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The Role of Tissue Engineering in Articular Cartilage Repair and Regeneration

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Abstract

Articular cartilage repair and regeneration continue to be largely intractable due to the poor regenerative properties of this tissue. The field of articular cartilage tissue engineering, which aims to repair, regenerate, and/or improve injured or diseased articular cartilage functionality, has evoked intense interest and holds great potential for improving articular cartilage therapy. This review provides an overall description of the current state and progress in articular cartilage repair and regeneration. Traditional therapies and related problems are introduced. More importantly, a variety of promising cell sources, biocompatible tissue engineered scaffolds, scaffoldless techniques, growth factors, and mechanical stimuli used in current articular cartilage tissue engineering are reviewed. Finally, the technical and regulatory challenges of articular cartilage tissue engineering and possible future directions are discussed.

Keywords

Articular cartilage; tissue engineering; regeneration; repair; scaffolds; cells; stem cells; self-assembly; bioactive factors; regulatory affairs

I. INTRODUCTION

Joint and articular cartilage injuries are frequent occurrences; over 6 million people visit hospitals in the U.S. each year for various knee, wrist, and ankle problems.[1] Progressive wear and tear on articular cartilage can lead to a progressive cartilage tissue loss, further exposing the bony ends, leaving them without protection. This finally deteriorates into the most common arthritis—osteoarthritis (or degenerative joint disease).[2] It has been reported that osteoarthritis affects 33.6% (12.4 million) of adults age 65 and older in the U.S.[3,4] The American Academy of Orthopaedic Surgeons (AAOS) reports that osteoarthritis is a primary diagnosis accounting for 67% of short-stay and nonfatal hospitalizations in 2004.[5] Considering the increasing population, especially in the elderly with longer life expectancies, occurrences of injuries and osteoarthritis will undoubtedly increase, not only in the U.S., but world-wide.

Unlike other self-repairing tissues, such as bone, cartilage has a low regenerative capacity. Consequently, once injured, cartilage is much more difficult to self-heal. Three types of cartilage exist in the human body: hyaline cartilage (e.g., within diarthrodial joints), fibrocartilage (e.g., knee meniscus and TMJ disc), and elastic cartilage (e.g., ear).[2,6]

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Specifically, articular cartilage covering bone surfaces is a soft and specialized hyaline cartilage that exhibits superior lubrication, wear, and low friction properties; it also reduces stresses in the joint.[7,8] Articular cartilage is composed of a small percentage of chondrocytes, but a dense extracellular matrix (ECM) prevents chondrocyte mobility. In addition, articular cartilage lacks vascular, neural, and lymphatic networks, as well as various local progenitor cells. It has also been described as having high levels of protease inhibitors, which may inhibit efficient tissue repair.[9,10] For these reasons, currently it is challenging to restore full tissue function in damaged or diseased articular cartilage.

Although traditional methods like autografts and allografts have been clinically employed to treat articular cartilage lesions, there still exist many shortcomings associated with these therapies. Autografts, which require the transplantation of a small portion of low-weight-bearing cartilage from the patient into defect sites, have disadvantages such as donor site morbidity and limited cartilage tissue availability.[11–13] Allografts, cartilage pieces obtained from tissue banks, may potentially induce immune responses.[13] For patients with severe joint damage and osteoarthritis, total joint replacement surgery is needed. However, many complications such as inflammation, infection, and implant loosening frequently occur after joint replacement and may lead to implant failure, necessitating future revision surgery.[14,15] In fact, nearly 36,000 revisions for 328,000 hip replacements (11%) and 33,000 revisions for 418,000 knee replacements (8%) were performed in the U.S. in 2003 due to failed hip and knee replacements.[5] Therefore, it is desirable to develop an efficient and simple method to successfully repair and regenerate articular cartilage tissues.

As a rapidly expanding field, tissue engineering may provide alternative solutions for articular cartilage repair and regeneration through developing biomimetic tissue substitutes. This review describes the anatomy of articular cartilage, traditional strategies and related problems, the current progress of articular cartilage tissue engineering, and future directions of articular cartilage repair and regeneration. In this context, the term “repair” is used to denote the restoration of normal function of cartilage regardless of the composition of new tissue that fills the defect sites. On the other hand, “regeneration” is defined as a process, which not only restores the normal functions of injured articular cartilage, but also results in the formation of new tissue that is indistinguishable from the native cartilage.

II. ARTICULAR CARTILAGE COMPOSITION AND STRUCTURE

II.A. Composition of Articular Cartilage

Articular cartilage is a thin connective tissue covering the surfaces in diarthrodial joints. For example, the thickness of articular cartilage in a normal human adult knee is roughly 1.5–3 mm.[16,17] It is composed of two phases – solid and liquid. Table 1 summarizes its components, contents in two phases, and their corresponding functions. Generally, 60–80% of total wet weight of articular cartilage is fluid (e.g., interstitial water and electrolytes), which contributes to many important physical and physiological characteristics of articular cartilage.[18,19] The remaining 20–40% of the tissue is mainly solid ECM and chondrocytes.[20]

Chondrocytes, the only cell type existing in articular cartilage, account for less than 5–10% of the total tissue volume.[21] Although chondrocytes do not directly contribute to the mechanical properties of cartilage,[20] they can sense and respond to various mechanical stimuli within their individual microenvironments.[22] In addition, chondrocytes from different zones of articular cartilage may respond to forces differently[22] and exhibit diverse morphologies (see section II.B for details). Mature chondrocytes are completely encapsulated in the dense cartilage ECM and are not able to migrate or proliferate in a significant manner, unlike cells in bone,[11,19] thus potentially limiting the regenerative capacities of cartilage after injuries.

Articular cartilage ECM, which includes various organic constituents like collagen, proteoglycans, and other noncollagenous proteins, accounts for most of the dry weight of the tissue (Table 1) since chondrocytes occupy but a small fraction of the tissue. As will be described below, the ECM and its interplay with the interstitial fluid play a critical role in cartilage biomechanics. A variety of collagens such as collagen II, VI, IX, X, and XI are the main components of articular cartilage ECM and contribute to the tensile properties of articular cartilage.[7] With maturation, the proportion of collagen II to other collagens increases from 75% in fetal cartilage to over 90% in adult cartilage.[7,23] Conversely, the proportion of collagen XI to all collagens decreases from 10% of fetal cartilage to 3% of adult cartilage.[23] The organization of collagens also changes from random distribution and uniform size in immature articular cartilage into oriented distribution and non-uniform size in mature tissues.[7]

Moreover, collagens IX and XI can crosslink with collagen II to form larger fibrils. These fibrils then interconnect into a mesh network, which is the main contributor to the tensile properties of cartilage.[7] Small amounts of collagen VI in the pericellular matrix surrounding chondrocytes[24] have been shown to play a role in mechanotransduction via cell-collagen interactions.[25] Moreover, by balancing proteoglycan swelling, the collagen fibers affect the degree of tissue hydration, thereby contributing to tissue compressive properties.[26–28]

A special class of glycoproteins, proteoglycans, is another main component in hyaline cartilage. Its biomechanical role is to provide compressive properties to the tissue. The majority of proteoglycans found in cartilage are associated in aggregates (aggrecan). Aggrecan is a large proteoglycan with long and unbranched glycosaminoglycan (GAG) chains that spread out like tubular brushes. These brush-like structures are chondroitin sulfate and keratin sulfate molecules attached to a high molecular weight protein core.[29] The aggregating structure is stabilized by aggrecan molecules being connected to hyaluronic acid GAG chains via link protein. Aggrecan is highly negatively charged due to abundant carboxyl (COO^-) and sulfate (SO_3^-) groups on chondroitin sulfate or hyaluronic acid GAG chains.[30] Since the collagen fibers prevent aggrecan from escaping from cartilage, the fixed negative charges associated with aggrecan attract freely mobile cations in the fluid phase into the tissue. The resultant high density of ions within the tissue creates what is termed the “Donnan osmotic pressure.” This osmotic pressure causes cartilage to swell and also manages water amounts within the tissue.[8,31] Aside from aggrecan, smaller proteoglycans like biglycan, fibromodulin and decorin also occur in minute amounts; many of these have been shown to contribute to matrix organization.[11,32]

II.B. Structure of Articular Cartilage

Articular cartilage is divided into four different zones, each with varying matrix composition, morphology, cellular, mechanical, and metabolic properties.[22] These are termed the superficial, middle (or transitional), deep (or radial), and calcified zones. Figure 1 illustrates the non-homogeneous distribution of cells and ECM in zones of mature articular cartilage. Each zone plays a different role in contributing to the functional properties of articular cartilage.

Starting from the articulating surface, the superficial zone only accounts for 10–20% of the total articular cartilage thickness, but contains the highest density of collagen within the tissue (Table 2). When compared to other zones, the collagen fibers here are the thinnest and most densely packed to form an oriented lamina splendens that covers the joint. Similarly, a relatively small number of fibroblast-like chondrocytes with few organelles[33] are flattened in the superficial zone and are oriented parallel to the surface and the direction of shear stress. The ECM in this zone has fewer proteoglycans compared to other zones. It is

believed that the composition and organization of this zone contributes to tensile strength, resists shear during articulation, and adjusts fluid permeability.[29]

The middle zone is a transitional zone between the superficial and deep zones. This zone has the highest proteoglycan content in the tissue. When examined from the superficial to the deep zone, the collagen and water contents gradually decrease, and the collagen fiber size increases in this zone (Table 2). Unlike the superficial zone, chondrocytes in the middle zone exhibit a rounded morphology and have synthetic organelles. In addition, the collagen fibrils transition from a tangential orientation in the superficial zone to a random orientation here, to finally reach a perpendicular orientation in the deep zone (Figure 1).

