

The Prospect of Stem Cell-Based Regeneration of the TMJ Disc: Where Have We Gone So Far?

SUMMARY

Conservative and surgical methods utilized so far to treat temporomandibular disorders have demonstrated inconsistent results. Tissue engineering is a new, rapidly expanding, multi-disciplinary field aiming to generate tissue and organ substitutes in vitro. Stem cells have already shown the potential to form multiple tissue types. Thus, their ability could be incorporated in contemporary paradigms to reconstruct the temporomandibular joint disc, though such attempts are rare. This review points out the unique histological and biomechanical character of the disc, trying to delineate the basic parameters that need to be considered in this process. In fact, the histological character of the disc cannot be defined as hyaline cartilage but fibro-cartilage. Particularly, the type I fibrillar collagen prevalence and relatively low glucosamino-glycan content, along with their specific topographic arrangement and distribution, correspond to the cellular content and correlate to the biomechanical characteristics of the disc, which mirror its functional role. Here, the various biomaterials, biochemical and biomechanical stimuli utilized for the reconstruction of hyaline cartilage are explored, since the experience and knowledge from these research fronts offer a useful guideline for the reconstruction of the temporomandibular joint disc. The cross-talk between these advancing scientific fields, together with a deeper comprehension of the physiology and pathophysiology of the temporomandibular complex can act as a catalyst, elevating the prospects of the disc's regeneration to new heights.

Keywords: Tissue Engineering; Temporomandibular Joint (TMJ); Temporomandibular Joint Disc; Mesenchymal Stem Cells; Cartilage

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Introduction

The temporomandibular joint (TMJ), a diarthrodial, synovial joint, is the only joint contained in the craniofacial region. Its importance is highlighted by its involvement in the processes of mastication, ingestion and speech. The TMJ is basically unique in the sense that it is composed by 2 separate joints, located on either sides of the mandible, whose coordinated function is necessary to perform the opening and closure of the jaw. The joint of either side consists of the articulating surfaces of the temporomandibular (or glenoid) fossa and the head of the mandibular condyle, as well as an intervening articular disc. The disc together with the

synovial membrane serves to lubricate and enhance the congruity of the articular surfaces, stabilize the joint and absorb shock. The articular surfaces of the TMJ and the articular disc normally experience dynamic loading during physiological functions, such as chewing and speaking. Parafunctional habits, such as clenching, grinding or bruxism, apply static loading to the joint and are considered as causal factors of a number of disorders. Cartilage has a low cellular content and is in short supply of progenitor cells from the blood or the bone marrow since it lacks vasculature. As a result, it has a low self-repair capacity which hinders therapeutic approaches. Temporomandibular disorders (TMDs) are manifested with cervical pain, headaches, impaired movement,

clicking and popping of the jaw, all of which eventually interfere with the physiological function of the TMJ and negatively affect the quality of life.

The etiology and clinical management of TMDs are subjects of considerable controversy¹. This broad category of diseases involves both TMJ and related musculoskeletal structures of the head and neck^{2,3}. Epidemiological data show that TMDs affect roughly a quarter of the general population and 70% of them suffer from TMJ dysfunction⁴⁻⁶. Abnormalities of the TMJ may develop as a part of systemic disease as well. Osteoarthritis mainly affects older people and involves the erosion of the articulating surfaces of joints. It is mainly caused by excessive stress on joints over time, leading to the gradual degeneration of the cartilage matrix, while genetic disorders of the molecular components of the matrix may also predispose individuals to osteoarthritis. Rheumatoid arthritis is another systemic disease that attacks joints including the TMJ. Its cause remains unclear, though autoimmune, bacterial antigen and genetic mechanisms have been proposed⁷⁻⁹. At a local level, several causal factors such as occlusal dysfunction or stress related parafunctional habits have been explored and found to play a key role in the pathophysiology of TMDs. These conditions, in combination with genetic predispositions, hormonal and inflammatory mediators, may participate in the rise and development of TMDs.

Nearly 3-4% of the population seeks treatment for TMDs and interestingly, significantly more women are treated for TMDs than men, with a female to male patient prevalence reported between 3:1 to 8:1^{4,10,11}. Conditions requiring treatment include internal joint derangement, ankylosis, arthritic conditions, anterior disc displacement, traumatic injuries and tumours. With this vast array of abnormalities and related symptoms, dental practitioners and surgeons have encountered a great dilemma over treatment selection.

Treatment methods range from conservative, such as physical therapy (including self-care exercises¹² and splints¹³) and medication, to various surgical procedures. The latter are nowadays considered as last options when non-surgical approaches fail to give satisfactory results¹⁴. Discectomy was initially performed to treat all kinds of disc derangement, regardless of their causality and severity¹⁵. Today, this procedure is used only in severe cases of TMDs to remove a damaged or dislocated disc and may involve the use of a disc replacement. Alternatively, as was first described by Farrar and McCarty⁵ in 1979, a surgical procedure called discoplasty is utilized for the repositioning of the disc, in a less aggressive approach. When removal of the disc does not succeed in relief of pain and recovery of functional joint mobility, the need of its replacement arises. This drove to the development of disc analogues from alloplastic materials, such as silicone rubber¹⁶ and Proplast/Teflon¹⁷. Though early reports showed promising

results¹⁸, both implant systems were found to undergo fragmentation gradually, due to their vulnerability to the repeated mechanical stresses and ultimately cause a foreign body reaction; this fact led to their withdrawal from the market¹⁹. Because of the disastrous results from the use of alloplastic materials, autogenous tissues, such as dermis²⁰ and the temporalis muscle²¹ were used to replace the disc but showed inconsistent results. In an approach for the reconstruction of the total TMJ complex, 3 implant systems, approved by the FDA, are currently commercially available (Christensen TMJ Implant, W. Lorenz/Biomet TMJ Implant, Techmedica/TMJ Concepts TMJ Implant).

Given the marginal success displayed by the methods used so far for the treatment of TMDs, tissue engineering (TE) arises as the perfect candidate for the regeneration and replacement of a tissue that, as mentioned above, has limited ability for intrinsic repair. Tissue engineering can be defined as an interdisciplinary field that applies principles of engineering and life sciences (trying to understand the mechanisms of tissue growth) toward the development of biological substitutes that restore, maintain or improve functions of tissues or whole organs²². In early attempts, transplantation of *in vitro* expanded autologous chondrocytes into defect sites underneath membranes (ACT), was used to regenerate cartilage in major joints, such as the knee joint giving promising functional results²³⁻²⁵. The isolation of mesenchymal stem cells (MSCs) from multiple tissue sources²⁶⁻³⁰ and identification of their ability to differentiate into multiple mesodermal cell lines³¹⁻³³ and form tissues of mesenchymal origin, combined with recent advances in biotechnology led to the evolution of TE approaches and raised therapeutic prospects (Fig. 1). Briefly, the field of TE comprises of 3 basic axes: the adequate cells, a biocompatible scaffold and the delivery of specific bioactive molecules. Contemporary concepts involve the use of cell lines capable to generate tissues with functional, biochemical and biomechanical properties similar to those of native tissues. In order to achieve such results, these cells must first be cultivated and conditioned with specific biological differentiating stimuli. As such, growth factors (GF) can not only induce differentiation of multipotent cells, but can also promote their proliferation and anabolic activity. The use of biomaterial scaffolds of synthetic or natural origin provides a hospitable environment for cellular attachment, proliferation and growth. Through cellular synthetic activity and concurrent scaffold degradation, the latter is eventually replaced by the newly formed tissue. The *in vitro* maturation process of the compound takes place in bioreactors, devices that provide the necessary biochemical and biomechanical micro-environmental features in a controlled manner. Alternatively and/or additionally, a period of *in vivo* maturation may be required. Scaffolds provide the means to deliver the cells to the site of the defect and ensure

after transplantation that they stay in place long enough to achieve the desired repair. Furthermore, incorporated bioactive molecules trigger the chemotactic migration of host cells in a means to enhance *in vivo* maturation and integration of the construct.

