD). The prevalence of TMJ dysfunction is surprisingly high; based on various epidemiological studies, 28-88% of the population exhibit some physical sign or symptom of a TMJ dysfunction (Solberg *et al.*, 1979).

Around one-fifth of patients exhibiting symptoms seek medical treatment for TMDs (Gray *et al.*, 1995). In the United States, there is an estimated 10 million TMD patients (TMJ Implants - A Consumer Information Update [FDA Report], 1999); around 70% of patients seeking treatment exhibit a displaced TMJ disc (Farrar and McCarty, 1979). Figure 1 illustrates the five stages of TMJ disc internal derangement as described by Wilkes (1989); the patient population from this study had an average age of 31 years and a female to male ratio of 7:1, common characteristics of the TMD patient population.

In addition to joint pain, TMD symptoms include headaches, earaches, jaw clicking, limited jaw opening, and jaw lock (Farrar and McCarty, 1979; LeResche, 1997; Solberg *et al.*, 1979). Unfortunately, TMD symptoms offer little aid in understanding the cause of TMDs. Numerous treatment options for TMD patients exist, but standard approaches and treatments are rarely agreed upon, even among experts. TMJ treatments and surgical approaches are presented in greater detail in reviews by Wong *et al.* (In Press) and Dimitroulis (2005). Briefly, non-surgical options are the first treatment modality and include pain medication and physical therapy. Minimally invasive surgery, like arthrocentesis or arthroplasty, may be attempted in dysfunctional joints with limited tissue degradation; these procedures aim to reduce inflammation or repair the disc/attachments. When the disc is beyond repair, it may be removed (discectomy). Post-discectomy the joint may be left empty or replaced with autologous tissue. Synthetic discs are no longer implanted due to extensive wear and immune response (Trumpy *et al.*, 1996). In the most extreme cases of degeneration, patients may opt for total joint replacement. Unfortunately, many TMDs are progressive, leading to extensive joint remodeling. Treatments primarily focus on the reduction of pain. This leaves the field of TMJ research primed for tissue engineering alternatives that have the potential to reduce pain and restore total function.

Disc characteristics

The TMJ disc is located between the mandibular condyle and fossa-eminence of the temporal bone (Figure 2). The joint is enclosed in a synovial capsule; the synovium serves to nourish and lubricate the joint (Piette, 1993). The TMJ is a ginglymo-diarthrodial

joint, meaning it exhibits both hinge-like and rotational motions. During normal movements, the disc translates anteriorly during jaw opening and posteriorly during closing. The presence of the disc's fibrous attachments is important to joint motions, but their exact mechanical function and location is heavily debated. The disc is believed to aid in joint lubrication as well as load distribution, jaw stabilization, and shock absorption.

The TMJ disc is divided into three regions: anterior band, posterior band, and intermediate zone (Figure 2). The posterior band is thicker than the anterior band; both bands are significantly thicker than the intermediate zone (Rees, 1954). The disc is generally divided into these three regions for characterization purposes. The bilaminar zone, a fourth element of the disc, exists between the posterior band and the posterior attachments, but generally, is not considered part of the disc. This region possesses some vasculature and is difficult to discern from the posterior attachment tissue.

While the disc is cartilaginous, it is very different from hyaline articular cartilage or even the knee meniscus (Almarza and Athanasiou, 2004a). A healthy TMJ disc is primarily avascular, although some vasculature can be found near the attachment regions. It is well hydrated, containing 70% water (Detamore *et al.*, 2006). Similar to the knee meniscus, the TMJ disc exhibits a mixed population of cell types. In the porcine disc, there are approximately 70% fibroblast-like cells and 30% chondrocyte-like cells (Detamore *et al.*, 2006). The percent of chondrocyte-like cells increases in the intermediate zone and decreases in the bands. This cell population is indicative of the disc's proper characterization as fibrocartilage.

