Osteoarthritis and Cartilage

Review

The clinical status of cartilage tissue regeneration in humans

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SUMMARY

Purpose: To provide a comprehensive overview of the basic science and clinical evidence behind cartilage regeneration techniques as they relate to surgical management of chondral lesions in humans.
Methods: A descriptive review of current literature.
Results: Articular cartilage defects are common in orthopedic practice, with current treatments yielding acceptable short-term but inconsistent long-term results. Tissue engineering techniques are being employed with aims of repopulating a cartilage defect with hyaline cartilage containing living chondrocytes with hopes of improving clinical outcomes. Cartilage tissue engineering broadly involves the use of three components: cell source, biomaterial/membranes, and/or growth stimulators, either alone or in any combination. Tissue engineering principles are currently being applied to clinical medicine in the form of autologous chondrocyte implantation (ACI) or similar techniques. Despite refinements in technique, current literature fails to support a clinical benefit of ACI over older techniques such as microfracture except perhaps for larger (>4 cm) lesions. Modern ACI techniques may be associated with lower operative revision rates. The notion that ACI-like procedures produce hyaline-like cartilage in humans remains unsupported by high-quality clinical research.
Conclusions: Many of the advancements in tissue engineering have yet to be applied in a clinical setting. While basic science has refined orthopedic management of chondral lesions, available evidence does not conclude the superiority of modern tissue engineering methods over other techniques in improving clinical symptoms or restoring native joint mechanics. It is hoped further research will optimize ease of cell harvest and growth, enhanced cartilage production, and improve cost-effectiveness of medical intervention.

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Introduction

Articular cartilage defects are commonly encountered in orthopedic practice but still represent a treatment challenge with inconsistent long-term results. Articular cartilage is an avascular tissue composed of chondrocytes dispersed within an extracellular matrix (ECM) comprised of collagen and proteoglycans. Found at the articulating end of bones, hyaline cartilage provides a low friction interface that also bears load. Formed initially from undifferentiated mesenchymal cells, chondrocytes synthesize cartilage matrix composed of 60% collagen (type II predominant), 25% proteoglycans, and 15% glycoproteins. The composition of cartilage matures during progression to adulthood, resulting in a zonal organization of superficial, middle and deep calcified layers that are anchored into subchondral bone. Overall, maturation results in a seven-fold increase in collagen cross-linking, and a 450% increase in the tensile and 180% increase in the compressive modulus of cartilage. While chondrocytes are primarily involved in articular cartilage maintenance through the synthesis of ECM, overall cartilage homeostasis is thought to be the product of a complex interplay between joint mechanics, growth factors, hormones and aging.

Although our understanding of these processes is evolving, a chondral lesion can simply be thought of as the inability of matrix synthesis to counter-act destructive forces placed on a joint.
trauma or disease provokes an intra-articular destructive process, human adult articular cartilage has a limited ability to spontaneously heal, especially for larger defects (>3 mm), defects that do not breach the subchondral plate, or in older patients. Reasons for the ineffective reparative response after damage are thought to include the inability of chondrocytes to migrate to the site of injury, the avascular nature of cartilage, and the absence of a fibrin clot scaffold.

While a range of clinical options exist for the treatment of cartilage defects, the majority of current treatment options are aimed at symptom relief and fall short of the goal of recreating pre-injury joint mechanics with the biologic capacity of long-term healing (see Table 1 for a summary of current management options). At one end of the spectrum, symptomatic relief may be obtained with oral analgesia, weight loss, physiotherapy to strengthen deconditioned muscles or arthroscopic chondroplasty, which aims to shave off the loose cartilage margins thought to be involved in mechanical joint irritation. These processes address pain, but fail to address the chondral lesion and thus are thought to not adequately address the longer-term sequela of cartilage injury: the development of osteoarthritis. At the other end, joint arthroplasty can be performed in most major synovial joints to replace a severe osteoarthritic process with a metallic prosthesis. While this procedure affords good quality of life (QOL), it is not appropriate for young individuals as the risk of failure increases over time and the functional limitations of a prosthesis are likely not adequate for an otherwise active or working individual. In between these two types of treatment modalities ‘biological’ cartilage treatments broadly attempt to fill the cartilage defect with stimulated fibrocartilage growth (i.e., microfracture) or a chondrocyte-containing plug (e.g., osteochondral transfer, mosaicplasty or autologous chondrocyte implantation (ACI).

The ideal treatment would reestablish the low friction properties of cartilage with the ability to resist wear over time by repopulating a lesion with chondrocytes able to produce a hyaline matrix that is fully integrated with surrounding host cartilage. The goal of creating integrative hyaline cartilage within a joint will theoretically improve joint mechanics and delay or even stop osteoarthritic progression within a joint. Hope lies in the area of tissue engineering to achieve this goal.

The purpose of this article is to describe the principles of tissue engineering in the context of cartilage regeneration in humans. Both the current status and future directions of tissue-engineered cartilage will be explored.