In this review, an attempt is made to present the state of knowledge in the rapidly advancing field of TE on cartilage reconstruction. This presentation will focus on the prospect of reconstructing the TMJ disc, highlighting

its unique histological and biomechanical characteristics. Taking into consideration the fact that articular cartilage and the TMJ disc share several functional and histological similarities and given the scarcity of attempts to reconstruct the TMJ disc³⁴⁻³⁶, a brief presentation of cartilage engineering research is going to follow. Knowledge of the anatomical and histological features of the TMJ gives the theoretical background for understanding these efforts.



Figure 1. Title: Stem cell-based tissue engineering

Anatomy and Histology of the TMJ

The TMJ is a diarthrodial synovial joint composed of the condylar process of the mandible on one side, the glenoid fossa and articular eminence of the temporal bone on the other and an articular disc located in

between (Fig. 2). Both the surfaces of the condyle and glenoid fossa are covered with fibro-cartilage. The glenoid fossa is an oval depression in the temporal bone's lower surface, anteriorly to acoustic meatus. The condyle is situated over the ascending mandibular ramus. A capsule encloses the joint and consists of

2 layers: an outer fibrous one and an inner synovial one. The latter contains the disc which together with its surrounding attachments divides the joint into superior and inferior joint spaces. Both cavities contain approximately one millilitre of synovial fluid each. The synovial membrane-disc complex serves to absorb shock, lubricate and improve the affinity of the joint surfaces. As the articular disc has little vasculature, the transfer of nutrients, oxygen and waste products occurs through the synovial fluid. The disc is biconcave in shape, with a thinner centre and a thicker periphery; it is subdivided into the anterior, intermediate and the posterior zones and additionally into lateral, central and medial zones. Dislocation of the disc is prevented by

its peripheral attachments: posterior to the retrodiscal tissue and the condyle, medially and laterally to the disc capsule and the medial and lateral poles of the condyle *via* triangular zones of connective tissue and anteriorly to the eminence, the head of the condyle and through a fibrous insertion to the lateral pterygoid muscle. The latter facilitates the disc's movement forwards, following the rotation of the condyle during jaw opening, while the repositioning of the disc is attributed to the elastic recoil of the superior lamella of the retrodiscal tissue pad. The masseter and temporalis muscles also have fibrous connections to the disc. The capsule and to a lesser degree the disc are innervated by small unmyelinated nerve fibres of the trigeminal ganglion³⁷.

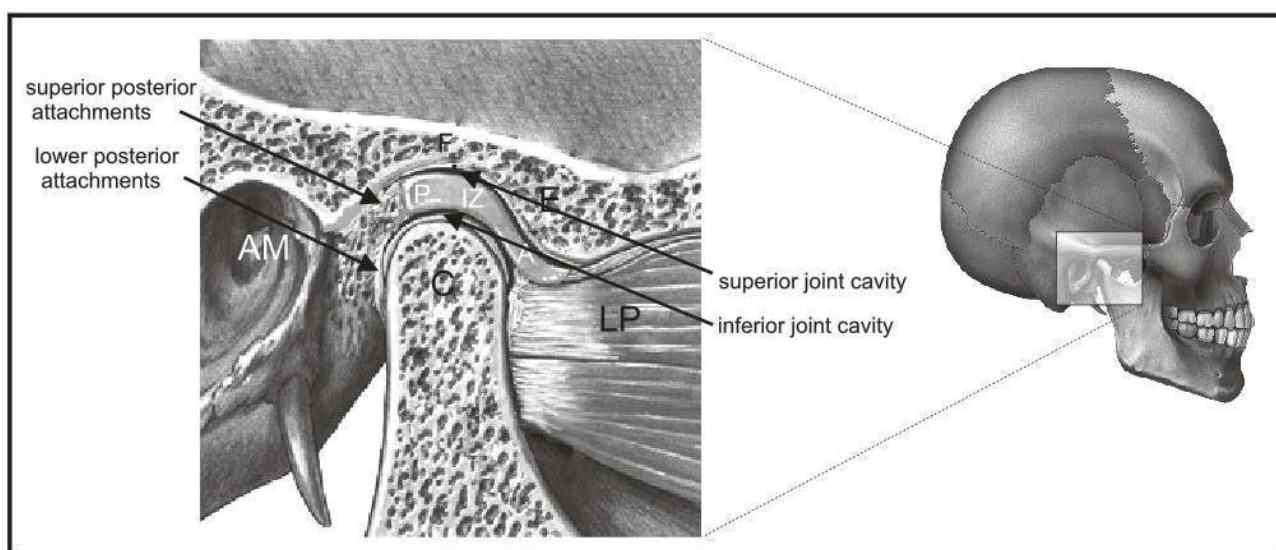


Figure 2. Graphic representation of the TMJ complex: Temporomandibular joint disc, located between the glenoid fossa [F], the anterior eminence [E], the mandibular condyle [C], is subdivided into posterior [P], intermediate [IZ] and anterior [A] zones. Lateral pterygoid muscle [LP], acoustic meatus [AM]

From a histological point, the TMJ condylar cartilage is different from other joints. There are 3 types of cartilage (hyaline, elastic and fibro-cartilage) present in adults, depending on function and location. The mandibular condyle's articular cartilage is fibroelastic³⁸, having characteristics similar to both hyaline and fibro-cartilage, mirroring the functional needs of the jaw movement. It consists mostly of type I fibrillar collagen unlike hyaline cartilage, which is made out of type II collagen³⁹. Specifically, the cartilage of the condyle displays 4 distinct layers:

The superficial or fibrous articular zone, forming the articular surface, is composed of dense fibrous tissue. On the contrary, most joint surfaces consist of hyaline cartilage. Most fibres are collagenous and are arranged parallel to the surface, running anteroposteriorly through the centre of the condyle and circumferentially around the periphery. Elastin fibres exist as well but in a low

extent. Chondrocytes are scarce in this region and appear flattened and elongated;

The cell-rich or prechondroblastic layer is the site where proliferation occurs, providing a source of cells to adjacent layers. This layer has been reported to thin down or even disappear with age;

The chondroblastic or fibrocartilagenous zone. Here, the round chondrocyte-like cells are irregularly distributed unlike in growth plates of axial bones. This arrangement allows growth and remodelling of cartilage in all three dimensions⁴⁰;

The endochondral ossification or hypertrophic zone, where hypertrophic chondrocytes, calcified cartilage blood and lymph vessels are observed.

The synovial membrane lining the inner surface of the fibrous joint capsule is composed entirely of loose connective tissue rich in type V collagen. Deep stromal cells and 2 types of lining cells: macrophage-like cells

(type A cells) and fibroblast-like cells (type B cells) populate the membrane^{41,42}. Type A cells are arranged so, as for their apex to protrude into the synovial space and therefore seem to participate in the formation of the viscous synovial fluid and removal of waste degradation products. Efficient lubrication is considered a necessity for proper joint function and is attributed to surface-active phospholipids and hyaluronic acid⁴³⁻⁴⁵. Surface-active phospholipids cover the articulating surfaces while hyaluronic acid forms a mucus film separating them to prevent friction^{43,44}. Phospholipids consist mainly of phosphatidylcholine and are subject to hydrolysis by phospholipase A2. Adherence of hyaluronic acid to phospholipid membranes (liposomes) was seen *in vitro* and protected those membranes from phospholipase A2 activity. This observation suggests that *in situ* hyaluronic acid probably plays a key role by adhering to the surface-active phospholipids and preventing their uncontrolled degradation from phospholipase A2⁴⁵.