The extracellular matrix (ECM) of the disc is essential to tissue function and important to thoroughly understand before attempting to engineer a construct. The TMJ disc is primarily collagen, and the collagen of the TMJ disc is nearly all collagen type I. Collagen type I makes up the majority of the disc's dry weight, approximately 85% (Nakano and Scott, 1989). Trace amounts of types II, III, VI, IX, and XII can be found in various animal models (Ali and Sharawy, 1996; Gage *et al.*, 1990; Gage *et al.*, 1995; Landesberg *et al.*, 1996; Milam *et al.*, 1991; Minarelli and Liberti, 1997). The fibers of the disc are primarily oriented circumferentially around the outer regions of the disc (Minarelli *et al.*, 1997). In the intermediate zone, fibers are more random but possess a primarily anteroposterior alignment. Collagen fibers in the porcine disc have an average diameter of 18 $\pm$ 9 μm with a range of 2.9 to 37.4 μm (Detamore *et al.*, 2005). Parallel to the collagen fibers are elastin fibers, which are found in all regions (Detamore *et al.*, 2005; Mills *et al.*, 1994; Minarelli and Liberti, 1997; O'Dell *et al.*, 1990).

Glycosaminoglycans (GAGs) and proteoglycans (PGs) are also important components of tissue ECM. The TMJ disc contains approximately 5% GAGs on a dry weight basis (Axelsson *et al.*, 1992; Detamore *et al.*, 2005; Nakano and Scott, 1989). Chondroitin sulfate is the most prevalent GAG in the disc, comprising 70-80% of the total GAG content (Detamore *et al.*, 2005; Kobayashi, 1992; Nakano and Scott, 1989). Aggrecan is an example of a chondroitin sulfate PG that is present in the disc and is important in hydration, lubrication, and compressive strength (Sindelar *et al.*, 2000). Dermatan sulfate is the next most abundant GAG in the disc, making up 15-25% of total GAG content (Kobayashi, 1992; Nakano and Scott, 1989; Nakano and Scott, 1996). Dermatan sulfate PGs include decorin and biglycan, which are important in controlling the collagen fiber lateral packing ability and diameter size (Sindelar *et al.*, 2000). Hyaluronic acid, which binds non-convanently to aggrecan, has been found in the range of 2.8-10% of the total GAG content (Axelsson *et al.*, 1992; Kobayashi, 1992; Nakano and Scott, 1989; Scott *et al.*, 1995). Heparan sulfate was found as 4.3% of total GAG content in the human disc (Axelsson *et al.*, 1992). Keratan sulfate GAGs are generally considered a trace component of the TMJ disc but have been measured up to 2% of the total GAGs (Detamore *et al.*, 2005; Kobayashi, 1992; Nakano and Scott, 1989; Nakano and Scott, 1996).

Mechanical properties of the TMJ disc are important to understand since engineered constructs must support the necessary load imparted on the native tissue. The tensile elastic modulus of the porcine TMJ disc is higher in the anteroposterior direction than the mediolateral direction at 76.4 MPa and 3.2 MPa, respectively (Beatty *et al.*, 2001). In the mediolateral direction, Detamore and Athanasiou (2003) found significant differences between the posterior band, anterior band, and intermediate zone with relaxation moduli of 23.4 MPa, 9.5 MPa, and 0.58 MPa, respectively. In the anteroposterior direction, the stiffest region was the central section followed by the medial section and then lateral section (Detamore and Athanasiou, 2003; Tanne *et al.*, 1991).

Several methods have proved useful in modeling the compressive properties of the TMJ disc. An elastic, compressive modulus for human discs was observed in the range of 211 kPa to 514 kPa, dependent on the strain rate (Chin *et al.*, 1996). The biphasic theory has been employed frequently since its conception to illustrate a tissue's viscoelastic characteristics (Mow *et al.*, 1980). Biphasic modeling of the porcine TMJ disc yielded properties of 20.1 kPa for the aggregate modulus, 0.45 for the Poisson's ratio, and 24.1×10^{-15} m^4/Ns for the permeability (Kim *et al.*, 2003). Most recently, unconfined compression, stress relaxation

tests were performed to give the surface-regional instantaneous and relaxation moduli of the porcine disc. These values were found to be strain dependent, ranging from 90-3870 kPa (instantaneous modulus) and from 16.9-74.6 kPa (relaxation modulus) for 10%-30% strain, respectively. The coefficient of viscosity was also strain dependent, ranging from 1.3-13.8 MPa*s (Allen and Athanasiou, 2005a).

Shear properties of the TMJ disc have recently received due attention. Tanaka *et al.* (2004a) found a storage modulus between 0.78-2.0 MPa depending on the compressive strain and percent shear. A loss modulus near 0.4 MPa and loss tangent ranging from 0.2-0.25 MPa was observed.