The collagen fibrils in the deep zone are the largest in diameter. They are organized in radial directions (perpendicular to the articulating surface) and are inserted across the tidemark (a visible basophilic line that separates deep and calcified zones). The functional role of these collagens is to strengthen the bond between cartilage and bone.[19,32] The chondrocytes are packed in columns parallel to the organized collagen fibers (Figure 1). Moreover, cells in the deep zone show 10-fold higher synthetic activities although they only have twice as much surface area and volume than cells in the superficial zone.[34] It was observed that cells from the deep zone attach and spread faster on tissue culture plastic (TCP) and synthesize more keratin sulfate than cells from the upper zones.[35]

The transitional zone from articular cartilage to subchondral bone is the calcified zone, which contains few inert chondrocytes embedded in a calcified ECM. It is the only zone having collagen type X, which helps cartilage mineralization and provides structure integrity.[36]

From a matrix point of view, articular cartilage is classified into three regions including territorial, interterritorial, and pericellular matrices based on their distances from the cells. The thin pericellular matrix is composed of proteoglycans, collagen type VI, and other non-collagenous proteins. This matrix closely surrounds individual or a column of chondrocytes and protects the cells from various mechanical loads.[36,37] The interterritorial matrix is farther from the cells and is made of organized collagen fibrils that are the largest in diameter when compared across the three types of matrices.[7,37] This matrix accounts for a large percent of the total matrix volume.[36] Finally, the territorial matrix is the farthest matrix from cells, and it consists of collagen fibrils that may be less organized than the other two matrices.[7]

II.C. Mechanical Properties of Articular Cartilage

Due to the small volume of articular cartilage, the amount of shock and energy that can be absorbed by cartilage during normal activities are far less than those taken up by surrounding muscles, tendons, ligaments and the underlying bones.[29] However, the fiber-reinforced, permeable articular cartilage plays a unique role in repeatedly dissipating compressive loads, redistributing loading forces, and lowering joint frictions.[38] According to the biphasic cartilage model,[39,40] cartilage is composed of liquid and solid phases, and the interactions between these two phases characterize the viscoelastic properties of this tissue. The incompressible interstitial fluid phase of cartilage encounters friction as it flows out of the porous collagen-proteoglycan solid matrix. This frictional drag counterbalances the compressive forces applied onto the tissue. Fluid exudation from the tissue also serves to lubricate the joint during loading.[41]

Aside from structure, cartilage composition is also important in determining the tissue's biomechanical properties (e.g., tensile, compressive, and shear). As mentioned above, collagen fibrils are the main contributors to the tensile properties of articular cartilage. Since

different zones have different collagen diameters and organization, the tensile properties vary significantly among zones. For example, Akizuki and associates[42] measured tensile moduli of human knee joint cartilages and found that the equilibrium tensile modulus value was higher in the superficial zone (10.1 MPa) as compared to the other zones (e.g., 5.4 MPa in the middle zone). This can be attributed to fact that collagen is the most abundant and organized in the superficial zone.[20] Within the same study, it was also shown that high weight-bearing areas have lower tensile modulus values than low weight-bearing areas.

Compressive properties of articular cartilage are important because the cartilage tissue is frequently compressively loaded during physiological use. Through confined compression, unconfined compression, or indentation methods,[20] the compressive properties of articular cartilage have been evaluated. Generally, compressive moduli change with the depth and location.[43,44] It was reported that the compressive modulus increased nearly 27-fold from the superficial zone (0.079±0.039 MPa) to the deepest zone (2.10±2.69 MPa) in bovine articular cartilage.[44] In addition, human articular cartilage's aggregate equilibrium compressive moduli, a measure of the solid ECM stiffness, may range from 0.1 to 2 MPa depending on location.[17,21,45–47]

III. TRADITIONAL STRATEGIES AND PROBLEMS FOR ARTICULAR CARTILAGE REPAIR

III.A. Articular Cartilage Injuries

Articular cartilage defects, which are caused by traumatic destruction or degenerative joint diseases, are primarily divided into two categories: partial-thickness and full-thickness cartilage defects.[48,49] The partial-thickness defects only damage the zonal articular cartilage but do not penetrate into the underlying subchondral bone, rendering the defect site inaccessible to blood cells, bone cells, and progenitor cells in bone morrow.[49] Thus, the defect site lacks fibrin clots and other self-healing responses. Although some metabolic and enzymatic activities occur and chondrocytes may begin to proliferate and synthesize ECM right after the creation of a partial-thickness defect, there are still not enough new chondrocytes to migrate into the injured sites to effectively repair the injury. Furthermore, the reparative activities of chondrocytes typically cease before the cartilage defect is healed, thus resulting in a lasting defect that reduces tissue function and can serve as a starting point for tissue degeneration.[29,49]

Full-thickness (or osteochondral) defects penetrate the entire thickness of articular cartilage, beyond the calcified zone, and into the subchondral bone. Unlike partial-thickness defects, full-thickness defects are accessible to mesenchymal progenitor cells, macrophages, and blood cells,[49] all of which are involved in a spontaneous immune response and a healing process after injuries as described elsewhere.[48–50] Briefly, immediately following injury, the defect void is filled with a fibrin clot and an inflammatory response is activated. Next, mesenchymal stem cells from bone marrow migrate into the defect, gradually replacing the fibrin clot and completely filling the defect after one week.[49] Many of these mesenchymal stem cells can differentiate into chondrocytes later, which secrete a proteoglycan-rich ECM and repair the damaged cartilage tissue. However, it has consistently been observed that fibrous, not hyaline, tissues with weaker mechanical properties and higher permeability are formed in defect sites.[51,52] Consequently, the spontaneous repair process in full-thickness defects is only transient and imperfect, and tissue degeneration eventually occurs several months later and proceeds continuously.[49,50,53] After this point, the cartilage tissue often becomes hypertrophic and is finally replaced by the progressive deposition of subchondral bone.[48,49] At this point, while chondrogenesis may still occur sporadically, complete

resurfacing is rarely observed, leading to bone to bone articulation, inflammation, significant pain, and disability.

III.B. Traditional Therapies and Problems for Articular Cartilage Repair

1. Microfracture—Microfracture surgery is one quick and common method to treat smaller articular cartilage defects. Inspired by the spontaneous repair process of full-thickness cartilage defects, this method aims to create microfractures in the underlying subchondral bone via drilling, shaving, or abrasion.[48] Microfracture causes the subchondral bone to release bone marrow progenitor cells and, as expected, repair occurs similar to full-thickness defects. This treatment is effective especially for small articular cartilage defects (e.g., < 2 cm^2), and is attractive due to its relatively minimally invasive nature, short surgery and recovery time, and low morbidity.[12] However, it should be noted that articular cartilage repair results using microfracture has high inter-patient variability. Younger patients, earlier treatment of defects,[12] or smaller lesions may yield better cartilage repair, particularly since mesenchymal stem cells, the cell type responsible for repair, are more abundant and active in younger patients.[54,55] In some cases, little or no hyaline cartilage is regenerated, and the generated hyaline cartilage may turn over into weaker fibrocartilage, thus resulting in high failure rates and limiting microfracture surgery effectiveness.[11,48,56]

2. Autologous Chondrocyte Implantation—As the first generation of cell transplantation techniques for cartilage repair,[57,58] autologous chondrocyte implantation (ACI, also known as the Carticel® procedure by Genzyme corporation, MA) has been accepted and used widely. It has been recommended for patients who have cartilage lesions between 1 cm^2 and 12 cm^2, or have had previously failed microfracture surgeries.[59] There are two surgeries involved in this technique. In the first surgery, a small piece of healthy cartilage is harvested from the low weight-bearing area of a patient's knee. Then, chondrocytes are retrieved from the cartilage tissue and further expanded *in vitro* for 3–5 weeks on monolayer to obtain sufficient numbers for reimplantation (approximately 12×10^6 cells).[60] A second surgery then occurs to inject the cells into the trimmed and prepped lesion, and a periosteal patch from the patient's shin bone is sutured as a cover to secure chondrocytes within the injured site.[60] Although many satisfactory results have been reported, this technique still has some limitations and disadvantages. For example, the invasive ACI procedure has a long recovery time and requires multiple surgeries to harvest healthy cartilage, to harvest a periosteal patch, and to re-implant the healthy cells.[61] In addition, the possibility of periosteal hypertrophy, dedifferentiation of patients' chondrocytes during *in vitro* culture, and decreased human chondrocyte number or cellularity with aging may impair or even result in the failure of repair using ACI.[12,62,63]

3. Autografts and Allografts—Autografts and allografts are two other popular therapies for repairing small cartilage lesions. For an osteochondral autograft, healthy, cylindrical cartilage tissue plugs are harvested from a patient's low weight-bearing area and are then implanted into defect sites to restore function.[12] Encouraging clinical results and excellent tissue integration associated with autografts have been reported.[64] However, there are some limitations related to autografts including insufficient donor tissues (both in quantity and quality), donor site morbidity, surface mismatch of the graft and implant sites, graft instability, and long-term survival of the implant at its new high weight-bearing location considering that it was harvested from a low weight-bearing region.[12,48] Using the autologous mosaicplasty technique that implants many small osteochondral autografts into one defect site, a smoother contour can be created for small or medium defects.[64,65] Smaller donor tissues have three significant advantages. First, smaller donor site defects are produced, and donor site morbidity is reduced. Second, more sites can serve to provide donor tissue as compared to only sites that are as large or larger than the defect to be filled.

Third, the smaller plugs address the surface congruity and contour problems seen with only one large plug. The technique has shown promising results for treating 1–4 cm^2 articular cartilage lesions at short, middle, and long term follow-ups.[66,67]

Osteochondral allografts adopt cartilage tissues from tissue banks, thus avoiding donor site morbidity, and alleviate the insufficient donor tissue supply. Allografts also circumvent the multiple step surgeries required in autograft procedures. However, it has similar limitations to autografts, such as contour matching and load-bearing capacity (which is typically reduced during processing). The use of allografts may also induce immune reactions such as inflammation or rejection. Finally, allografts contain dead cells that cannot maintain the articular surface. Whereas cartilage has been shown to secrete proteins to lower the friction of its articulating surface, the dead cells of allografts do not replace this function.

4. Total and Partial Joint Replacements—For severe joint injuries, disease, or advanced osteoarthritis, articular cartilage cannot be recovered by any of the above discussed treatments. In these cases, total or partial joint replacements are performed to help patients restore normal function. In joint replacement therapies, the damaged osteochondral tissue is partially or totally removed and resurfaced. An artificial implant composed of a metal shell (such as titanium, stainless steel, or alloys), a polymer piece (such as polyethylene in order to glide smoothly), and a metal stem is implanted to replace the damaged joint.[14] As the average age of the population increases, there is a potentially large market for total knee and hip replacements. However, due to frequently reported complications including infection, implant loosening, osteolysis, implant wear and tear, and relatively short life spans of current implants, revision surgeries are often a necessity which burdens the patient with increased pain and health insurance costs.[14]

IV. PROMISE OF TISSUE ENGINEERING FOR ARTICULAR CARTILAGE REPAIR AND REGENERATION

IV.A. The Concept of Tissue Engineering

Tissue engineering (sometimes called regenerative medicine, though the latter refers primarily to the use of stem cells) is an emerging interdisciplinary research field initially defined in the early 1990s.[68–70] It uses principles and methods in engineering, material science, biology, and chemistry to develop biological substitutes that restore, maintain, or improve functionality of damaged tissues and organs. In the ensuing years, this discipline quickly developed to encompass a variety of cell types (e.g., stem cell, chondrocytes, osteoblasts, endothelial cells, fibroblasts, and smooth muscle cells), scaffolds (e.g., biodegradable, natural or synthetic materials, polymers, and nanocomposites), bioactive factors (e.g., various growth factors and cytokines), and physical stimuli (mechanical, electrical, etc.) to form biomimetic tissues (Figure 2). In the following sections, cells, scaffolds, bioactive factors, and mechanical stimuli for articular cartilage tissue engineering will be discussed in detail.