The articular disc of the TMJ is a dense fibrous tissue and its histology has not been investigated thoroughly as for its cell distribution and extracellular matrix composition in humans⁴⁶. Several animal models have been used instead, including mice^{37,47}, rabbits^{48,49}, bovines⁵⁰ and porcine⁵¹. The porcine model is suggested to be the most suitable animal model for biomechanics research and tissue engineering studies. A study by Detamore et al⁵² on porcine TMJ discs investigated the cell types and distribution. Cells were classified either as fibroblasts (spindle-shaped with no discernable cell boundary) or chondrocyte-like (polygonal, in lacunae, therefore described as fibro-chondrocytes). Predominant in total cell count were fibroblasts (70% \pm 11%, mean \pm SD). Cells were unevenly distributed through the anteroposterior and mediolateral axes. The anterior and posterior bands contained fewer cells than the intermediate zone, which in turn had fewer cells than the lateral and medial regions. This contradicts previous studies on rabbits⁵³ and primates⁵⁴ reporting that cells were more numerous in or near the anterior and posterior bands compared to the intermediate zone. The cell type regional distribution varied significantly; fibroblasts were predominant in the anterior and posterior bands. Fibro-chondrocytes showed higher relative numbers in the centre and through the superoinferior axis in the inferior layer of the disc. Regarding the ultra-structure of the cells, Detamore's group reported that fibroblasts appeared to have large nuclei and few organelles, poorly developed smooth and rough endoplasmic reticulum (ER) and rarely observed Golgi apparatus and mitochondria. Chondroblast-like cells also had relatively large nuclei, small amounts of rough ER and Golgi apparatus, but often displayed several mitochondria, a sign of high metabolic activity.

The extracellular matrix (ECM) of the disc consists of tissue fluid (65-85%) and macromolecules which

mainly refer to collagen (85-90%) and proteoglycans (10-15%)^{55,56}. Smooth muscle has also been detected, mainly in the anterior section of the disc, implying the presence of vasculature⁵². The primary glucosaminoglycan (GAG) was Chondroitin Sulphate followed by Dermatan sulphate. The high content of both GAGs (as components of the large proteoglycan, aggrecan) found in the intermediate and anterior parts of the disc^{51,55,57} corresponds well with the increased compressive stiffness of the area reported in other studies^{58,59} and is attributed to the abundance of fibro-chondrocytes in that region. Proteoglycans (PGs) attribute significant compressive strength, because they have the ability to trap amounts of water, which is rather incompressible, due to their highly anionic nature. Regarding the prevalence of type I collagen and noticeable amounts of elastin found in the central parts of both the anterior and posterior bands⁵¹, a correlation with the predominance of fibroblasts in those regions is indicated⁵². Furthermore, the same research group observed the collagen fibres' arrangement in the disc using scanning electron microscopy and reported fibres running in a circular manner around the periphery and anteroposteriorly through the intermediate zone⁵¹. These characteristics are similar to those of condylar cartilage and demonstrate their close functional relation.

Concluding, the TMJ disc is both fibrous and cartilaginous in nature; its cellular and histological composition shares characteristics with articular cartilage, tendon and meniscus (Tab. 1).

Table 1. Summary of TMJ disc key characteristics:

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| <ul style="list-style-type: none"> • Predominance of fibrillar type I Collagen (not type II). • Collagen fibers arranged parallel to the surface, running circularly around the periphery and anteroposteriorly through the intermediate zone. • Main GAG is Chondroitin sulphate, total GAG content is lower than hyaline cartilage. • High content of GAGs in anterior and intermediate regions attribute increased compressive stiffness. • Main PG is aggrecan that consists of Chondroitin sulphate. • Uneven distribution of fibroblasts and fibrochondroblasts through its zones. |
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Stem Cells

A stem cell (SC) is one that, through asymmetric mitotic division, is able to differentiate into several specialized cell lineages (multipotency), while retaining the potential for self-renewal. Such division results in 2 daughter cells, 1 of which follows the mothers' genotype while the other (either by genetic or environmental influence) targets a specialized lineage⁶⁰. Stem cells exist

at different hierarchical levels during the development of an organism. At one end of the spectrum are SCs that can give rise to all cell types in the body, which are called pluripotent. Other SCs may be partially committed and, thus, only retain ability to differentiate into a more restricted subset of different cell types; these are called multipotent. Sources of stem cells can be either embryonic or adult. Embryonic stem cells are obtained from the inner cell mass of blastocyst stage embryos⁶¹; however, their use has given rise to ethical considerations. Although adult stem cells can be found in children, adolescences and adults, and can be obtained from multiple tissues, the question “how age might affect their capacity?” is still unclear⁶².

In the early sixties Petrakova et al⁶³ were the first to show that fragments of bone marrow had the potential to form osseous tissue when implanted ectopically. This was the first indication that bone marrow contains some kind of cells with osteogenic capacity. Isolation of such cells from bone marrow was performed by Fridenshtein⁶⁴ in the late sixties, and they were described as “colony forming fibroblast-like cells”. Almost 2 decades later, Caplan⁶⁵ gave them their current name (mesenchymal stem cells, MSCs) and identified their full potential to differentiate into multiple mesodermal cell lines, forming tissues of mesenchymal origin⁶⁶. This revelation broke new ground for the prospect of tissue regeneration. Multiple sources of adult stem cells have been identified since then, including adipose tissue^{27,67}, heart²⁸, umbilical cord blood⁶⁸, skeletal muscle⁶⁹, dermis of skin^{30,70}, the bulge region of the hair follicle⁷¹ as well as brain^{72,73}. In the craniofacial region, stem cells can be isolated from orofacial bone⁷⁷, dental pulp^{74,75} exfoliated deciduous teeth⁷⁶, and periodontal ligament⁷⁸. The 2 most extensively examined stem cell sources for their capacity to differentiate into various cell lineages are bone marrow derived stem cells (BMSCs) and adipose derived stem cells (ASCs).

BMSCs are considered the premier source for tissue engineering. They can be easily obtained from various locations⁷⁹ and have the ability to form mesenchymal and connective tissues³² such as bone, cartilage, ligament, fat and muscle⁸⁰⁻⁸². Moreover, BMSCs have been reported to differentiate into hepatic, cardiac, renal and neural cells^{33,83-85}, although these pathways are not normally utilized to prove multipotency of isolated MSCs as will be described later in this paper. At this point, it should be noted that clonal studies showed that adhering cell populations isolated from bone marrow are functionally heterogeneous, containing undifferentiated stem progenitors and lineage restricted precursors^{86,87,95}. This heterogeneity can partially explain the variations of the differentiation capacities observed in related studies composing a hurdle in their comparative evaluation.

Though being considered primarily as a metabolic reservoir, the adipose compartment appears to be a good and plentiful source of MSCs as first Zuk⁶⁷ found. ASCs

can be readily obtained *via* lipoaspirate, a minimally invasive procedure, of great need nowadays. Several groups reported that ASCs undergo adipogenesis, chondrogenesis, osteogenesis, and myogenesis^{27,67,88,89}, while others even suggest that ASCs are able to differentiate into non-mesodermal tissues, such as hepatic and neuronal-glial⁹⁰⁻⁹².

Even though there are no definite markers of MSCs, different methods have been used for their identification. One of the main characteristics of MSCs, still used in contemporary identification processes, is their ability to adhere to the plastic plate surfaces when maintained in standard culture conditions, as was first observed by Friedenstein⁶⁴. Currently, more sophisticated procedures that researchers utilize include positive selection with microbeads combined with fluorescence-activated cell-sorting or magnetic-activated cell-sorting that enable the defined isolation and precise characterization of MSCs^{93,94}. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy⁹⁶ has listed the minimal criteria required for a cell to be regarded as an MSC, providing a general consensus (Fig. 3). The list includes several markers that cells should lack or exhibit additionally to the ability to differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*. However, new ones are proposed over time.