Tissue engineering

Tissue engineering is a potential option for the future treatment of diseased or injured discs. The general approach to tissue engineering involves selection of a cell source, seeding these cells on an appropriate scaffold, and applying external stimuli to encourage ECM production and organization. These external stimuli may be grouped into two general categories: biochemical and mechanical. Tissue engineering approaches may commence *ex vivo* or *in vivo* and may exclude one or more of the aforementioned factors (cells, scaffold, and stimuli). For example, skin therapies have been successful using acellular collagen scaffolds. However, all tissue engineering therapies aim to replace the native tissue characteristics through tissue remodeling or regeneration. TMJ tissue engineering has focused on the combination of scaffolds, cells, and stimuli *in vitro* as illustrated in figure 3.

Scaffolds

Scaffolds, an important part of a construct's initial mechanical integrity, provide surface area for cell attachment. The earliest tissue engineering study used a porous collagen scaffold; after two weeks the construct appeared similar to the disc in gross morphology and cell shape (Thomas *et al.*, 1991). Later, researchers attempting to create a replacement for the TMJ disc used fibers of polyglycolic acid (PGA) and polylactid acid (PLA) and concluded that both scaffold materials were able to support cell attachment, matrix production, and retain testable mechanical properties after 12 weeks (Puelacher *et al.*, 1994). Another study compared PGA, polyamide filaments, expanded polytetrafluoroethylene (ePTFE) filaments, and bone blocks (Springer *et al.*, 2001). While all these scaffolds supported cell attachment and a small amount collagen production, they were unable to form neotissue after 4 or 8 weeks. Tissue engineering studies in our lab have primarily used PGA non-woven meshes (Almarza and Athanasiou, 2005; Almarza and Athanasiou, 2006; Bean *et al.*, Accepted November 2005; Detamore and Athanasiou, 2004; Detamore and Athanasiou, 2005b). While PGA supports cell attachment and matrix production, it degrades very rapidly, leaving constructs with limited mechanical integrity after only a few weeks. PLA non-woven mesh, however, has shown promise in retaining tensile and compressive integrity over a similar time scale (Allen and Athanasiou, 2005b).

Some researchers have investigated novel materials for TMJ disc engineering that would allow custom-shaped scaffolds to be implanted through minimally invasive surgery (Poshusta and Anseth, 2001). Acrylated collagen type I scaffolds were successfully photopolymerized through a layer of rat skin; in this study, viability of osteoblasts in a photopolymerized poly(ethylene oxide) dimethacrylate was demonstrated, suggesting this process could be accomplished with other cell types. However, corresponding data for TMJ disc cells encapsulated in alginate showed a drastic decrease is cell numbers at 4 and 8 weeks of culture with no ECM production at any time point, suggesting TMJ disc cells may not survive an encapsulated environment (Almarza and Athanasiou, 2004b).

Cell source

The cell source for a tissue engineering study is tremendously important, but limited research has been conducted in TMJ disc engineering studies. The most commonly used cells for these experiments are derived from the TMJ disc (Almarza and Athanasiou, 2004b; Almarza and Athanasiou, 2005; Almarza and Athanasiou, 2006; Almarza and Athanasiou, Accepted August 2005; Bean *et al.*, Accepted November 2005; Detamore and Athanasiou, 2004; Detamore and Athanasiou, 2005a; Springer *et al.*, 2001; Thomas *et al.*, 1991) or articular cartilage (Girdler, 1998; Puelacher *et al.*, 1994; Springer *et al.*, 2001). A major hurdle to overcome in tissue engineering is that tissue engineering generally requires a large cell population to create a construct. While passaged cells may seem appealing, chondrocytes have been found to de-differentiate to a more fibroblastic phenotype after only a couple of passages (Darling and Athanasiou, 2005). Additionally, TMJ disc cells showed a decreased expression for ECM proteins with the exception of decorin and biglycan due to passage (Figure 4) (Allen and Athanasiou, Submitted 2006). Thus, for the future of TMJ disc engineering, a cell source that can yield a large population of TMJ disc cells, or a population of cells that rapidly fill a scaffold, must be identified.