IV.B. Cell Sources for Articular Cartilage Repair and Regeneration

1. Chondrocytes—Chondrocytes are the sole cell type in articular cartilage and are 10–13 μm in diameter.[71] Clinically, these have served as the only cell source for articular cartilage repair. Autologous chondrocytes have been extensively used in articular cartilage repair and regeneration; however, there are some limitations. For example, autologous chondrocyte availability is limited and cannot satisfy the high cellular demand of articular cartilage repair. Although *in vitro* cell expansion methods, such as those used in ACI, have been adopted to increase cell numbers for transplantation, chondrocytes may dedifferentiate during *in vitro* culture.[72]

Other readily available alternative chondrocyte sources (such as allogeneic or xenogeneic chondrocytes) have also been widely studied. However, these chondrocytes can potentially induce immune responses and transmit diseases. Thus, the field of allogeneic and xenogeneic chondrocyte sourcing requires further investigations to mitigate such concerns. Another area of investigation is the use of separately seeded zonal chondrocytes toward regenerating biomimetic functional cartilage tissue,[29,73] since chondrocytes from each of the four zones have been shown to exhibit different properties.[74,75] To more efficiently form different sizes of cartilage tissues with suitable mechanical properties, the various cell sources described above have also been grown in numerous biocompatible scaffolds and treated with growth factors for articular cartilage tissue engineering applications, which will be discussed later.

2. Stem Cells—Due to the many aforementioned limitations related to chondrocyte sources, there is much effort to explore better alternative cell sources. Desirable characteristics for such sources include accessibility, availability, and chondrogenic capacity. Consequently, stem cells such as adult mesenchymal stem cells (MSCs) and embryonic stem cells (ESCs) have emerged as promising cell sources for articular cartilage tissue engineering.

Functionally, the broad definition of stem cells originates from two unique properties: the self-renewal capabilities that can generate numerous descendant cells identical to the mother cells while maintaining an undifferentiated state throughout, and the potent ability to differentiate into multiple types of specialized cells. Figure 3 illustrates the hierarchical structure of stem cells. According to the number of cell types that can be differentiated from them, human stem cells can be classified into four types: totipotent, pluripotent, multipotent, and unipotent stem cells.[76] Morula cells are totipotent stem cells, which have the ability to differentiate into any tissue in human body, while ESCs, usually harvested from the inner cell mass in a 5–6 day old blastocyst from artificial *in vitro* fertilization, are pluripotent stem cells. Pluripotent stem cells are almost totipotent; however, they cannot differentiate into placental cells. Multipotent stem cells including adult stem cells derived from many parts of the body have the ability to differentiate into multiple closely-related cell types only. For example, MSCs, as shown in Figure 3, are able to differentiate into cartilage, bone, muscle, etc., while hematopoietic stem cells can create all blood cell types such as red and white blood cells, but not cartilage, bone, and muscle. Lastly, unipotent cells are the non-strictly defined stem cells that only differentiate into one cell type but have self-renewal capabilities.

a. Mesenchymal Stem Cells: Autologous MSCs from a variety of human tissues including bone marrow, fat, synovium, periosteum, skeletal muscle, skin, etc., have been widely investigated in regenerative medicine for small and large cartilage defect repair.[77–79] These autologous MSCs have a high enough proliferative capacity to expand to enough cell numbers without losing their MSC phenotype, and they do not induce immune responses as allografts and xenografts do. In addition, they can be easily isolated from many mesenchymal tissues (especially the minimally invasive procedure to isolate MSCs from adipose and skin tissues), which may decrease donor site morbidity and patient pain compared to autograft and ACI therapies.[71]

Many methods have been studied to induce chondrogenesis of MSCs (typically marked by GAG and collagen type II production).[71] Various transforming growth factors (e.g., TGF-β1 and TGF-β3), insulin-like growth factors (e.g., IGF-I), dexamethasone, bone morphogenetic proteins (e.g., BMP-2 or BMP-6), and fibroblast factors are supplemented in media,[77,80–85] and mechanical stimuli such as hydrostatic pressure[86] and cyclic compression[87] have all been reported to improve the chondrogenic differentiation of MSCs.

Chondrogenic potentials of MSCs from different tissues have also been investigated and compared.[88–91] Specifically, MSCs from bone marrow (Figure 4) are the most popular considering they are easily harvested (via the iliac crest) and have good chondrogenic potential. Many *in vitro* and *in vivo* studies have revealed promising results of marrow-derived MSCs combined with various biomaterials or growth factors for repairing cartilage defects.[92–96] For example, Koga and associates[88] embedded MSCs isolated from bone marrow, synovium, adipose tissue, and muscle of adult rabbits in collagen gels and then implanted them into full-thickness cartilage defects in rabbits. Their results demonstrated that MSCs from bone marrow and synovium had greater chondrogenic capability *in vivo* than those from other mesenchymal tissues. Another study showed that synovium-derived MSCs proliferated faster than bone marrow-derived MSCs when cultured in autologous human serum, thus serving as another promising cell source for cartilage regeneration.[97]

Although *in vitro* studies have shown that adipose-derived stem cells (ASCs) may have lower chondrogenic potentials than bone marrow-derived MSCs,[89–91] ASCs still attract increasing attention for cartilage tissue engineering because of their abundance and ease of procurement (e.g., high yields of ASCs obtained from waste adipose tissues via liposuction).[98] In addition, the minimally invasive acquisition of ASCs from subcutaneous adipose tissue circumvents donor site morbidity and pain. Chondrogenic growth of human ASCs has also been reported to occur on different biomaterial scaffolds including agarose, alginate, and biologically active gelatin to create tissue engineered cartilage constructs.[99]

It is important to note that most of the current MSC transplantation studies are still in preclinical trials. Only a few results are available from clinical trials of MSC-based articular cartilage repair on patients.[100–103] Wakitani and colleagues[102] evaluated clinical results of autologous bone marrow-derived MSC transplantation into the knees of three patients. The bone marrow-derived MSCs were harvested from the iliac crests of the patients, expanded *in vitro*, embedded into collagen gels, and then reimplanted into 9 full-thickness articular cartilage defects of the patients' patello-femoral joints. The patients' clinical symptoms were improved after 6-month transplantation and maintained satisfactory performance during 17–27 months. In addition, as a leading stem cell company, Osiris Therapeutics Inc. has developed a manufacturing process to expand human bone marrow-derived MSCs for clinical use. Their stem products, such as Chondrogen, have shown significant therapeutic potential for preventing osteoarthritis during Phase I or II clinical trials.

Based on the results from preclinical and clinical studies, MSC transplantation exhibits tremendous promise for promoting articular cartilage repair and regeneration. Thus, more work is needed to optimize MSC culture conditions, understand underlying chondrogenic differentiation mechanisms, regenerate biomimetic MSC-based cartilages, and explore clinical therapies for successful human cartilage regeneration.

b. Embryonic Stem Cells: When compared with multipotent adult MSCs, ESCs have features of unlimited proliferation (seemingly immortal) and almost universal differentiation potential into any somatic cell type.[71] These features make them promising for tissue regeneration demanding large numbers of cells (e.g., traumatic cartilage defects). To date, *in vitro* and *in vivo* studies have provided some evidence of direct chondrogenic differentiation of ESCs via growth factors such as BMP-2, BMP-4, TGF-β1[104] and TGF-β3,[105] or via co-culture with primary chondrocytes,[106] embryonic limb bud cells[107]. For human ESCs, Koay and associates[108] developed a novel scaffold-free, modular approach that first differentiates human ESCs in serum-free, chemically-defined conditions and then assemble them into neocartilage constructs for cartilage tissue engineering applications (Figure 5). This scaffold-free, modular approach has also been applied to fibrocartilage tissue engineering.[109] In addition, Hwang and colleagues derived MSCs from human ESCs and

demonstrated *in vivo* commitment and cartilaginous tissue formation from the MSCs by using chondrocyte-secreted morphogenetic factors.[110] ESCs have also been seeded into various biocompatible scaffolds such as polycaprolactone,[111] 3D fiber-deposited scaffolds,[112] and poly(ethylene glycol)-based (PEG) hydrogels[113] to induce their chondrogenic differentiation. One of the current challenges in scaffold development is in fabricating materials that can improve differentiation efficiency via controlled release of growth factors, linked peptides, and other biochemical methods. As ESCs may also respond to mechanical forces by shifting their lineage, scaffold load-shielding effects should be considered, but are yet to be investigated, in the area of scaffold development for stem cell use. In short, there is a plethora of biochemical methods being pursued in conjunction with scaffold effects on differentiation, but few investigators are examining the effects of scaffold mechanics on differentiation.

Since ESC research is still in its infancy stages, there are many unexplored areas and ethical concerns related to their clinical applications. For example, we still do not know the best method to selectively differentiate ESCs into desirable cell lineages at injury sites to regenerate desirable tissues. It is possible that multiple tissues are formed out of ESC-differentiation, resulting in an undesirable teratoma.[114] Due to the allogeneic nature of ESCs, potential immunogenicity problems also exist for clinical transplants. Additionally, since a layer of feeder cells like mouse embryonic fibroblasts are normally adopted to culture ESCs, animal pathogens may potentially be introduced.[115] It is, however, possible to use human fetal and adult fibroblasts as safer alternative feeders to support human ESC growth.[116] More importantly, there are concerns about the sources of blastocysts, the safety of ESCs, and so on[115] for ESC research and clinical applications. Obviously, these issues require more investigations to fully explore the medical potential of this flexible cell source.

3. Other Cell Sources—An equally exciting potential cell source is the dermis of the skin.[117–122] Considering its relative abundance and accessibility, the dermis is considered one of the best autologous source organs to isolate stem/progenitor cells for future therapeutic applications. This is true not only in the replacement of skin,[123–125] but also as an alternative cell source for several other organs. Human dermal fibroblasts cultured with demineralized bone powder have been shown to acquire a chondroblastic phenotype.[117–121,126–132] Chondro-induction has also been shown for the human foreskin fibroblast cell line Hs27 and the adult rabbit dermal fibroblast cell line RAB-9 when cultured on aggrecan-coated surfaces.[133] Several types of fibroblasts exist in the dermis,[134–136] and not all dermis subpopulations may possess latent chondro-induction potentials. From these, a dermis-isolated, aggrecan-sensitive subpopulation has also been shown to yield engineered constructs containing cartilage specific matrix.[122]

IV.C. Tissue Engineering Scaffolds for Articular Cartilage Repair and Regeneration

For tissue engineering applications, biomaterial scaffolds play a critical role in providing a 3D environment to support cell growth, matrix deposition, and tissue regeneration. An ideal tissue engineering scaffold should satisfy several essential criteria: it should (1) be biocompatible to minimize local tissue response but maximize cell growth and tissue integration; (2) be biodegradable with a favorable resorption rate, which can provide structural support for the initial cell growth and then gradually degrade after new tissue formation; (3) have suitable porosity and interconnectivity to allow cell migration and efficient exchange of nutrients and wastes; (4) possess appropriate mechanical properties to support tissue growth under native mechanical loads.[71,137] To date, a range of biomaterial scaffolds including natural polymers extracted from living organisms and synthetic materials obtained from various chemical processes have been widely investigated for tissue repair and regeneration.[71] The most extensively used natural or synthetic scaffolds and the

emerging nanostructured scaffolds in cartilage tissue engineering will be described in detail next.