Today, despite the fact that multiple sources of stem cells have been identified, certain drawbacks related to the harvesting process of BMSCs such as pain, stigma and low yield⁶² as well as the debate surrounding the use of embryonic stem cells led to the elevation of ASCs as an attractive option for a wide range of medical applications. Additionally, alternative orofacial sources of SCs and their capacities were also investigated. As mentioned, SCs can be isolated from the dental pulp of extracted humans 3rd molars^{74,75}, exfoliated deciduous teeth⁷⁶ and the periodontal ligament⁷⁸. These sources present tempting alternatives through minimally invasive harvesting methods. Although small in number, dental pulp SCs (DPSCs) demonstrated higher proliferation rates compared to BMSCs in a study by Shi et al⁹⁷. DPSCs can not only form dentin/pulp-like structures when implanted in immunodeficient mice⁹⁸, but have also shown the ability to differentiate into neuron-like and glial-like cells (expressing nestin and glial-fibrillar acid protein)⁷⁴. Furthermore, DPSCs are often mentioned in recent discussions about regenerative endodontics^{99,100}. Accordingly, when SCs from exfoliated deciduous teeth were implanted in immunodeficient mice in a HA/TCP carrier, they formed dentin-like structures⁷⁶. Periodontal ligament SCs (PDLSCs) generated cementum/PDL-like structures, containing a thin layer of cementum interfaced with dense collagen fibres similar to Sharpey's fibres in a small animal *in vivo* model⁷⁸. A recent review article by Huang et al¹⁰¹ discussed availability of dental derived stem cells in regenerative medicine.

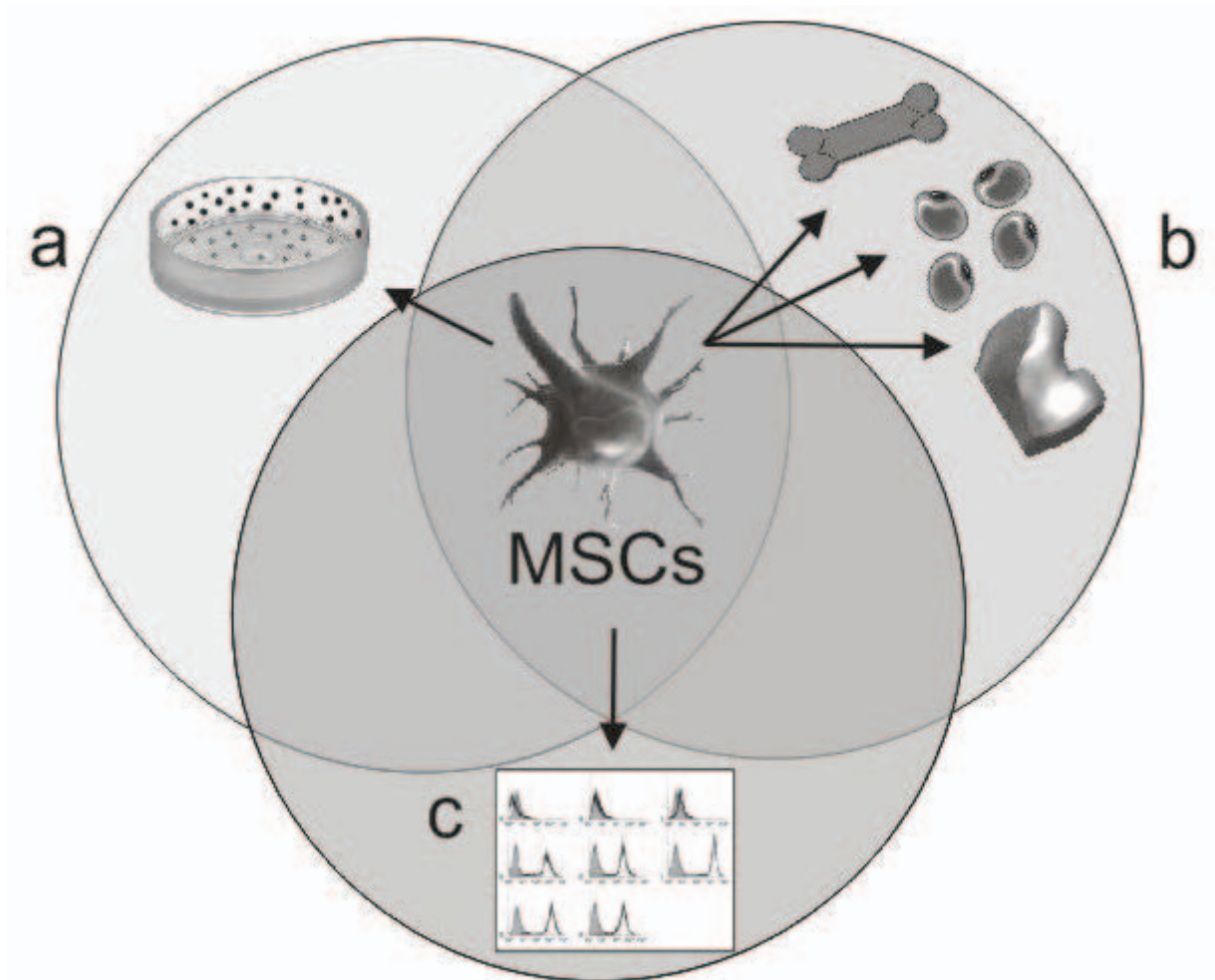


Figure 3. Minimal criteria for defining multipotent mesenchymal stromal cells. MSCs must: (1) be plastic-adherent when maintained in standard culture conditions; (2) differentiate to osteoblasts, adipocytes and chondroblasts in vitro; (3) express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules

Based on the great potential of tissue engineering and other stem cell based therapies, storage banks were established to isolate and freeze-store stem cells from multiple sources, such as extracted 3rd molars and the umbilical cord. These banks provide donors a prospect for stem cell-based therapies in the future if such need arises and given that stem cell science has evolved. However, there is the need to fully certify and standardize the procedures utilized for the isolation and particularly the maintenance of cells in a viable state.

But to what extent do stem cells, originating from different tissue, differ in their potential? Moreover, does their potential lie with their intrinsic features, or with the fact that stem cell populations might include lineage restricted precursors? A study by Im et al¹⁰² suggested that ASCs have inferior chondrogenic potential than BMSCs, a view supported by the work of Vidal et al¹⁰³ and Kisiday et al¹⁰⁴. In addition, a comparative study between orofacial and iliac crest derived MSCs reported that the

latter showed more proliferative and fewer senescence properties and different chondrogenic and adipogenic potentials⁷⁷. In a comparative study by Wagner¹⁰⁵ in 2005, however, no phenotypic differences between MSCs from bone marrow, adipose tissue and umbilical cord blood were observed. Thus, research must focus on identifying and comparing the distinct capacities of stem cells from different sources.

Scaffolds

In order to provide a hospitable 3D environment for cell attachment, proliferation and differentiation to form a tissue analogue, the use of biomaterial scaffolds was introduced in tissue engineering. Scaffolds must satisfy a number of requirements, primarily to ensure their safe usage and secondarily to fit the specific

characteristics of the tissue we intend to reconstruct. For example, scaffolds used for bone modelling must have comparable mechanical properties to bone, while for engineering of soft tissues (i.e. dermis, oral mucosa), a much softer scaffold is often required. It is essential that biocompatibility of the material and its subsequent degradation products is ensured¹⁰⁶. Additionally, scaffolds must be biodegradable, with a controlled, predetermined degradation rate; after implantation, they must act as a temporary framework securing the initial stability and mechanical support of the construct itself, protecting the cells contained within, and surrounding tissues. Gradually they should give space to the newly formed tissue, allowing its ingrowth. Ideally scaffold materials should also demonstrate good handling characteristics (the ability to be shaped and fit into complex defects).

Moreover, scaffolds must have sufficient porosity to enable cellular colonization and tissue ingrowth (pore size is dependent to the specific cell type needs), as well as pore interconnectivity¹⁰⁷⁻¹¹¹ to facilitate the transport of nutrients and oxygen inwards, degradation and cellular metabolic by-products outwards. It is possible to modulate the pore topography and size to suit a particular cell type. Research has shown that pore shape can have a profound effect on both cell attachment and long term survival of cells¹¹². Current developments in 3D computerized-tomography, 3D computer-aided design and rapid prototyping techniques are used for the design and engineering of customized cartilaginous implants made of synthetic polymers in defined pore architecture^{113,114}. Porosity can, however, adversely affect important mechanical characteristics of the scaffold, thus precise computational design techniques are needed to predict and ultimately optimize the microstructure to achieve the desired balance¹⁰⁶.