As mentioned previously, after a discectomy, surgeons may replace the disc with some type of autologous tissue, such as skin, auricular cartilage, dura mater, temporalis muscle, or temporalis fascia (Puelacher *et al.*, 1994). Any of these tissues may serve as potential cell sources for the TMJ disc, but one of the most appealing in terms of clinical feasibility and patient comfort is dermis. Adult dermal fibroblasts have been

shown to produce matrix indicative of a chondrocytic phenotype when seeded on aggrecan-coated plates (Figure 4) (French *et al.*, 2004).

Biochemical factors

Growth factors are commonly used in tissue engineering studies. Four studies have demonstrated the potential of growth factors for TMJ disc tissue engineering. This potential was first observed using transforming growth factor-β_1 (TGF-β_1) and prostaglandin E_2 (PGE_2) on bovine TMJ disc cells in monolayer. TGF-β_1 increased cell proliferation by 250%, while PGE_2 had no significant effect (Landesberg *et al.*, 1996). Also in monolayer, the effects of platelet derived growth factor (PDGF), insulin like growth factor (IGF) and basic fibroblast growth factor (bFGF) on porcine TMJ disc cells demonstrated that lower concentrations of these growth factors favored biosynthesis, while higher concentrations favored proliferation (Detamore and Athanasiou, 2004). The most beneficial growth factors were IGF-I and bFGF, which both showed significant increases in collagen synthesis and cell proliferation. The effects of IGF-I, bFGF and TGF-β_1 on porcine TMJ disc cells in PGA scaffolds showed increased collagen production when exposed to low concentrations of IGF-I and TGF-β_1 (Detamore and Athanasiou, 2005b), but no other significant differences between the experimental groups existed. In the end, IGF-I was recommended for future tissue engineering studies due to low cost and beneficial collagen production. Of course, the native tissue is exposed to a variety of growth factors; so, it is possible growth factor combinations will be more beneficial than any single factor. IGF-I, bFGF, and TGF-β_1 have been investigated in combinations of two to determine if synergistic effects exist (Almarza and Athanasiou, 2006). All constructs exposed to growth factor combinations improved in structural integrity compared to a no growth factor control, but no combination was statistically significant in terms of biochemical or mechanical properties. While synergistic effects were not observed, improved overall cellularity of the constructs was noted when both growth factors were used at a high concentration.

Although growth factors have received the most attention, positive biochemical stimulation is also likely to come from culture conditions and cellular interactions as well. An ascorbic acid concentration of 25 μg/mL has been shown to produce constructs with higher total collagen content and higher aggregate modulus relative to concentrations of 0 μg/mL or 50 μg/mL (Bean *et al.*, Accepted November 2005). This was likely associated with improved seeding observed for the constructs cultured in 25 μg/mL of ascorbic acid. Initial cell seeding is another important consideration in any tissue engineering construct due to cell-to-cell interactions and signaling. Almarza and Athanasiou (2005) showed that PGA scaffolds seeded at saturation increased cellularity and ECM content relative to scaffolds seeded below saturation.

Mechanical stimulation

The native TMJ disc undergoes significant loading, which is often broken down into compression, tension, and shear components (Tanaka *et al.*, 2003). While cells proliferate and produce ECM in static culture, mechanical stimuli may be required to produce an optimal tissue engineered construct. A variety of mechanical stimuli may be beneficial including compression, tension, hydrostatic pressure, and fluid shear stress. Darling and Athanasiou (2003) have published an extensive review of the mechanical bioreactors that have been used in engineering cartilaginous tissues.

Three recent studies have investigated the effects of mechanical stimulation on TMJ disc constructs. A low-shear fluid environment by means of a rotating wall bioreactor created constructs with dense matrix and cell composition (Detamore and Athanasiou, 2005a); however, when the biochemical content of these constructs was compared to those grown in static culture, no clear benefit of the bioreactor was observed. When disc cells were exposed to hydrostatic pressure in monolayer or PGA scaffolds, constant hydrostatic pressure at 10 MPa increased collagen production compared to static culture (Almarza and Athanasiou, Accepted August 2005). In contrast, intermittent hydrostatic pressure from 0 to 10 MPa at 1 Hz frequency was detrimental to the constructs, producing less collagen and GAGs than unloaded controls. These results were consistent in both two and three-dimensional culture. In another recent study, dynamic tensile strain significantly reduced interleukin-1 β induced up regulation of matrix metalloproteinase (Deschner *et al.*, 2005). This may have implications on future tissue engineering studies since MMPs play an important role in ECM degradation and remodeling.