1. Natural Scaffolds—Natural biomaterials are the popular scaffolds for cartilage repair and regeneration due to their good biocompatibility for cell attachment and differentiation. Specifically, natural scaffolds used in articular cartilage tissue engineering include carbohydrate-based hyaluronic acid, agarose, alginate, and chitosan, and protein-based collagen or fibrin glue.[137]

As a non-sulfated glycosaminoglycan derived from ECMs of many tissues, hyaluronic acid (or hyaluronan) has been used to support chondrocyte growth or stimulate MSC chondrogenesis.[138,139] For example, a hyaluronan-based scaffold (Hyaff-11) seeded with autologous chondrocytes has shown to be effective in regenerating cartilage tissues *in vivo*.[138] In addition, a minimally invasive surgical technique using hyaluronan as an injectable material has shown promise in healing cartilage defects.[71]

Agarose and alginate are polysaccharides derived from seaweed and used as biocompatible 3D scaffolds to encapsulate cells for cartilage tissue engineering. Agarose gel is obtained through changing temperatures, and a cross-linked alginate matrix can be formed via ionic bonding in the presence of Ca^{2+}. Both of these scaffolds have exhibited excellent cytocompatibility for cell growth[140–142]: however, the poor degradation properties and the difficulty to modify the scaffolds' life[71] may hinder their clinical applications for tissue regeneration.

Collagens are main protein components in natural cartilage, bone, and other connective tissue ECMs. They contribute to cell adhesion, proliferation and differentiation,[143] and, thus serve as one of the most common scaffold materials for cartilage tissue engineering. Many studies have demonstrated that a combination of collagens (such as type I and type II collagens) with chondrocytes and stem cells facilitated cartilage tissue growth *in vitro* and *in vivo*.[51,144–146] Specifically, a clinical therapy named the Matrix-induced Autologous Chondrocyte Implant (MACI® implant, Genzyme) has been developed. In this method, chondrocytes are expanded in a collagen membrane and then reimplanted into articular cartilage defects without suturing. Moreover, other natural scaffold materials like the biodegradable fibrin, chitosan or composites thereof are widely studied and have shown potential to enhance cartilage tissue regeneration.[71]

2. Synthetic Scaffolds—Due to the ease of fabrication and chemical modification, good biocompatibility, high versatility, suitable mechanical properties, and controllable biodegradability, polymers currently elicit increasing interest from scientists who are investigating their potential as synthetic cartilage tissue engineering scaffolds.

The most popular synthetic polymers for cartilage tissue engineering scaffolds are poly lactic acid (PLA, which is present in both L and D forms), poly-glycolic acid (PGA), and their copolymer poly-lactic-co-glycolic acid (PLGA). These FDA approved biodegradable polymers can be fabricated into 3D matrices via particulate leaching, textile technologies, or three-dimensional (3D) printing techniques, etc.[14,147] The fabricated polymer scaffolds have a controllable porosity and a suitable surface structure for cell attachment, proliferation, and differentiation. In particular, it has been shown that PGA improved proteoglycan synthesis when compared to collagen scaffolds.[148] In addition, increasing chondrogenesis was observed in a chondrocyte/PGA/bioreactor system over 40 days of cultivation.[149] PLLA has a slower degradation rate than PGA, and, thus is suitable for those applications requiring a longer duration of matrix structural supports. As a derivative copolymer of PLA and PGA, PLGA has high biocompatibility, an ability to degrade into harmless monomer units, a

useful range of mechanical properties, and controllable degradation time depending on the copolymer ratio.[14] Studies demonstrated that nonwoven PLGA scaffolds are suitable for the chondrogenesis of human adipose-derived stem cells.[150] Additionally, PLGA scaffolds have been loaded with various chondrogenic factors like TGF-β1 and dexamethasone to improve chondrogenic differentiations of bone marrow-derived MSCs.[151] Other polymers including poly(ε-caprolactone) (PCL) and PEG have also received substantial attention for articular cartilage tissue engineering.[71]

However, some disadvantages related to using synthetic polymers in cartilage engineering applications are still present. For example, although synthetic polymers have flexibility in design, they may lack the optimal cytocompatibility properties that natural materials possess for cell growth and may elicit a host response caused by the release of toxic byproducts during degradation. Therefore, there is a desire to design composite scaffolds combining the respective advantages of synthetic and natural materials to improve cartilage tissue repair and regeneration. For instance, fibrin glue, alginate, and hyaluronan have been used to modify various PLGA, PGA, PCL scaffolds,[152–155] and the results revealed that these composite scaffolds can stimulate the chondrogenesis of different chondrocytes or progenitor cells, thus warranting further investigations.

3. Nanostructured Tissue Engineering Scaffolds—Conventional natural or synthetic scaffolds still require improvement to yield better biocompatibility and functional properties for cartilage regeneration. Since natural cartilage tissues are nanometers in dimension and chondrocytes directly interact with (and create) nanostructured ECMs, the biomimetic features and excellent physiochemical properties of nanomaterials play a key role in stimulating chondrocyte growth as well as guiding cartilage tissue regeneration.[156] Although it is a field in its infancy, many investigators are currently seeking to fabricate biomimetic nanostructured tissue engineering scaffolds encapsulating cells (such as progenitor cells and chondrocytes) for repairing and regenerating cartilage tissues.

Nanofibrous or nanoporous polymer matrices can be fabricated via electrospinning, particulate leaching, chemical etching, 3D printing techniques, and phase separation. For cartilage applications, there has been great interest in incorporating chondrocytes or stem cells into the 3D polymer or composite nanofibrous scaffolds through electrospinning.[111,157,158] For example, *in vitro* chondrogenesis of bone marrow-derived MSCs was evaluated in an electrospun PCL nanofibrous scaffold and compared with an established cell pellet culture.[157] The electrospun nanofibrous PCL scaffold effectively induced chondrogenic differentiation of MSCs and finally formed a tissue engineered construct with plentiful cartilaginous matrices. In addition, the easily fabricated and modified nanofibers possessed much better mechanical properties compared to the cell pellets, and thus the electrospun nanofibrous PCL scaffold presented itself as an ideal candidate for stem cell transplantation during clinical cartilage repair. Because the small pore sizes of nanofibers may inhibit cell infiltration, uneven cell distributions may occur throughout the electrospun nanofibrous scaffolds. Therefore, a recent study improved chondrocyte seeding technology and created a more homogeneous cell–PLLA nanofiber composite.[158] It was observed that chondrocytes were uniformly present throughout the entire cell-nanofiber composite, and the scaffold developed into a smooth, cartilage-like tissue with more total collagen and improved mechanical properties in a dynamic bioreactor relative to one obtained in static culture. Moreover, another study observed significantly increased chondrocyte functions (adhesion, proliferation and matrix synthesis) on 3D nanostructured PLGA created via chemical etching.[159]

Besides research effort of pursuing optimum cytocompatibility properties of the above mentioned biomaterial scaffolds, mechanical characteristics of biomaterials are another

critical consideration for designing cartilage tissue engineering scaffolds. Due to the different tissue loading environments, different natural or synthetic scaffolds should be chosen to provide appropriate mechanical properties for cell adhesion and tissue regeneration. For example, metal, ceramics or ceramic reinforced polymer composites with robust mechanical properties have been used for bone repair, which requires more rigorous mechanical loading.[156] Since articular cartilage is under continuously excessive loading environments, the mechanical mismatch between implanted scaffolds and surrounding tissues may frequently deteriorate cartilage regeneration at defect sites and then lead to implant failure. Thus, biomaterial scaffolds with both superior biocompatibility and suitable mechanical properties similar to cartilage are desirable for articular cartilage tissue engineering.

IV.D. Scaffold-free Cartilage Tissue Constructs

1. Scaffold-free Methods—Aside from using scaffolds, several scaffold-free techniques for generating neocartilage have been investigated including organ, pellet, aggregate cultures, and, more recently, a self-assembling process.[160–167] These techniques do not employ exogenous materials at all and were initially used to study chondrocyte phenotype, metabolism, development, and disease. Within these methods, the state of the art in chondrocyte culture by the late ‘80s was severely limited by diffusion, and, with few exceptions,[165] resulted in tissues less than 500 μm in size.[165,168–170] Replacement cartilage would need to be of native articular cartilage thickness (1–3mm or thicker),[17,171] and, as described below, recent techniques are capable of delivering constructs of similar size.

In the past few years, resurgence in scaffold-free culture has benefited from the knowledge developed using scaffold systems (e.g., growth factor and mechanical stimuli) to culminate in the production of thicker, more clinically relevant sized cartilage constructs that demonstrate functional characteristics.[172–174] As an example, chondrocytes were seeded on (instead of into) a non-adhesive hydrogel mold in a process termed self-assembly.[167] As the system minimized its free energy, the cells associate and coalesce to form neocartilage free of exogenous biomaterials and unaffected by adhesion to any surface other than each other (Figure 6).[175] Several other forms of scaffoldless culture techniques exist. For example, high density culture of chondrocytes on tissue culture plastic, culture insert membranes, and silicon molds have been used to create constructs ranging from 0.5 mm to 2.9 mm in thickness.[176] Some scaffoldless constructs have also been examined *in vivo*.[177,178]

For scaffold-free constructs formed on an adhesive surface, the aggregate modulus values of engineered constructs have been reported at 41.6 kPa at 8 wks.[176] In contrast, when chondrocytes self-assemble over an agarose gel (of 98% water), cell-biomaterial interactions are greatly reduced; engineered cartilage formed thusly has been shown to reach aggregate modulus values of over 150 kPa.[179] The differences in the properties observed may be due to whether seeding occurs onto an adhesive or non-adhesive biomaterial. Surfaces where proteins may adsorb to allow for cell attachment or spreading can alter cartilage tissue formation, which has been shown to be mediated by cadherin[175,180,181] and integrin binding.[182–184] These recent observations serve to explain why, in a comparison between chondrocytes self-assembled over an adhesive TCP surface versus over agarose, the spreading and attachment onto TCP resulted in constructs with a corrugated appearance and significantly lower mechanical properties.[167]

Other scaffold-free methods include aggregate[165,168–170] and pellet culture.[185–189] In these cases, mechanical forces are generally present during construct formation. Aggregates are often formed by orbital shaker culture, and pellet cultures are formed under centrifugal forces. Shear forces present in orbital shaker culture can be detrimental to chondrocyte culture.[190,191] Driven to quickly form constructs via centrifugation, pellet culture not only

applies forces during construct formation, but may also alter cell-cell interactions that characterize other methods of tissue formation more akin to cartilage development.[175,180–184,192] Rather than using centrifugation or rotational culture to form aggregates, chondrocyte self-assembly allows for minimization of free energy by only allowing cell-cell interactions, e.g., N-cadherin binding; in morphogenesis this is described as the Differential Adhesion Hypothesis.[192–194]

2. Advantages of Scaffold-free Culture—Scaffold-free cultures offer certain advantages over scaffold use. Morphological change, brought on by spreading, has been strongly linked to chondrocyte dedifferentiation via cytoskeletal changes.[195,196] In retrospect, it is now known that diminished levels of collagen II and superficial zone protein (SZP) expression seen immediately upon monolayer culture due to chondrocyte dedifferentiation are unrecoverable.[72] Similar to monolayer culture, cells seeded onto polymer biomaterials spread,[197–200] and thus may dedifferentiate.