In an attempt to enhance the attachment of cells onto scaffolds, scientists have incorporated small peptide sequences (such as the RGD: Asp-Gly-Asp sequence¹¹⁵ and the IKVAV sequence¹¹⁶) in scaffold biomaterials. These peptides are recognized by cell surface receptors,

called integrins. In physiological cell-ECM interactions, integrins play an important role in tissue homeostasis. Binding of ECM proteins to these receptors promotes focal adhesion. Additionally, this process leads to activation of intracellular signalling pathways that mediate changes in gene expression and thus affect cell proliferation, survival and differentiation¹¹⁷⁻¹²⁰. Though results from their use are so far controversial^{121,122}, integration of such signalling molecules in scaffolds is of great interest and strikes promise for the use in chondrogenic induction of MSCs.

For tissue engineering purposes, a wide variety of different scaffold materials exists, which can be natural, synthetic or composite. Natural scaffold biomaterials can be further classified to protein-base and polysaccharide-based, while synthetic materials can be subdivided into ceramics (hydroxyapatites or HA), calcium phosphates (CaPs) and synthetic polymers. In cartilage engineering, the main scaffold materials found in literature are summarized in table 2; so far however, none seems to possess the ideal characteristics to rise up as the perfect candidate. Particularly natural biomaterials could stimulate cell attachment, proliferation, differentiation and synthetic activity as they home in the cell's natural environment. On the contrary, synthetic scaffold biomaterials offer significant advantages over the purely biological ones in terms of unlimited supply and manipulation of their mechanical properties, macro, micro and nano-topography and degradation behaviour.

Looking to the future, a minimally invasive surgical technique, such as an injection of a gel or paste material, seeded with MSCs or committed cells into a single or multi-tissue defect and shaped *in situ* seems ideal. In this direction several promising materials are being developed including, calcium phosphate cements^{123,124}, *in situ* polymerizable and cross-linkable materials^{125,126}, stimulus responsive systems^{127,128}, and self-assembling materials^{129,130}. A detailed review of injectable materials focusing on the regeneration of complex craniofacial tissues is available by Kretlow et al¹³¹.

Table 2. Scaffold materials used for cartilage tissue engineering

Type	Natural	References	Synthetic	References
Material	Agarose	132, 133, 134	PLA, poly(lactic acid)	155, 156, 157
	Alginate	133, 134, 135, 136	PGA, poly(glycolic acid)	158, 159, 160
	Cellulose	137, 154	PLGA, poly(L-lactic-co-glycolic acid)	161, 162, 163
	Chitosan	138, 139, 140	PEG, poly(ethylene-glycol)	164, 165, 166
	Hyaluronic Acid	141, 142, 143, 144	PCL, poly(capro-lactone)	167, 168, 169, 170
	Collagen	145, 146, 147, 156	POC, poly(1,8-octenadiol citrate)	171, 172
	Fibrin	147, 148, 149, 150	PU, poly(urethane)	173, 174
	Silk	151, 152, 153		

Bioreactors

Bioreactors can be defined as devices in which biological and/or biochemical processes are re-enacted under controlled conditions¹⁷⁵, providing an environment that is advantageous for the creation of a desired product, whether it is wine or ECM as in cartilage tissue engineering. A number of parameters such as pH, temperature, pressure, nutrient supply, oxygen supply and waste removal should be carefully controlled and kept in optimal levels in the challenging attempt to mimic *in vitro* the dynamics of *in vivo* tissue growth. The *in vitro* generation of large three dimensional tissue analogues requires the development of sophisticated and complex systems that allow cells to locate them in a 3D scaffold, differentiate, proliferate and produce ECM, at the same time satisfying their physiochemical demands. The accurate simulation of the native physiological microenvironment will not only enhance the quality of the tissue engineered construct, but also hopefully ensure cell survival, tissue integration and proper function following implantation.

The simplest and most commonly used bioreactors are culture discs and flasks. They are easy to handle, affordable and through mechanisms of passive diffusion of nutrients and oxygen allow rapid multiplication of cells when cultured in monolayer. When multilayer approaches are adopted, transport of these substrates in and out of the cell containing microenvironment is mediated by inducing fluid flow, and/or passive diffusion along concentration gradients.

The advances in technology of bioreactor systems for the hosting of scaled-up scaffolds, trying to facilitate improved cell survival, reach new levels of complexity in tissue engineering techniques. Current standard culture conditions seem inadequate to distribute nutrients and oxygen throughout the construct and removal low molecular metabolites and waste products from it, which occurs mainly through diffusion phenomena from the center of the scaffold. This leads to migration of cells, initially located in the inner areas, to more superficial areas of the scaffold, where the nutrient concentration is higher. Eventually a steep fall in the cell density in the deeper zones of the construct is observed, affecting the homogeneity of the ECM and cell distribution in the final tissue equivalent¹⁷⁶.

As summarized by Depprich *et al*¹⁷⁵ in an excellent review, the concept of improving the bioreactor and scaffold design, in order to reduce diffusional limitations and ensure the viability of inner areas of the three dimensional construct, requires advances in both bioreactor and scaffold technology. From this point of view a dynamic laminar flow environment, achieved by perfusion directly through bioreactor inherent tubes and prefabricated interconnecting pores of the scaffold, would seem an ideal prospect¹⁷⁵. Further advances in bioreactor

technology should include developments of automated closed systems equipped with sophisticated tools, providing the means to both monitor physiochemical variables and enable adjustment of their values, thus allowing reproducibility and standardization of culture conditions. Aiming to predict the maturation level of the engineered construct advanced non-destructive analysis techniques (such as micro computerized tomography) could be integrated.

Mechanical stimulation seems to be a factor that greatly influences the developmental process. Research has shown induction of multipotent cells, seeded in engineered constructs subjected to physiological loads, towards various cell lines depending on the type of mechanical stimulation as it will be discussed later in this paper. Different stress protocols have been incorporated in bioreactor systems and applied to cell-loaded scaffolds. These include perfusion flow, shear, compression and tension among others. When these forces are applied in an advantageous combination with specific magnitudes and frequencies, they may not only enable controlled induction of differentiation but also enhance the quality of the generated construct. Schulz and Bader have recently reviewed different bioreactor systems used for the cultivation and stimulation of chondrocytes for the purposes of cartilage tissue engineering¹⁷⁷.

Signalling Molecules

In the processes of tissue engineering, numerous hurdles need to be surpassed, as different approaches, referring to the selection of cell types, can be adopted. For example, when dealing with MSCs, apart from an appropriate 3D scaffold, the use of a series of signaling molecules is required for the *in vitro* proliferation, guided differentiation of the cells and the induction of neo-tissue formation. *In vivo*, MSCs' differentiation is initiated through interactions of molecular signals, emitted from neighboring cells, transduced via extra or intra-cellular pathways and induced by cytokines, growth factors and ECM proteins (such as PGs and collagens), or through direct interaction with surface proteins of neighbouring cells. Trying to mimic the *in vivo* environment, researchers provide controlled delivery of stimulating factors to stem cells cultured *in vitro*, but furthermore have to ensure the long term maintenance of their phenotype. This last requirement also stands for the *in vitro* culturing of differentiated cells, such as autologous chondrocytes. A series of excellent reviews of the growth factors used for those purposes is available in the literature¹⁷⁸⁻¹⁸⁰ and a brief presentation of them follows. Among the growth factors investigated, some families of molecules have demonstrated a great impact on articular cartilage TE.

The transforming growth factor (TGF) superfamily, include 5 members of TGFs- β (1-5), the BMPs, activins and inhibins which interact with type I and II cell surface receptors and activate intracellular mechanisms. TGF- β 1, 2 and 3 are predominantly produced in bone and cartilage and are considered to be potent stimulators of proteoglycan and type II collagen production and induce chondrogenic differentiation of MSCs *in vitro*¹⁸¹. It seems that TGF- β , as part of a serum-free differentiating medium, is one of the most widely used GFs and, in addition to promoting chondrogenesis, has also been shown to inhibit osteogenic and adipogenic differentiation^{32,182,183}. BMPs compose a large subgroup of 20 polypeptides mainly investigated for their role in bone repair and seem to be key regulators of skeletal development. There is evidence indicating that BMP-2, -4, and -6 can stimulate chondrogenic differentiation of MSCs¹⁸⁴ and synthesis of type II collagen and aggrecan by chondrocytes *in vitro*¹⁸⁵. BMP-7 (osteogenic protein-1) was reported to enhance repair articular cartilage defect in animal models^{186,187}.