Future directions for TMJ disc tissue engineering

While TMJ disc tissue engineering is in its infancy, other musculoskeletal tissues have been studied to a greater extent. These tissues include articular cartilage, bone, and tendon. TMJ disc tissue engineering should build on not only past TMJ research but also successes in these other tissues, while keeping in mind the disc's structural and functional differences.

The issue of scaffold certainly requires further investigation. Scaffolds that degrade too quickly are unable to provide the necessary mechanical integrity; thus, future research may focus on polymers with longer degradation times or that encourage rapid ECM production. Alternatively, using natural polymers like

collagen may be effective since cells would simply remodel existing matrix instead of forming a new collagen network, thereby decreasing the time until the scaffold reaches a functional state. A third option is a scaffoldless or self-assembling process. Such approaches have been examined in both tendon and articular cartilage (Calve *et al.*, 2004; Hu and Athanasiou, March 2006). While these methods require refinement to increase mechanical strength, data suggest these approaches may offer a new direction in soft tissue engineering. Futhermore, by eliminating the scaffold material within an engineering construct, concerns over mechanical integrity and cell toxicity due to the scaffold degradation process are diminished.

An optimal cell source is necessary for tissue engineering to be realized. To date, no such source has been identified that is likely to be clinically sound. However, research in other musculoskeletal tissues like cartilage, tendon, and bone has explored the possibility of using mesenchymal stem cells for tissue engineering (Altman *et al.*, 2002; Awad *et al.*, 1999; Funakoshi *et al.*, 2005; Hankemeier *et al.*, 2005; Juncosa-Melvin *et al.*, 2005; Li *et al.*, 2005; Mao and Nah, 2004; Mauck *et al.*, 2005; Moreau *et al.*, 2005; Tanaka *et al.*, 2004b; Wang *et al.*, 2005; Wayne *et al.*, 2005). Using progenitor cells may also be desirable for the TMJ disc, since bone marrow or adipose tissue could potentially yield a large population of autologous, pluripotent cells. Alternatively, research on other potential cell sources, such as embryonic stem cells and dermis-derived fibroblasts, continues to demonstrate promise.

The inclusion of biochemical signaling will be an integral part of producing a TMJ disc tissue engineering construct. Significant work has been performed in both two- and three-dimensional cultures to determine optimal growth factor signaling for TMJ disc engineering. Recent work showed the growth factors IGF-I and TGF-β_1 used alone produced increases in collagen production (Detamore and Athanasiou, 2005b). This provides a basis for growth factor selection in future TMJ disc tissue engineering studies. Beyond growth factors, the media used for culturing should also be further investigated. Ascorbic acid concentration has influenced the outcome of engineered constructs (Bean *et al.*, Accepted November 2005); thus, other media supplements may need further optimization as well. Cell-to-cell interactions are important, and seeding the cells in scaffolds at saturation was shown to produce constructs with significant increases in ECM production (Almarza and Athanasiou, 2005). This is clearly vital for fabrication of an optimal TMJ disc construct.

Cartilage is a mechanical tissue; thus, mechanical stimulation should be expected for regeneration of any cartilaginous tissue. The most successful mechanical stimulation used to date for the TMJ disc has been constant hydrostatic pressure (Almarza and Athanasiou, Accepted August 2005). Hydrostatic pressure should certainly be pursued further, because there are likely to be other beneficial loading regiments. Tension has shown promise in monolayer culture and should be pursued for future three-dimensional tissue engineering studies (Deschner *et al.*, 2005). Success in engineering the knee meniscus has been seen using direct compression; these results may apply to the TMJ disc due to the fibrocartilaginous nature of both tissues (Aufderheide and Athanasiou, Submitted 2005). Additionally, perfusion increased cellularity and ECM production in articular chondrocytes and may hold the same potential for the TMJ disc (Davisson *et al.*, 2002). Perfusion may also create larger constructs due to increased nutrient circulation.

In conclusion, while the field of TMJ disc engineering remains young, significant progress has been achieved. With this progress have come new, challenging questions and a wealth of knowledge on the disc's characteristics. Related research may begin to merge with TMJ disc engineering due to the increased knowledge of TMJ disc design criteria. Tissue engineered TMJ constructs may now be validated with the increased fund of information on the tissue's native characteristics. With these tools at hand, TMJ research will continue to rapidly progress to, hopefully, a viable tissue engineering implant.