Though chondrocytes are allowed to retain their spherical morphology, gel encapsulation can limit cell-cell communication[188,201] to inhibit ECM synthesis. This is in contrast to the high cadherin and integrin activity, shown to be active during cartilage development, observed during scaffold-free culture.[175,182]

Additionally, as chondrocytes are highly mechanosensitive, scaffold materials may result in stress shielding that limits beneficial mechanotransduction.[202] Finally, as with any implanted biomaterial, there are concerns regarding potential toxicity of degradation byproducts and immune responses.[203]

IV.E. Growth Factors and Mechanical Stimuli for Improving Cartilage Tissue Repair and Regeneration

1. Growth Factors—The hormonal and growth factor regulation of chondrocyte aggregation,[204] adhesion,[205] growth,[206] and metabolism[207] have been investigated since the late ‘60s and ‘70s. Cartilage tissue engineering using growth factors coincided with the development of scaffold materials in the ‘90s. From this point, the literature can be separated into two categories: techniques to incorporate growth factors into biomaterials and the effects of growth factors on chondrocytes. For the former, specific techniques on retaining activity, controlled release, and other parameters particular to different biomaterials constitute a major area in materials research. As the technologies applicable to different scaffold systems are unique in themselves, it is impossible to present a comprehensive overview (the reader is instead directed to a recently published textbook that elaborates in depth on this subject).[71] Nonetheless, the development of techniques that combine growth factors with scaffolds remains an active and populous area of research. For the latter, research in cartilage tissue engineering has reached a consensus via progress from the ‘90s and beyond that growth factors are beneficial in improving functional properties. IGF-I, basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and platelet-derived growth factor (PDGF) and the TGF-β superfamily are some of the most actively researched growth factors today.

Various growth factors that have shown to have effects on proliferation, differentiation, and synthesis include IGF-I, bFGF, HGF, and PDGF.[208] IGF-I has been shown to mitigate injurious response of impacted cartilage by limiting the loss of matrix components.[209] This may be due to IGF-I reducing apoptosis caused by a disruption of the collagen network.[210] Applied to chondrocytes, IGF-I has been shown to increase collagen and proteoglycan deposition,[211] though their effects are different across cartilage zones.[212] Research on HGF has been limited as it is now considered to be ineffective toward chondrogenesis.[213] PDGF enhances chondrocyte migration[214] and increases SZP expression.[215] However, it also

changes chondrocyte morphology to a spindle-like shape,[216] which, as shown in other studies, detracts from the chondrogenic phenotype.[196] Because of this, PDGF has been used in fibrocartilage tissue engineering, though its effects have been limited.[217] Aside from these growth factors, the most dramatic results have been seen with members of the TGF-β superfamily. Involved in repair and inflammation,[218] the TGF-β superfamily contains several isoforms, such as TGF-β1 and TGF-β3. These have been shown to promote collagen formation and increase construct wet weight.[219,220] Scaffold-free constructs stimulated using TGF-β1 showed approximately 1-fold increases in both aggregate modulus and tensile modulus over controls.[221] Both TGF-β1 and TGF-β3 have been employed in combination with mechanical stimuli, to be discussed later.[86,173,222,223] BMPs are also part of the TGF-β superfamily. These growth factors influence endochondral bone formation, proliferation, matrix synthesis, and defect healing *in vivo*.[218,224] Increased matrix (e.g., proteoglycans and collagen) synthesis, and cell proliferation have been demonstrated using BMP-2,[221,224–226] BMP-4,[224,227] BMP-7,[224,228] BMP-12, and BMP-13.[225] Of these, BMP-1 and -2 have been shown to be particularly beneficial over other growth factors (e.g., other BMPs[225] or TGF-β1[229]). Like TGF-β, BMPs have also been investigated in conjunction with mechanical forces.[173,230]

2. Mechanical Stimuli—*In vivo*, the synovial fluid reduces friction of articulating surfaces, and tissue shear is minimal. Shear has nonetheless been examined as a tool to induce matrix production. Oftentimes, the application of shear requires that a small amount of compressive strain be applied to maintain contact between the two surfaces.[231–233] Dynamic shear of 1–3% at 0.01–1 Hz has been shown to increase ECM synthesis.[233] Within this range (dynamic shear of 2% at 1 Hz) it has been shown that a 6-fold higher equilibrium modulus in constructs compared to unstimulated controls can be achieved with just a short (6 minutes every other day) application of this stimulus.[234] Shear has also been shown to increase cartilage oligomeric matrix protein expression.[235] Shear, applied onto chondrocyte monolayers using a cone viscometer at 1.6 Pa, resulted in a 10- to 20-fold increase in prostaglandin E2 release and 9-fold increase in tissue inhibitor of metalloproteinase mRNA,[236] and shear also increases interleukin-6 and nitric oxide levels.[191,237] These proinflammatory mediators and signs that are observed in osteoarthritis have indicated to some researchers that a shear force may not be the best stimulus for cartilage tissue engineering. Since a method to increase diffusion is via fluid flow, efforts have thus been directed toward reducing shear in systems that attempt to employ fluid flow in increasing nutrient and waste transfer.[238–242] Rotating wall bioreactors are capable of applying shear on the order of ~0.15 Pa,[238] as compared to the 1.6 Pa shown to increase proinflammatory mediators.[236] Rotating wall bioreactors have been shown to increase GAG content within engineered constructs beyond physiological levels, while maintaining collagen levels.[240]

Direct compression, as applied to tissue engineered constructs, has been mostly dynamic, as native cartilage has been shown to respond negatively to static loading.[142,243–247] As previously described, compressive loading has been suspected to increase solute transport[248] in addition to mechanically stimulating the cells.[245] For this reason, it may not be the static force that is causing decreased synthesis, but the lack of diffusion under static compression conditions. It has also been shown that static compression causes a decrease in the pH of the local environment,[245] which may also inhibit synthesis. As with other forms of mechanical stimuli, the main dynamic compression parameters that have been optimized over the past decade are frequency, the duty cycle, the strain or force used, and the duration of the experiment. Over the past 15 years, frequencies ranging from 0.0001 to 3 Hz, strains from 0.1 to 25%, loads from 0.1 to 24 MPa, and durations lasting hours to weeks have been examined at various duty cycles and waveforms.[87,222,243,246,249–257] Many of these experiments were performed on mature native tissue, though the developing environment in engineered constructs can be vastly different. For instance, it is well known that the

pericellular environment serves a unique role in mechanotransduction,[25,37] and this environment is rapidly changing during culture. The effects of ECM or scaffold stress-shielding thus become an apparent obstacle in comparing direct compression studies for tissue engineering. Nonetheless, certain results have been shown across several systems. Dynamic compression at 1 Hz or lower and 10% or lower have typically shown beneficial effects in agarose,[243,250] poly(L-lactide-co-epsilon-caprolactone),[256] and other scaffolds.[257] Particularly, since the local microenvironment is so important in mechanotransduction, it would be interesting to examine the effects of nanomaterials on mechanotransduction. This has been investigated, preliminarily, with shear systems (though the effects of shear itself were not quantified in conjunction with the nanomaterial).[158] However, the combination of nanomaterials with direct compression remains an open field with respect to direct compression.

As described previously, fluid flow out of cartilage is inhibited by the dense matrix and Donnan osmotic pressure. During compression, this inhibition results in elevated hydrostatic pressures. Physiological levels of hydrostatic pressure have been determined to be 7–10 MPa,[258,259] and tissue engineering studies have employed magnitudes within this range as well as hypo- and hyper-physiological forces. Constant hydrostatic pressure applied for long periods has been shown to have a negative impact on matrix secretions and cell viability.[260–262] Above the physiological range, static pressure at 30 MPa in chondrocyte monolayers inhibited proteoglycan synthesis.[261] Low frequencies of hydrostatic pressure (akin to a static application) have also been shown to similarly deter synthesis in isolated cells and explants in physiological and hypo-physiological magnitudes, but the same study also showed that the ECM may alter cellular perception of hydrostatic pressure.[260] From these studies, it was initially presumed that static hydrostatic pressure would not be useful in engineering cartilage, but recent studies have proven otherwise. Application of hydrostatic pressure has thus far resulted in tissue engineered constructs with aggregate modulus values approaching 300 kPa,[172] and its combination with growth factors has shown both additive and synergistic effects in improving construct properties.[173] In this case, the developing, scaffoldless construct contained rounded cells (contrasted with monolayers[261]) and immature ECM that is distinctly different from the previously examined cartilage explants.[260] Aside from static pressure, a window of pressures and frequencies between 0.1 and 15 MPa and 0.05 and 1 Hz have been shown to yield positive results toward cartilage tissue engineering.[263–269]

In addition, mechanotransduction has been well examined as a tool in tissue engineering, as metabolic responses to mechanical forces can precipitate via several coupling mechanisms (Figure 7). For instance, membrane deformation due to direct compression or shear can result in the activation of mechanosensitive ion channels.[270,271] Hydrostatic pressure-sensitive changes to intracellular ion concentration have also been observed in chondrocytes.[272] These changes in intracellular ionic concentrations can activate or suppress various genetic responses. Mechanical forces can also be coupled via integrins and the cytoskeleton. Tethering to the mechanical environment, activated integrins initiate the formation of a focal adhesion complex (FAC). The FAC is formed by recruiting not only other integrins, but also adaptor proteins and several kinases, and these proceed to activate or suppress genes and transcription factors.[273]

3. Combinations of Stimuli—BMP-2 application with IGF-I have been shown to increase the functional properties of engineered constructs.[174] In this case, continuous versus intermittent growth factor treatments were compared, along with the combination of two and three growth factors (BMP-2, TGF-β1, and IGF-1). Neither synergistic nor additive response was observed, and the authors pointed to prior results showing that BMP-2 signal transduction can be inhibited by TGF-β1.[274] TGF-β alone is known to cross-talk with the

mitogen-activated protein kinase (MAPK), P13K/Akt, Wnt, and various other pathways.[275] Application of multiple growth factors continue to be an intense area of research for cartilage tissue engineering.