The fibroblast growth factor (FGF) family comprises 22 members that bind to one of 4 FGF receptors (FGFRs). The importance of FGFs in skeletal development is highlighted by number of dysplasias that have been attributed to specific mutations of genes encoding FGFRs. Research showed that FGF-18 stimulated cell proliferation, differentiation and cartilage ECM formation both *in vitro* and *in vivo*^{188,189}. Expansion of MSCs in medium containing FGF-2 has also shown to promote chondrogenesis¹⁹⁰⁻¹⁹². In a rabbit model, FGF-2 stimulated cartilage restoration in temporomandibular or articular cartilage defects¹⁹³.

The insulin-like growth factor (IGF) family comprises of IGF-1, IGF-2, their respectful receptors (IGF1R, IGF2R) and several IGF-binding proteins and proteases that regulate their activity. Their anabolic role is supported by severe growth retardations observed in mice with IGF-1 mutations. Expression of IGF-1 and IGF1R in adults occurs in chondrocytes, osteoblasts and osteoclasts¹⁹⁴. Additionally, it was shown that IGF-1 induces proteoglycan synthesis, chondrocyte survival and proliferation¹⁹⁵ and promotes articular cartilage regeneration¹⁹⁶, a fact that supports its role as a mediator of cartilage growth and homeostasis. IGF-1 has also been shown to stimulate chondrogenic differentiation of MSCs¹⁹⁷.

The Wingless (Wnt) family contains more than 20 members involved in skeletal development and the control chondrogenesis. Church et al¹⁹⁸ investigated the role of Wnts in chondrogenic development and found that Wnt-5a, Wnt-5b and Wnt-11 regulate chondrocyte proliferation and hypertrophic maturation in embryonic and post natal growth plates, while it seems that deregulation of Wnt signalling might lead to disease, in particular osteoarthritis. A recent review on the role of Wnts in cartilage

development and degeneration is provided by Chun et al¹⁹⁹.

The Hypoxia induced factor (HIF) family consists of 3 subgroups (HIF-1a, HIF-2a and HIF-3a) of transcription factors capable of sensing and responding to alterations of the oxygen tension in the cell's microenvironment. Recent studies show enhanced chondrogenic differentiation of rat MSCs, in hypoxic conditions, through up-regulation of the HIF-1a levels²⁰⁰. The importance of HIF-2a induction of the human articular chondrocytic phenotype in hypoxic condition has been suggested, whereas HIF-1a has been shown to be essential for growth arrest and survival of chondrocytes²⁰¹. Another study showed that chondrocytes produce increased quantities of collagen type II *in vitro*, through HIF-1a²⁰².

Other substances used for the induction of chondrogenesis include dexamethasone, β -glycerophosphate and ascorbic acid. Dexamethasone, a synthetic glucocorticoid, stimulating chondrogenesis by enhancing expression of cartilage extracellular matrix genes is used in standard cultivation media²⁰³. In animal studies, it is reported to be a potent stimulant of chondrogenesis²⁰⁴. In a study by Bean et al²⁰⁵, the use of 25 μ g/ml concentration of ascorbic acid in culture media showed increased production of collagen by TMJ disc cells and is considered to be effective for the regeneration of the TMJ disc.

Even though extensive research is being conducted in the field of growth factors, the sometimes contradictory results for their potential role in *in vitro* chondrogenic differentiation of MSCs, and *in vivo* chondrogenic development, cartilage homeostasis and function, highlight the need for a better characterization of their signalling pathways. Currently, in the effort to simulate physiological *in vivo* processes of tissue development and maintenance through the series of interactions of biological factors, researchers seem to be trying to decode and reproduce an unknown tongue. In fact this is the richest and most complex language that exists, the one of nature. So far in this challenge, we have succeeded in identifying only a few words (could be GFs) and their likely meanings (could be their role), but are still unable to generate meaningful sentences. Thus, exploring the vocabulary and understanding the syntax, based on which these words are used (put in order), are essential to both understand and speak this language.

Accordingly, a profound understanding of the underlying biological and physiological background, accurately delineating the stages and defining the molecular markers involved in chondrogenic differentiation and development, can facilitate coordinated supply of growth and differentiation factors. Furthermore, sophisticated materials have to be developed to enable controlled spatial and dynamic release of these factors.

Alternative approaches for the delivery of GFs, involving gene therapy techniques, have also been investigated. The concept of gene therapy, as first

described by Evans²⁰⁶, involves the delivery of a cDNA specific to a GF or signalling molecule into target cells which then produce that factor *in vivo*. Gene therapy can be performed directly, through delivery of transfection vectors in the targeted site or indirectly, where cells are transfected *in vitro* prior to their *in situ* implantation. The vectors can be either non-viral or viral which include lentiviruses, adenoviruses and adeno-associated viruses. Each approach displays different advantages and disadvantages. For example: the use of non-viral vectors is relatively simple but their expression is generally transient. On the other hand, the use of viral vectors results in prolonged transgene expression, but carries the risk of insertional mutagenesis. The incorporation of plasmid-DNA into scaffold materials is a similar idea that has also been explored in a number of studies^{207,208}. Recently, the concept of gene delivery in cartilage repair has been reviewed by Steinert et al²⁰⁹.

Since our clinical experience with GF technology is relatively new, issues such as the long-term effects of implanting materials containing supra-physiological doses of GFs and the safety of gene therapy approaches, have to be addressed.

Research in Cartilage Tissue Engineering

In the literature, reports for reconstruction of the TMJ disc using stem cell-based TE approaches are scarce³⁶. On the other hand, substantial work has focused on the TE of the hyaline cartilage, which is not surprising taking into consideration its significantly higher commercial interest. The experience and knowledge from the research in hyaline cartilage TE could provide an insight for attempts to reconstruct the TMJ disc, given the similarities of those tissues, while not overlooking their compositional and functional differences. Thus, a brief presentation of cartilage engineering will follow.

In vitro studies have attempted to determine the optimal mechanical and biochemical stimuli required for proliferation and differentiation of stem cells into the chondroblastic line, as well as for the maintenance of their phenotype and ECM production. As stated above, a number of different techniques can be used including: perfusion flow, cyclic strain and/or compression, bending, controlled pH, controlled oxygen levels and growth factor delivery. Elder²¹⁰ demonstrated an enhanced chondrogenic differentiation under cyclic mechanic compression (9.25 KPa, 0.33 Hz, 2 h, for 3 days) of BMSCs in agarose gel. The same group reported that cyclic hydrostatic compression induced chondroinduction of MSCs²¹¹. Cyclic hydrodynamic stimulation has been shown to promote the chondrogenic commitment of hBMSCs in

pellet cultures²¹². It seems that dynamic compression can further stimulate chondrocyte metabolism and enhance cartilage ECM production²¹³⁻²¹⁵. However, these specific effects are highly dependent on the magnitude and frequency of the applied stimuli. Furthermore, engineered tissues in different stages of development, progressing from scaffold occupied towards ECM occupied constructs, may require different mechanical stimuli. Osteogenesis, on the other hand, appears to be promoted by cyclic mechanical stretching²¹⁶ and continuous perfusion²¹⁷.

Articular cartilage experiences low oxygen concentrations due to its avascular nature. However, chondrocytes are adapted to these hypoxic conditions and produce ATP through anaerobic glycolysis mediated by the HIF-1 α transcription factor^{218,219}. Based on that fact, studies have investigated the effects of oxygen levels on cartilage regeneration *in vitro*. Reports have shown hypoxia to induce the chondrogenic differentiation of MSCs^{220,221} as well as the synthesis of ECM proteins in cultured chondrocytes²²², while higher oxygen tension leads to osteogenic differentiation of MSCs²²³. Studies suggest that low oxygen levels inhibit the expression of type X collagen, a marker of hypertrophic chondrocytes leading to bone formation²²⁴. Recently, Wuertz et al^{225,226} showed that low pH inhibits the expression of aggrecan by MSCs, while in another study they reported that, when cultured in an environment containing low glucose, low pH and high osmolarity, MSCs displayed lower proliferation rates and lower expression of matrix genes compared to standard conditions.