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Figures

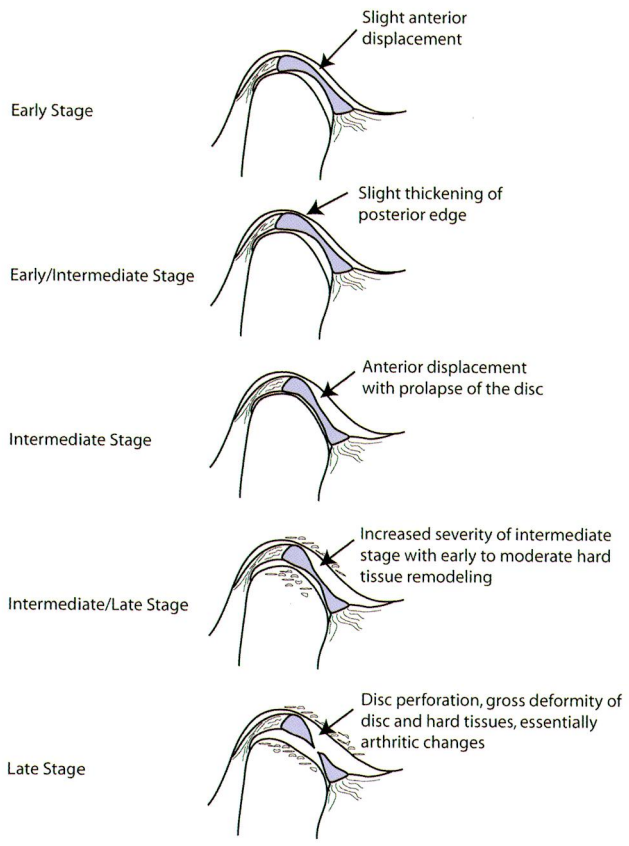

Figure 1: The stages of TMJ internal derangement as described by Wilkes (Wilkes, 1989).
Schematics describe the progression of TMJ internal derangement; these schematics were created based upon radiologic findings described by Wilkes (Wilkes, 1989). In early stages, clinical symptoms are limited (no significant pain or mechanical symptoms); however, a slight anterior displacement of the disc can be observed. As the derangement progresses towards the intermediate stage, a few episodes of pain along with occasional joint tenderness, headaches, and mechanical problems are reported. Here, the disc displacement is slightly more forwards and the posterior edge thickens. At the intermediate stage, pain intensifies along with other clinical symptoms; anterior displacement of the disc is significant and coupled with disc prolapse. As the disorder progresses toward late stages, chronic pain develops; disc displacements are severe and hard tissue remodeling ensues. In late stages, joint scraping and difficulty in function are evident. The disc may be out of position, degenerated, or perforated. Hard tissue remodeling is severe; the joint is essentially arthritic.

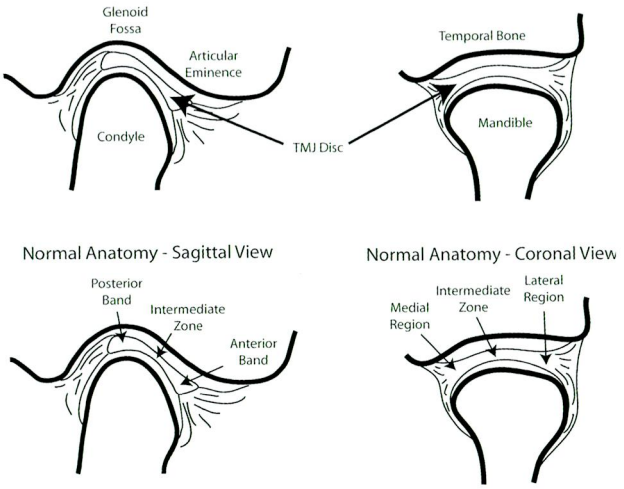

Figure 2: Joint anatomy and disc regions
The TMJ disc is located between the mandibular condyle and fossa-eminence of the temporal bone. The disc is fibrocartilaginous and has a biconcave shape in both sagittal and coronal views. Thickness variations are evident in the sagittal view, where the thick posterior and anterior bands differ significantly from the intermediate zone. In the coronal view, thickness variations are less pronounced; however, the medial and lateral extents of the disc are slightly thicker than the intermediate zone.