Growth factors have been examined with shear, compression, and hydrostatic pressure. For instance, shear and increased diffusion combined with BMP-2 induced chondrogenic gene and protein expression when dedifferentiated chondrocytes were cultured.[276] It was also deduced in a separate study that shear, when combined with IGF-I, produce a synergistic effect on chondrogenic synthesis.[232] IGF-I also combined with direct compression to increase proteoglycan and collagen synthesis by 180% and 290%, respectively.[246] TGF-β3 combined with direct compression[223] and TGF-β1 with hydrostatic pressure[173] both increased construct functional properties to native tissue ranges. In the latter case, the growth factor and mechanical stimulus were found to have additive effects on functional properties but synergistic effects on collagen content.[173]

V. CHALLENGES AND FUTURE DIRECTIONS

1. Technical

a. Design Specifications—Cartilage regeneration aided by tissue engineering faces varied challenges, and among them are differences in anatomical geometries and loads for different joints. Large, weight bearing joints require the replacement cartilage to have great compressive stiffness. The patellofemoral problem requires that a high shear-bearing construct be fitted. Clinical intervention in small, non-weight bearing joints suffer from the challenges of tight joint spaces and small radii of curvature. Added to these considerations is the fact that differences in integration have yet to be evaluated across different joints.

Actually, while cartilage must be engineered for functionality, a resultant engineered construct dense in collagen fibers will prove difficult to integrate. An optimal maturity for engineered constructs that balances construct stiffness with ease of integration has yet to be determined. In addition, integration techniques must be developed to ensure that the interface between engineered and native cartilages will not have stress concentrations. Along these lines, new surgical techniques may need to be developed to implant engineered cartilage.

It is difficult to design scaffolds while the design specifications for cartilage therapy are still evolving. Due to the integration issue, it is unclear whether a cartilage product should be designed to be mature (stiff but difficult to integrate) or immature (possibly easier to integrate, but with lower weight bearing capacity). Without clear design standards, the design process for scaffolds may be unclear. For instance, a parameter in scaffold design, degradation rate, will greatly influence the maturation and integrative properties of a resulting construct. It is recommended that, as researchers develop new biomaterials for cartilage engineering, they should also consider how an engineered product using such technology will be put to clinical use.

b. Stimuli interactions—Currently, the pressing questions that drive the area of multiple growth factor use are how best to employ them in sequence and in combination. The interplay of growth factors presented to chondrocytes (or progenitor cells) during cartilage formation is varied and delicate. If sequenced stimuli were to be necessary, controlled release of multiple growth factors at different sequences can be challenging in scaffold development.

Likewise, current challenges in the application of mechanical stimuli are 1) to systematically combine several mechanical stimuli at once, and 2) to investigate the coupled effects of

different classes of stimuli (e.g., growth factor with mechanical stimuli). The roles that growth factors play in altering mechanotransduction constitute an area of intense interest and challenge. Past studies that have combined the two are exciting in their abilities to increase construct functional properties. Some studies have even observed synergism for these different classes of stimuli.[173,232] How such synergism came about, why synergism is not seen for all properties, and, finally, what one should do in order to obtain additional synergistic effects are both interesting and perplexing questions. These must first be addressed before exogenous stimuli can be efficiently applied for cartilage tissue engineering.

Lastly, cartilage tissue engineering studies thus far have focused on anabolic processes, i.e., with creating more cartilage matrix. Recent studies using chondroitinase-ABC (C-ABC) have shown that increased functional properties can be obtained with the selective and timed application of a catabolic enzyme.[277,278] This counter-intuitive method is proof that matrix turnover in engineered constructs should be examined. Thus, catabolic processes can be elucidated and harnessed in producing functional constructs.

2. Regulatory

The progress in cartilage tissue engineering during the past two decades is rapid and humbling. As with other rapidly maturing technologies, standards and regulation have not caught up with the advancements seen in this field. Clearance by the Food and Drug Administration (FDA) now poses as a significant hurdle for companies seeking to translate cartilage engineering technologies to clinical use. The different Centers of the FDA that are relevant to cartilage tissue engineering and potential pathways to market are described below.

a. Structure of the FDA—Of the seven product-oriented centers within the FDA, two are particularly relevant to articular cartilage tissue engineering: the Center for Biologics Evaluation and Research (CBER) and the Center for Devices and Radiological Health (CDRH). For cartilage tissue engineering, a product that is a combination of both biological product and a device will be assigned by The Office of Combination Products to one of the two centers, where primary jurisdiction over the product will reside.

The CBER regulates products whose primary mode of action is metabolic. Past products regulated by this center include blood, allergenics, tissues, and other cellular products derived from living sources. Manufacturers of biological products must follow current good manufacturing practices (cGMP), as described by 21 Code of Federal Regulations (CFR) Part 211[279] and report adverse events to the Adverse Event Reporting System (AERS). Engineered cartilages derived from autologous, allogeneic, and xenogeneic products are produced by cells and will be regulated by CBER if their primary mode of the effects is metabolic.

For implants, the FDA has had a history of classifying most orthopaedic implants as medical devices, which are regulated by the CDRH. The CDRH regulates firms that manufacture, repackage, relabel, and/or import medical devices. A medical device is defined as “an instrument, apparatus, implement, machine, contrivance, implant, *in vitro* reagent, or other similar or related article, including any component, part, or accessory, which is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease.”[280] In these cases, the primary mode of effect for devices is mechanical or electrical.

b. Center Assignment—Potential cartilage therapies can be assigned to CBER or CDRH, depending on the therapy’s primary mode of action. Assignment to different centers

translates to different filing requirements, which result in different financial burdens and time to market.

CBER oversaw the approval of ACI, whose primary mode of action is the metabolic production of cartilage matrix after implantation. The metabolic agents are manipulated, expanded autologous cells. In this case, a Biologics License Application (BLA) was filed for the permission to introduce a biologic product into the market, and the BLA is regulated under 21 CFR 600 to 680. Other requirements include complying with requirements set forth by Form 356h, which includes the applicant information, product and manufacturing information, pre-clinical studies, clinical studies, and labeling. For clearance by the CBER, clinical studies, which can be slow and costly, are required, and these are quire burdensome for companies that seek to introduce an engineered cartilage product whose primary mode of effect is metabolic.

Implants, whose primary function is to bear mechanical load (as tissue engineered cartilage is designed to function), may have CDRH as its primary regulator. Contrast this with the case of ACI: while ACI may eventually result in tissue that bears load, the initial implant does not act through a mechanical effect; instead, a metabolic process takes place where tissues are formed *in situ*. Another example is Medtronic's InFuse Bone Graft/LT-Cage, which is regulated as a medical device, despite containing recombinant human BMP (rhBMP). In this case, the primary mode of action for the product is mechanical. Other products regulated as devices include bone void fillers and demineralized bone matrix, both of which can be biologically derived, but serve mechanical functions during implantation.

Researchers and companies should take into consideration the Center assignment as they develop cartilage therapies, because the pathways to clinical usage are substantially different for each Center. Furthermore, if a company would like to be regulated under CDRH, it should have a plan for which class a product falls into and project the time and financial burdens from there. It is expected that tissue engineered cartilage implants would be examined by either CBER or CDRH. Center assignment is both an industrial and a scientific question. While the industrial aspect is obvious, scientists should likewise be cognizant of how safety and efficacy are demonstrated in designing their studies.

c. Device Classes—Orthopaedic device manufacturing companies, where tissue engineered cartilage therapies are likely to arise, are typically familiar with the CDRH. Devices regulated within the CDRH fall into three classes, each with different requirements that the manufacturer must fulfill prior to introducing a product to market. Again, the relevance to tissue engineering is that the time and money spent under each class differs and will affect whether and how long a therapy takes to reach the clinic.

Class I devices are low risk and pose minimal potential harm. For a Class I device to be approved, a company must demonstrate that it has implemented "general controls," which include quality system regulation (QSR), as described by 21 CFR 820,[281] to ensure adherence to predefined design controls and good manufacturing practices (GMP), label requirements to prevent product mislabeling, and the use of Medical Device Reporting (MDR) (in contrast with the AERS for biological products) to maintain records for the reporting of adverse events. Medical devices must use forms FDA-2891 and FDA-2892 for establishment registration and medical device listing.

Tissue engineered cartilage is more likely to be regulated as Class II or Class III devices. Class II devices are of moderate risk and often require a Premarket Notification 510(k) pathway to market. A manufacturer must notify the FDA 90 days before marketing a Class II device to show that it is substantially equivalent to a predicate device legally in

commercial distribution in the US before May 28, 1976. Either a traditional, special, or abbreviated 510(k) may be filed. A traditional 510(k) takes about 90 days to review. When a device is modified without changes to the intended use, a special 510(k) can be filed, which generally takes 30 days. An abbreviated 510(k) relies on use of guidance documents or special controls to provide a summary report that describes adherence to the relevant guidance document, and can also take less than 90 days. Class II devices may require special controls, such as postmarket surveillance, patient registries, guidances, and standards, after it has been marketed. Most joint arthroplasty components are cleared as Class II devices.

Devices that support or sustain human life, are of substantial importance in preventing impairment of human health, or present a potential, unreasonable risk of illness or injury are classified as Class III. Particularly relevant to tissue engineering, devices for which substantially equivalent predicates are non-existent also fall into this class. New device that are deemed to be substantially equivalent to a predicate Class III will also be classified as Class III. Finally, new devices determined to be substantially equivalent to Class I or II devices that were developed after 1976 will also be classified as Class III. Before legal distribution can occur, a company must submit a premarket approval application (PMA) or Product Development Protocol (PDP). Both preclinical and clinical data are needed to demonstrate safety and efficacy. A new device must first have an investigational device exemption (IDE) (see 21 CFR 812[282]) before it can be used in humans to collect clinical data. A company may petition to have a new device reclassified to Class I or Class II.

Additional pathways to market include the Humanitarian Device Exemption (HDE), which is intended for the development of devices to treat rare (<4,000 patients per year) conditions, and the PDP (for Class III devices). An alternative to the PMA, companies seeking to purse the PDP pathway, will work with the FDA in designing preclinical and clinical studies, protocols, assessment methods, and acceptance criteria.

VI. CONCLUSIONS

In summary, there is a great promise to advance current cartilage therapies toward achieving a consistently successful approach for addressing cartilage afflictions. Tissue engineering may be the best way to reach this objective via the use of promising cell sources such as stem cells, novel biologically inspired scaffolds or scaffoldless approaches, emerging nanotechnology, chondrogenic factors, and physical stimuli. Undoubtedly, there are challenges and a significant number of unanswered questions about cartilage pathophysiology that may hinder the progress. Furthermore, the regulatory pathways that future cartilage therapies may need to follow are still unfolding. Nevertheless, significant evidence exists now supporting the idea that tissue engineered articular cartilage represents a potentially cogent approach to effectively treat cartilage injury or trauma.