As described previously, TGF- β , FGF-2 and IGF-1 are the most commonly used growth factors for chondrogenesis. However, in physiological tissues, none of these substances acts independently, they rather participate in an orchestrated sequence of interactions that mediate physiological function of tissues.

Consequently, researchers have attempted to deliver simultaneously various signalling molecules implicated in chondrogenesis. In particular, a combination of TGF- β 3 and BMP-2 gave improved chondrogenic differentiation compared to either GF alone, or the combination of TGF- β 3 with either BMP-4 or BMP-6¹⁸⁴. Likewise the combination of TGF- β and application of cyclic mechanical load better promoted chondrogenesis of MSCs of either stimulant alone²²⁷. Indrawattana et al²²⁸ demonstrated how combinations such as TGF- β 3/BMP-6 and TGF- β 3/IGF-1 and the cycling of these combinations can induce chondrogenesis in MSCs. Additionally, a study by Chua et al²²⁹ demonstrated that the combination of IGF-1, bFGF and TGF- β 2 increased cartilage-specific ECM expression and enhanced the histological features of the engineered cartilage. IGF-1 could be involved in synergism with TGF- β 1, as the expression of chondrogenic-specific transcription factor SOX9 and production of type II collagen and cartilage specific PGs

by stimulated MSCs were comparable to those of mature chondrocytes, as shown by Longobardi et al²³⁰. Addition of the parathyroid hormone PTHrP to TGF- β -stimulated MSCs has been shown to inhibit the expression of type X collagen and suppress cells' terminal differentiation²³¹. At the same time, other reports show negative and no response to the use of the combinations IGF-1/FGF-2²³² and IGF-1/TGF- β ²³³, respectively.

Current experimental data give us a glimpse of the complexity of interactions involved in the processes of tissue growth and homeostasis; and as they emphasize the limited extent of our knowledge, they indicate a need for deeper understanding of the background of this field.

Discussion

To what extent can we really hope that TE techniques can result in a viable disc analogue? The idea of seeding multipotent cells in specially designed scaffolds with concurrent orchestrated delivery of biological factors, creating an environment for directed differentiation and tissue maturation for the reconstruction of a fully functional tissue, TMJ disc in this case, seems to be an ideal prospect. In this process, however, a number of issues still remain unsolved.

For example, which is the most favourable cell source? A cell source of chondrogenic and/or fibroblastic cells is needed. The most apparent choice would be native tissue cells, already possessing the tissue specific differentiated phenotype; however, in our case either physiological or degenerated disc cells are in short supply. Furthermore, their harvesting process is associated with an additional surgical procedure and post-operative donor site morbidity. The use of mature chondrocytes from either non-load bearing or non-articular cartilage, such as nasal, rib and auricular cartilage, has also been proposed, an approach that reduces the site morbidity due to the lower levels of physical forces it experiences. An alternative choice could be tissue specific cells of xenogenic origin, which would be limitless in number and easy to obtain, but their use could potentially implicate the possibility of disease transmission and histocompatibility issues.

The major obstacles in the utilization of mature chondrocytes for TE purposes is their low expansion rates and de-differentiation leading to loss of function when cultivated *in vitro*²³⁴. The de-differentiation phenomenon is accompanied by a shift towards a fibroblast-like phenotype characterized by the adoption of a spindle-like shape and type I collagen production²³⁵. De-differentiation seems to be reversible when these cells are relocated in a 3D environment^{222,236,237}. This observation points out significance of the 3D scaffold architecture in promoting the chondrocytic phenotype. Also, stimulation

by TGF- β , IGF-1 and FGF-2 has been shown to direct re-differentiation of de-differentiated cells^{238,239} and increase the cells' proliferation rate^{238,240}.

Stem cells were introduced early in the field of TE, as they possess the potential to differentiate into almost all types of cells. Multipotent stem cells, as mentioned before, can be obtained easily from various tissues. BMSCs have already demonstrated their tendency to undergo osteogenic and chondrogenic differentiation when cultured under certain conditions. However, it should be noted that adhering cell populations isolated from bone marrow are functionally heterogeneous, containing undifferentiated stem progenitors and lineage restricted precursors, with varying differentiation capacities^{86,87,95}. Moreover, chondrogenic differentiation of MSCs in standard *in vitro* culture systems routinely results in expression of hypertrophic markers, such as type X collagen and alkaline phosphatase, which correlate with calcification and vascular invasion²⁴². This undesirable phenomenon can be restrained by conditioning the cultured cells with biological, biochemical and biomechanical stimuli.

A vast number of animal studies have explored their ability to repair mainly osseous defects displaying controversial results. Bone marrow derived MSCs have been, combined with HA/TCP scaffolds to help build calvarial and alveolar bone defects in canines^{243,244} and mice²⁴⁵, seeded into hyaluronan-based polymers for reconstruction of orbital rim defects in pigs²⁴⁶. They have been also loaded onto gelatin sponges for repair of calvarial defects in mice²⁴⁷ and onto poly(caprolactone) (PCL)-based scaffolds to repair cranial defects in rabbits²⁴⁸. Adipose derived MSCs have been used in combination with gel foam scaffolds into rabbit calvarial defects²⁴⁹ and seeded onto PLGA scaffolds for rat critical-sized calvarial defects²⁵⁰, and showed moderate and significant results, respectively. In a worth mentioning human case by Warnke²⁵¹, a gross and fully vascularized mandibular construct was created through the combined use of a custom designed titanium mesh scaffold, loaded with hydroxyapatite (HA) blocks and a bovine type I collagen coating containing BMP-7 and bone marrow aspirate. The compound was allowed to mature in a prefabricated muscle flap and revascularized through vessels' ingrowth from the thoracodorsal artery. After 7 weeks of maturation, the construct with its accompanying soft tissue envelope (including the adjacent vessel pedicle) was transplanted into the defect. The thoracodorsal artery and vein were anastomosed onto the external carotid artery and the cephalic vein, respectively. The patient's ability to eat and speak improved greatly, but unfortunately the researchers were able to follow up only for 15 months when the patient passed away from cardiac arrest.

The repair of osteochondral defects has also been addressed through the use of MSCs in scaffold and

scaffoldless approaches. A combination of soluble scaffolds with MSCs have been injected in knees of rabbits and goats and showed controversial effects²⁵²⁻²⁵⁴. A biphasic PCL/TCP scaffold with MSCs was used in condylar defects of pigs²⁵⁵ and a PLGA/nano-hydroxyapatite (NHA) composite loaded with MSCs was used in rats' knees²⁵⁶ to repair osteochondral defects; both gave promising results. Accordingly, Wakitani et al²⁵⁷⁻²⁵⁹ investigated the effect of transplantation of MSCs embedded in collagen gel for repair of human full-thickness articular cartilage defects with promising results. Taking into consideration that there is no single method regarded as a standard for isolation and expansion of MSCs, in field of TE; it is important to realize that these varying approaches make it difficult to directly compare experimental results.

These efforts for osteochondral tissue reconstruction could potentially play a key role in TMJ TE, especially when dealing with defects involving both condylar bone and articular cartilage. Furthermore, as pointed out during the TE session of the TMJ Bioengineering Conference, surgical implantation and attachment of a TMJ disc alone is considered difficult or even impossible in practice by most. Thus constructing and attaching an engineered disc/condyle composite would be the most rational approach for implanting an engineered disc. This approach would better fit cases where disc abnormalities are concurrent or induced by primary degenerations of the condyle. Alhadlaq and Mao^{260,261} succeeded in generating constructs with the shape and dimensions of the human mandibular condyle. They used rat MSCs, independently stimulated towards the chondrogenic and osteogenic lines, seeded onto a photo-polymerizable, 2-layered polyethylene-glycol diacrylate (PEGDA) scaffold and implanted into the dorsum of immunodeficient mice for 12-week maturation. Each layer of the construct demonstrated tissue distinctive microscopic characteristics. Infiltration between osseous and cartilaginous compartments was enhanced by increasing cell encapsulation density. However, integration of the condyle analogue to the ascending ramus and surrounding attachments still composes a hurdle.