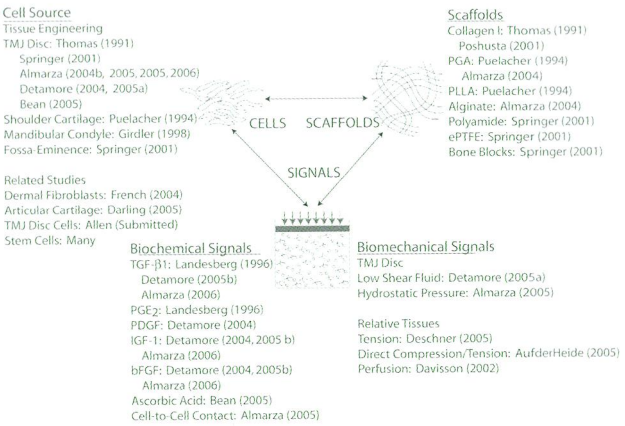

Figure 3: A tissue engineering paradigm: history of TMJ disc engineering
Tissue engineering, generally, is conducted by combining cells and signals on an appropriate scaffolding material. This approach has been the standard thus far in TMJ disc engineering. References to significant studies of scaffolding, signals, and cell source for the TMJ disc are placed within the classic paradigm figure. Clearly, TMJ disc engineering is very young; however, it is apparent that the field is rapidly expanding.

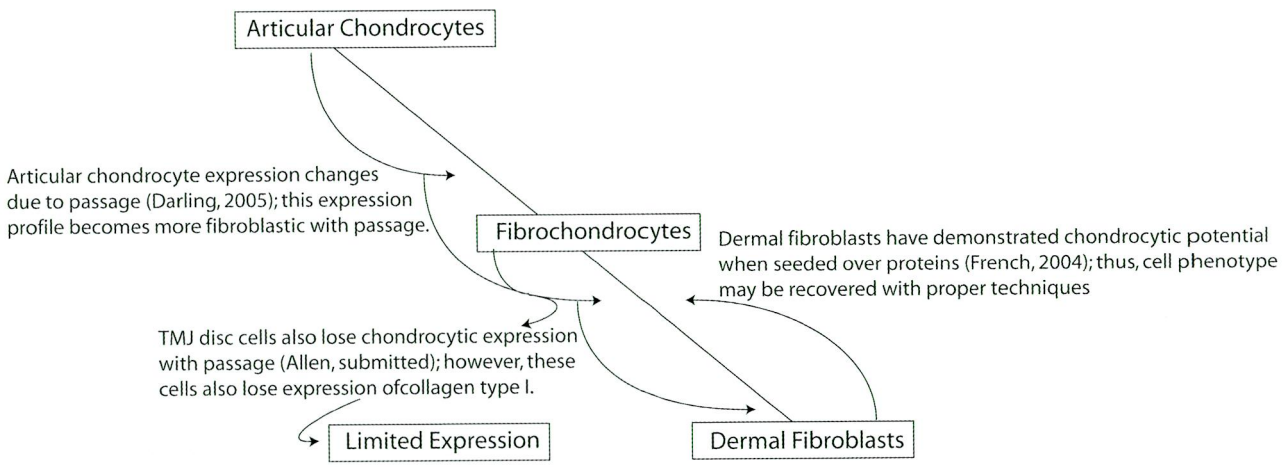

Figure 4: From chondrocyte to fibroblast
In our laboratory, we have investigated the relationship between chondrocytes, fibrochondrocytes, and dermal fibroblasts. First, chondrocytes progressively dedifferentiate as a function of monolayer culture (Darling and Athanasiou, 2005). As these cells are passaged, they become more fibroblastic in nature, characterized by a loss of chondrocytic ECM gene expression and a gain in fibroblastic expression. Fibrochondrocytes follow a similar loss in gene expression; however, fibroblastic gene expression is also lost as a function of passage (Allen and Athanasiou, Submitted 2006). However, it may be possible to regain these losses by seeding passaged cells over proteins; dermal fibroblasts have demonstrated a chondrocytic response when seeded over specific extracellular matrix proteins (French *et al.*, 2004).