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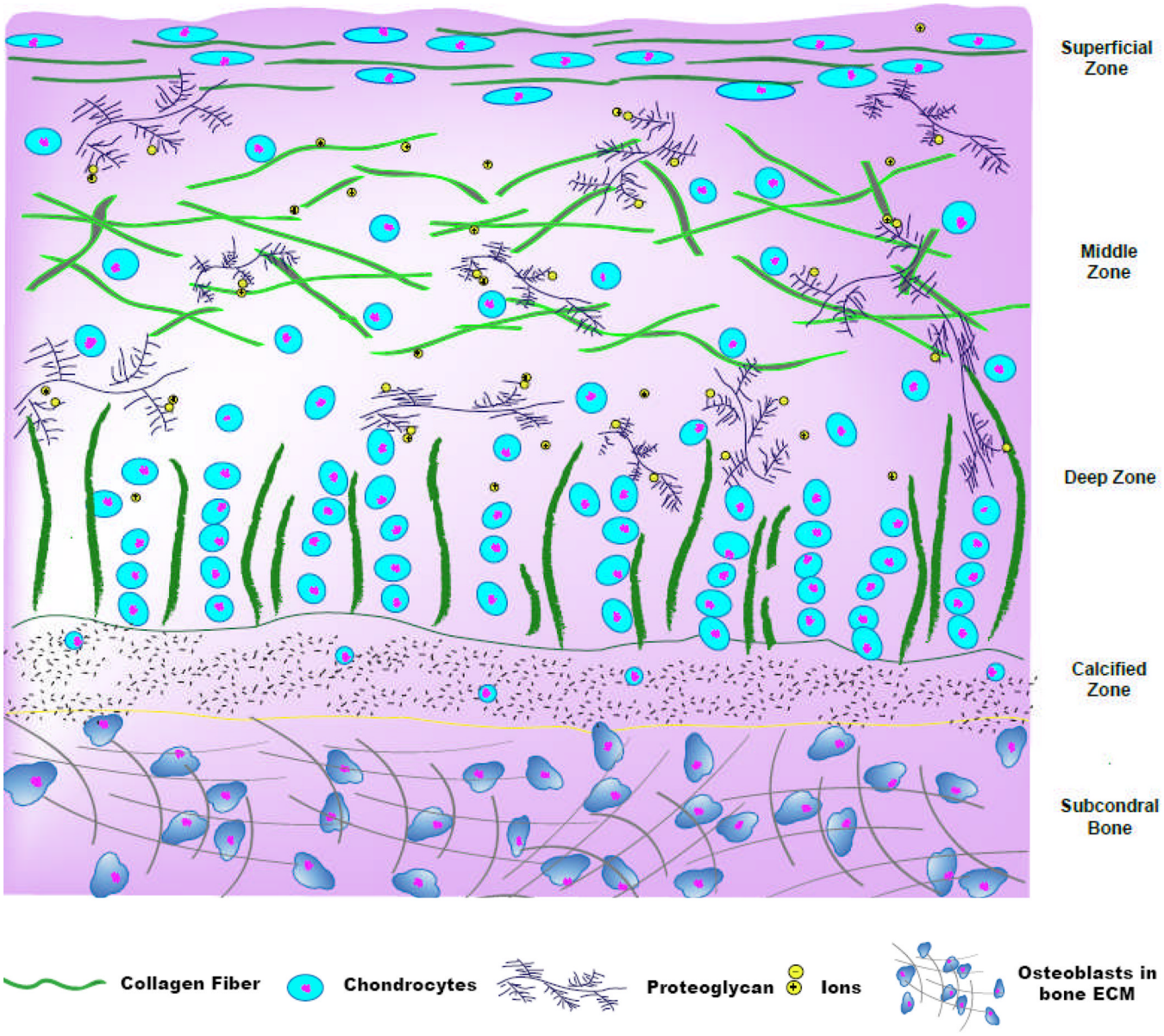

Fig 1.

Schematic illustration of composition and structure of articular cartilage lining the bone (not drawn to scale). There are four zones with different structures in articular cartilage: superficial, middle, deep, and calcified.

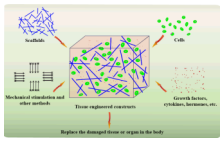

Fig 2.

The concept of tissue engineering. Tissue engineering incorporates many critical factors including cells, scaffolds, bioactive factors, and physical stimuli to assemble biomimetic tissue engineered constructs for replacing damaged tissues in humans.

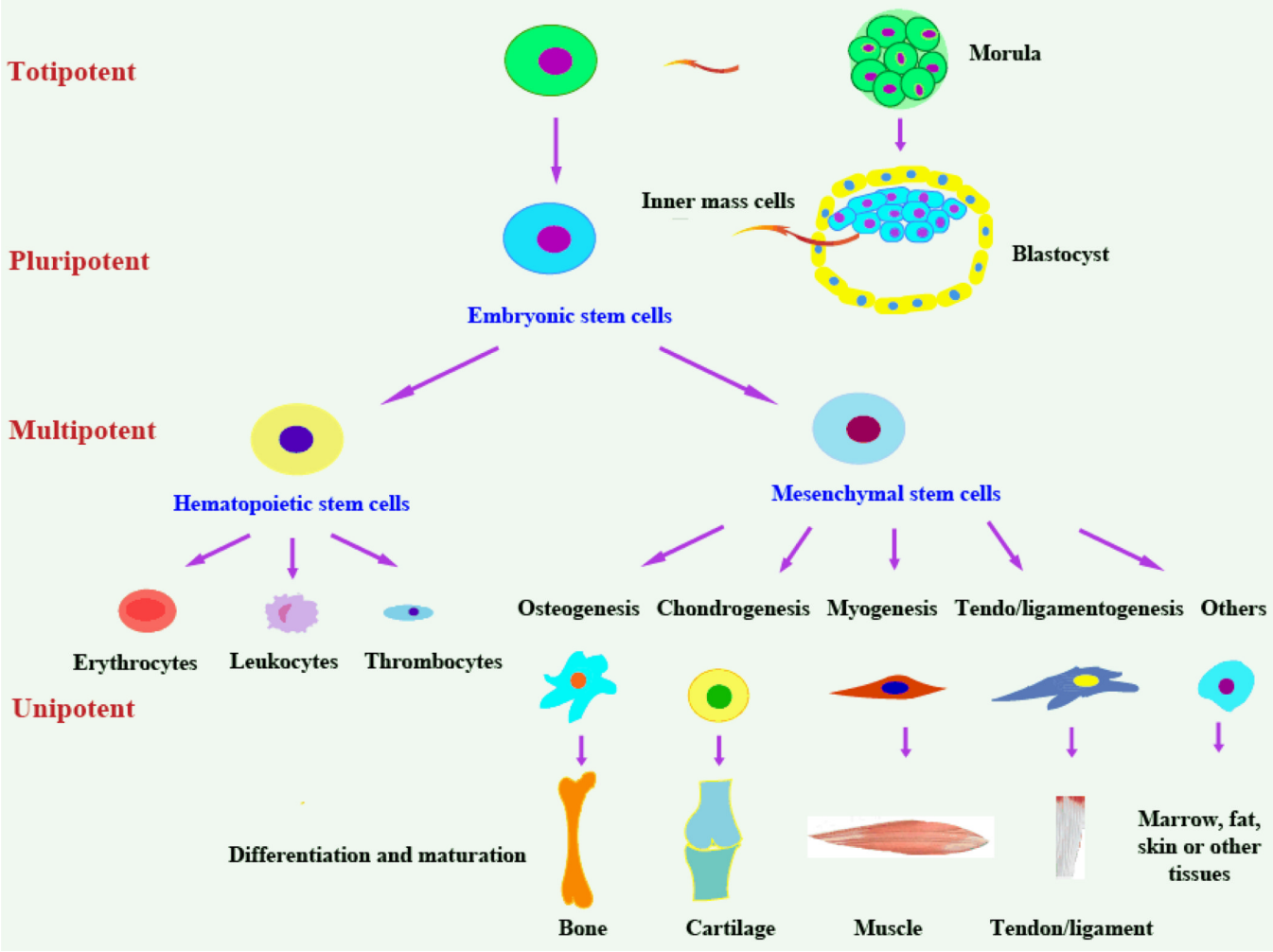

Fig 3.
The totipotent, pluripotent, multipotent, and unipotent stem cells.

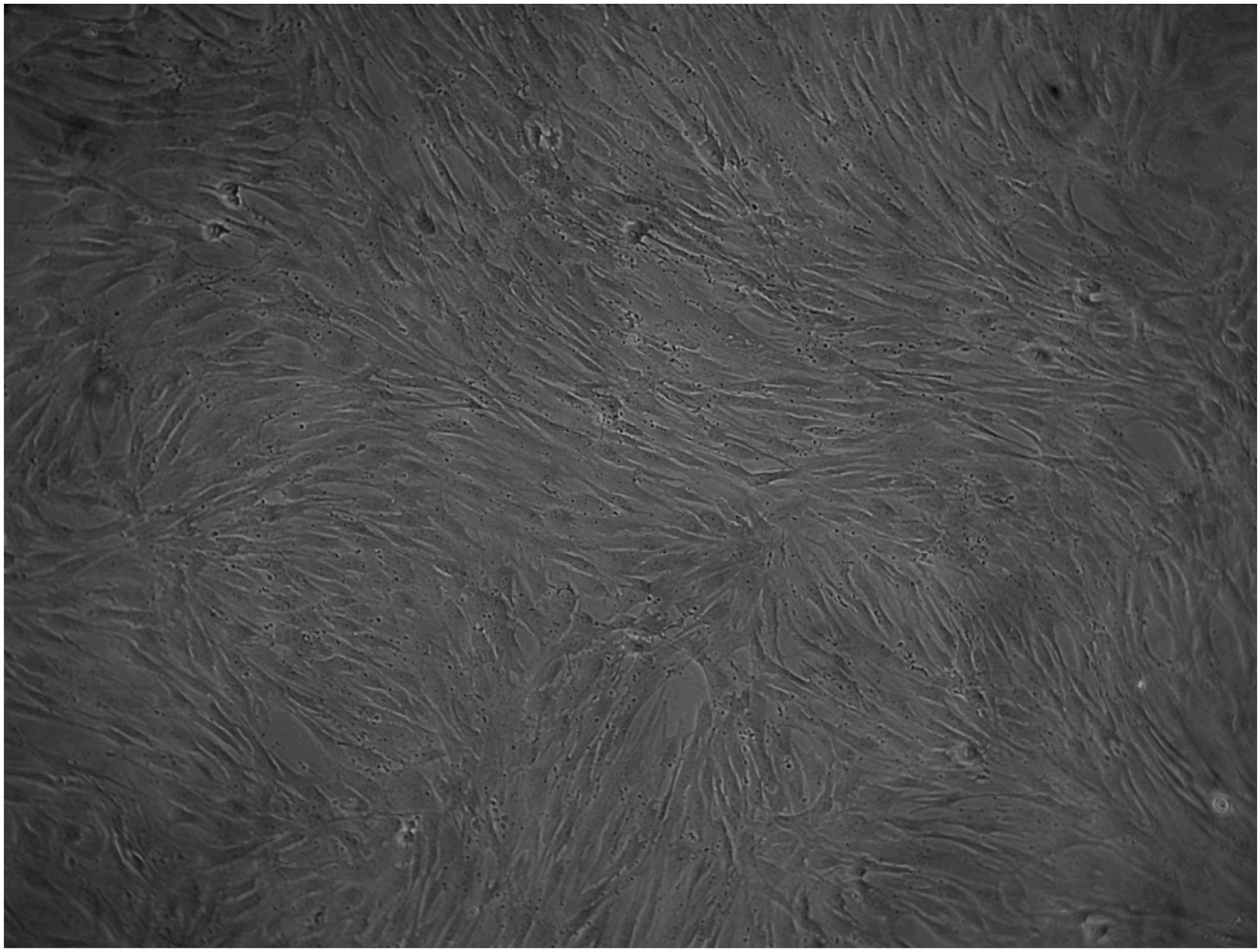

Fig 4.
Human bone marrow-derived mesenchymal stem cells after 6 days of culture, exhibiting a fibroblastic morphology.