Furthermore, a number of other issues need to be addressed in the challenge of implanting an engineered condyle. The tissue engineered condylar graft should be adequately vascularized whereas its attachments and mechanical properties should ensure the ability to withstand the early shear and torque stresses. Mechanical properties of the condylar construct depend highly on the level of *in vitro* maturation. Longer periods of maturation seem to lead to a better developed cartilaginous ECM, displaying better mechanical properties and enhanced success rates when subjected to physiological mechanical loads. However this prolonged maturation could negatively affect the construct's capacity to integrate with the adjacent tissues. In addition, proper construct

maturation and conditioning *in vitro* should diminish the incidence of undesirable metaplasia, meaning mineralization of the chondral part towards bone. A number of TE studies for the mandibular condyle are available as the number of scientists in the field is growing exponentially²⁶².

Nevertheless, these efforts did not incorporate current data regarding histological and biomechanical characteristics of the mandibular condyle's cartilage. This is not surprising since our experience on TE is limited. Particularly, the cartilage of the mandibular condyle contains a heterogeneously distributed ECM. The type and arrangement of collagen fibres and the type and distribution of GAG-associated PGs through its regions mirrors the function of the TMJ. For the construction of a viable and fully functional mandibular condyle we should not only attempt to mimic the macroscopic gross anatomy and composition of the targeted tissue. The construct should also display the same detailed organization of its cellular and non-cellular components to meet the requirements of native tissue. So far, efforts proposed for engineering composite constructs are based on specific strategies for selection of scaffolds and cell sources. These include single homogenous and biphasic scaffolds in cell-free approaches or loaded with chondrogenic, osteogenic or multipotent cells²⁶³.

Issues similar to the ones of tissue engineered osteochondral constructs complicate the disc reconstruction. The TMJ disc histology has not been investigated thoroughly as for its cell distribution and extracellular matrix composition in humans⁴⁶, but several animal models have been used, including mice^{37,47}, rabbits^{48,49}, bovines⁵⁰ and porcine⁵¹. It has been suggested, based on a TE study where no differences were observed between results from porcine and human cells²⁶⁴, that the porcine model is suitable for characterization and TE studies of the TMJ disc. Furthermore, the first TMJ Bioengineering Conference held in May 2006 in Broomfield, Colorado²⁶³ proposed that, given the high cost of primate research and the similarity of the gross morphology of the constituents of the TMJ including the disc²⁶⁵⁻²⁶⁹, the porcine model is the most suitable animal model for biomechanics research and TE studies. Data obtained from the histological, biochemical and biomechanical analysis of TMJ discs of animal models, such as the porcine, show that the disc contains a vast population of fibroblasts and relatively less fibrochondroblasts (chondrocyte-like cells); accordingly, it is composed mainly of type I collagen and its total elastin and GAG content gives it a unique character that falls in between hyaline cartilage and meniscus. The compressive and tensile properties, demonstrated by the disc, are attributed to its composition and beyond that, to the topographic arrangement of its macromolecules. The high GAG concentrations, located in the intermediate zone and anterior band of the disc^{51,57,270} seem to contribute

to the high compressive strength of these parts^{58,59}; collagen fibres running circularly around the periphery and anteroposteriorly through the intermediate zone of the disc⁵¹ also correspond to the high tensile stiffness reported by a previous study²⁷¹ and to the taut tension, applied through the disc's anteroposterior axis by its anterior and posterior attachments. Another arising issue is a theory of a more mechanically demanding environment in the inferior joint space, which is supported by the increased degeneration of the inferior disc surface observed in TMJ disorders²⁷²⁻²⁷⁴ and the high relative number of fibrochondrocytes found in the inferior layer, compared to the superior and middle layers of the disc⁵². However, it must be taken into consideration that though porcine TMJ discs are approved for characterization, some early research data for human discs²⁷⁵ show controversy regarding the GAG distribution. In addition, differences existing between functional parameters of the TMJ, such as the frequency of mastication, indicate that they are not completely identical. These facts ultimately lead to the conclusion that human discs need to be better investigated and characterized, while efforts to generate a disc construct *in vitro* for the purpose of realistically treating humans, must incorporate the compositional and architectural features found in the native tissue.

However, investigation of etiology of temporomandibular disorders' pathogenesis is also of great importance, as a large fraction of TMD's causes remain unclear. For example, why are more women treated for TMDs than men^{6,276}? Is it a matter of oestrogen receptors²⁷⁷? Is it just a matter of transmission or perception of pain²⁷⁸? Better understanding of TMD pathogenesis will not only help the prevention and treatment of TMDs, but will also give significantly better prospect to TE disc therapies. In fact, if the underlying degenerative disorder remains uncured, how can we hope that a tissue engineered disc equivalent can function for a long time in a viable and normal state unlike the disc it replaced?

Arthrocentesis, a procedure in which the fluid of a joint cavity (in our case the synovial fluid) is aspirated with a needle and/or a therapeutic substance is injected²⁷⁹, can be used as a diagnostic and therapeutic tool for TMDs, with a low incidence of associated complications²⁸⁰⁻²⁸³. Aspiration of synovial from a symptomatic joint, for example in cases of septic arthritis, will not only instantly relieve symptoms but also enable identification of the microorganisms involved through cultivation and antibiotic sensitivity testing. Microscopic analysis of the synovial fluid provides information on compositional changes of the fluid of diseased joints and thus is a valuable diagnostic tool. For example, identification of crystals allows innocuous effusion caused by osteoarthritis to be differentiated from that caused by crystal induced inflammation²⁸⁴. Borenstein et al²⁸⁵, using gas-liquid chromatographic analysis of the synovial fluid,

found that the presence of succinic acid and lactic acid is a reliable marker of septic arthritis. Lavage of the upper compartment of the TMJ washes away degraded particles and inflammatory components, such as radical oxygen species (ROS), phospholipase A2²⁸⁶ and interleukins 1 β and 6²⁸⁷ among others, concurrently decreasing the intra-articular pressure. So far, diagnostic criteria in various TMDs, through biochemical analysis of the synovial fluid, are insufficiently defined, leading to inefficient treatment. Progressive evaluative analysis of changes occurring in different developmental stages of the pathogenesis of TMDs can offer an insight on the ongoing pathogenetic mechanisms. This could facilitate a deeper understanding of the etiology of these pathoses and subsequently enable the development of new successful treatments.

The ultimate goal is reconstruction of a fully viable, rapidly integrating disc equivalent possessing the biomechanical and histological characteristics of a physiological human disc. In this direction, data derived from engineering research of relevant tissues (such as the knee joint) can be incorporated in TMJ disc TE paradigms. Concurrently scientists have to focus on the TMJ for a better comprehension of its physiology, pathophysiology and biomechanics. Advances in scientific fields, such as molecular and cellular biology, biomaterials and bioengineering, constitute a basis of collective wisdom. The cross-talk between specialties, forming interdisciplinary and multidisciplinary partnerships, will be the catalyst in moving the field of TE to new heights. Adult stem cells are a powerful tool for regenerative medical applications. TMJ tissue engineering is an opportunity that dentistry cannot afford to miss.

Abbreviations:

TE: tissue engineering

TMJ: temporomandibular joint

TMD: temporomandibular disorder

MSC: mesenchymal stem cell

BMSC: bone marrow derived stem cell

ASC: adipose derived stem cell

DPSCs: dental pulp derived stem cells

PDLSCs: periodontal ligament derived stem cells

ECM: extra-cellular matrix

GAG: glucosaminoglycan

PGs: proteoglycans

GF: growth factor

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