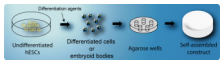

Fig 5.
A scaffold-free, modular approach for the engineering of articular cartilage from stem cells.

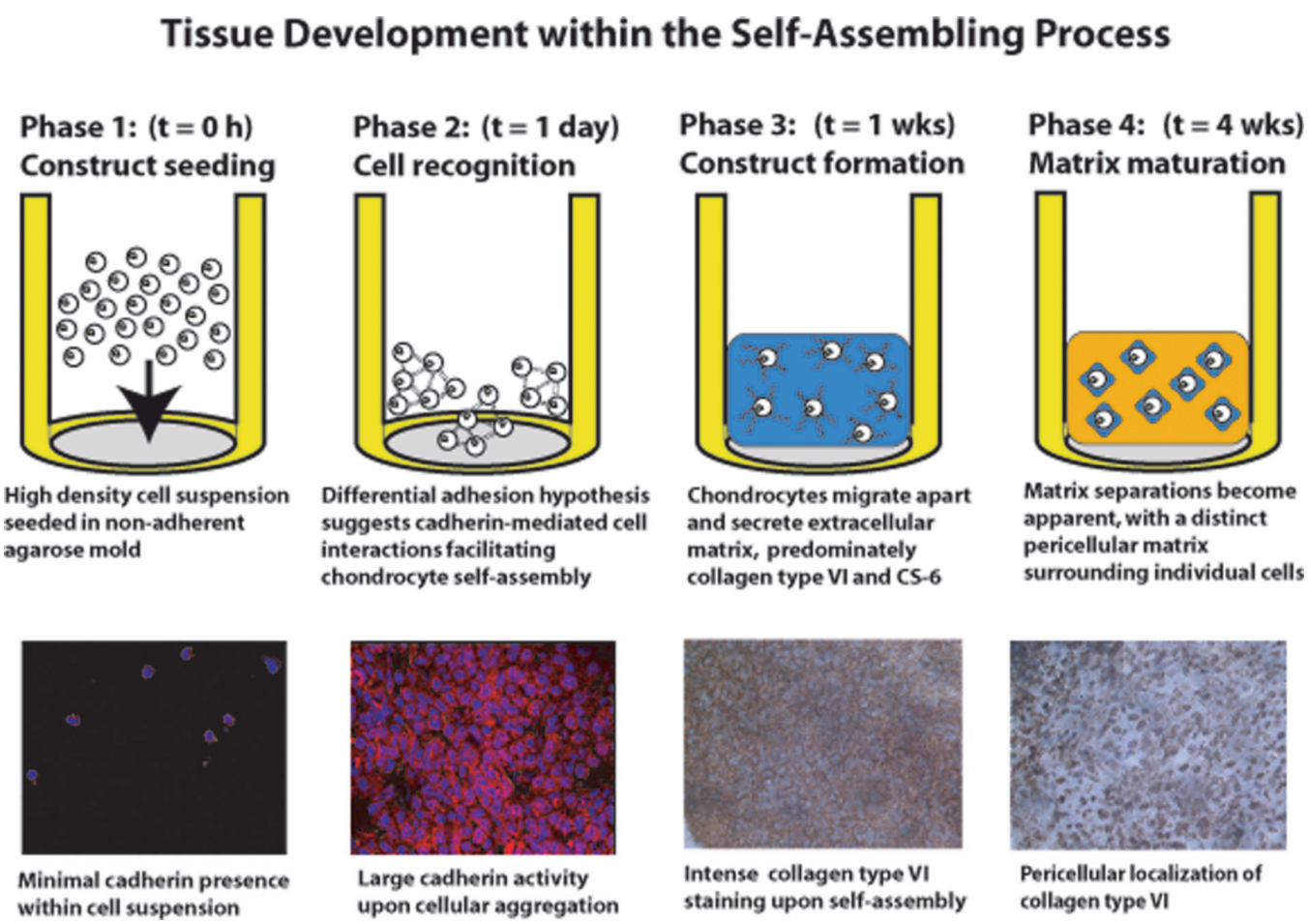

Fig 6.

Chondrocyte self-assembly and ECM development in a scaffold-free process. Sequences of cadherin and collagen VI expression and distribution parallel those seen in native cartilage development. (Used under the Creative Commons Attribution License, from Ofek et al., PloS ONE, 2008[175])

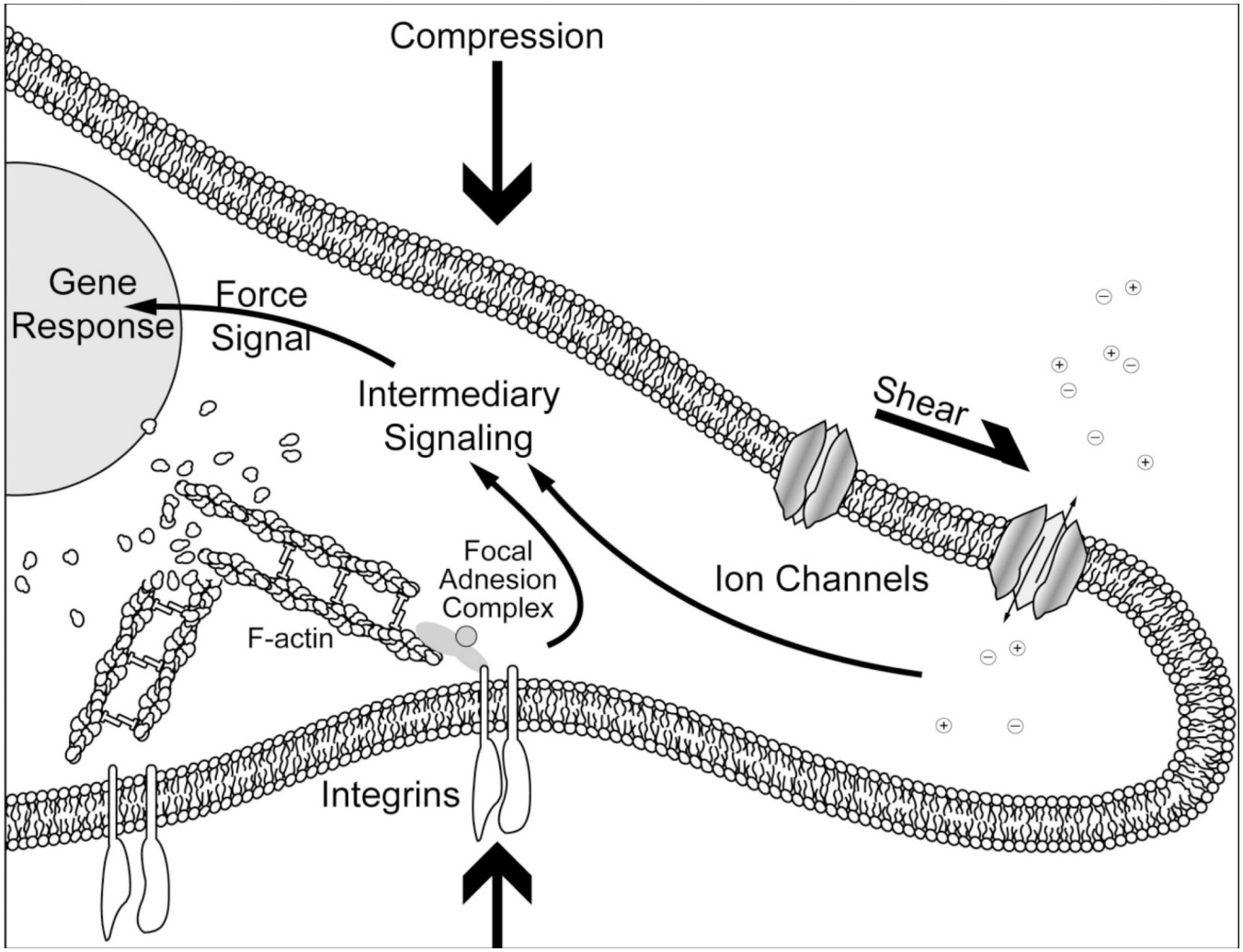

Fig 7.
Cells can respond to mechanical forces via several coupling mechanisms, such as stretch activated ion channels and focal adhesion complexes.

Table 1

The composition of articular cartilage

Articular Cartilage		% wet weight[19,20]	% dry weight[21]	Functions
Solid Phase (ECM)	Collagen	Type II collagen is 15–20% All other collagens are < 2%	50–75%	Contributes to tensile properties and macromolecule entrapment[19,11]
	Proteoglycan	10%	20–30%	Contributes to compressive and flow-dependent viscoelastic properties[283]
	Other glycoprotein, fibronectin etc.	Small amount	Small amount	Contributes to cell-ECM interaction and the stability of ECM
Solid Phase (Cells)	Chondrocytes	< 5–10% of total tissue volume		Modify ECM and maintain suitable tissue size
Fluid Phase	Interstitial water and electrolytes	60–80%	—	Exchanges nutrients with synovial fluid, lubricates the joint, and contributes to compressive resistance and deformation[19]

Table 2

The different morphology, size and distribution of components in four articular cartilage zones

Name		Superficial Zone	Middle Zone	Deep Zone	Calcified Zone
Chondrocytes	morphology	flattened	rounded	rounded or ellipsoid[284]	small and inert
Collagen fibrils	% dry weight[7]	86%	between	67%	ND
	diameter[7,285,286]	30–35 nm	between	40–80 nm	ND
Proteoglycan	% dry weight[29]	15%	25%	20%	ND
Water	% wet weight[284]	84%	between	40–60%	ND
Total thickness	% total tissue[21]	10–20%	40–60%	20–30%	ND

*Data obtained from[7,11,21,29,284–286]; ND = no data.