Principles of cartilage tissue engineering

Tissue engineering principles emerged in the late 1980s with the goal of reconstituting the structure and function of human tissues. This approach has since been investigated intensively and there is proof-of-concept evidence to support cell-based regeneration of cartilage tissue. With tissue engineering, researchers have been able to create biologically active, two or three-dimensional cartilage-like tissue complete with chondrocytes and supporting matrix that can fill a chondral lesion. Although complex, the overall process can be distilled down to three basic components: cells, scaffolds/matrix, and/or growth stimulators. Cells must be capable of maintaining the articular chondrocyte phenotype or stimulate the differentiation of other cell types into chondrocytes and accumulate hyaline cartilage matrix. A structural matrix or scaffold will facilitate the formation of a cartilage matrix. Finally, growth or matrix stimulators in the form of biological, chemical or mechanical stimulation will encourage appropriate cellular growth and matrix synthesis on the scaffold in vivo or vitro.

Cell sources for chondral repair

First and foremost, cartilage tissue engineering necessitates a large number of chondrocytes capable of creating hyaline cartilage. Unfortunately, the cell source also serves as the main limiting factor to clinical translation as, due to low cellularity, only a small number of primarily obtained autologous chondrocytes can be directly harvested from an individual. As a result, several other sources of chondrocytes have been identified including passaged chondrocytes, induced pluripotent cells (iPSCs), mesenchymal stromal cells (MSCs), and human embryonic stem cells (hESCs).

Table 1 Current clinical options for the treatment of cartilage defects

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>Benefits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-surgical</td>
<td>Oral analgesia, weight loss, physiotherapy</td>
<td>May avoid surgery</td>
<td>Only masks symptoms, chronic use of pain medications</td>
</tr>
<tr>
<td>Arthroscopic chondroplasty Microfracture</td>
<td>Minimally invasive resection of loose cartilage to decrease mechanical joint irritation and to encourage fibrocartilage growth</td>
<td>Simple procedure, immediate weight bearing</td>
<td>Only masks symptoms</td>
</tr>
<tr>
<td>Mosaicplasty/osteochondral autograft transfer</td>
<td>Multiple osteochondral autographs harvested from the patients femur to fill an osteochondral defect</td>
<td>No allograft, theoretically fills in with hyaline cartilage</td>
<td>Fibrocartilage biomechanically inferior to hyaline cartilage, brief period of non-weight bearing, unclear impact on development of arthritis</td>
</tr>
<tr>
<td>ACI</td>
<td>Harvested chondrocytes are cultured prior to being re-implanted into the defect</td>
<td>May produce hyaline cartilage, can treat lesions 2–10 cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Graft-site mismatch may not recreate native joint mechanics, graft-site morbidity, cannot treat large lesions, lack of integration with surrounding tissues</td>
</tr>
<tr>
<td>Osteochondral autograft transfer</td>
<td>Uses allogenic (cadaveric) osteochondral tissue to fill defect</td>
<td>Can treat large lesions, no graft-site morbidity</td>
<td>Allogenic tissue (potential for disease transmission), size/depth mismatch, questionable ability to produce hyaline cartilage</td>
</tr>
<tr>
<td>Joint arthroplasty</td>
<td>Resects and replaces arthritic bone with an artificial joint, most commonly metal implants (i.e., cobalt chrome) separated by a polyethylene liner</td>
<td>Pain relief, variable return to function</td>
<td>Variable return to function/activity limitations, infection, Implants wear out over time (need for re-operation), cannot completely recreate native anatomy or mechanics</td>
</tr>
</tbody>
</table>

A list of currently available treatment options for cartilage defects, most commonly used to treat lesions in the knee. The list is ordered from least to most invasive, and for the treatment of smallest to largest defects. The bulk of this article will focus on ACI and its evolution due to research into cartilage tissue engineering.

Note: no therapy has been shown to alter the natural history of a chondral lesion (i.e., progression to osteoarthritis), thus serving as a limitation for all therapies.
Stimulatory procedures, ACI, minced and passaged chondrocytes

Marrow stimulation techniques, which can be considered a precursor to tissue engineering, include osteochondral drilling, abrasion chondroplasty and microfracture. These techniques all seek to stimulate the release of chondroprogenitor cells into the defect to encourage the formation of fibrocartilage (composed of type I and type II collagen). While often the simplest option for small isolated defects, fibrocartilage is mechanically inferior to hyaline cartilage (composed of type II collagen). For that reason, marrow stimulation techniques can be considered a pain-relieving procedure that at most slows the progression towards osteoarthritic.

Osteoarticular transplant procedures use native chondrocyte-containing cartilage with underlying bone. Given the described complexity of the structure of cartilage, the allure of repairing a chondral lesion with structurally mature tissue obtained from either a cadaver (allogenic transplant) or non-weight bearing zone of the articular surface from the patient’s own body (autologous transplant) is understandable. Although very useful, concerns over donor site morbidity, chondrocyte viability, disease transmission from allogeneic tissue, and lack of integration with the margins of a transplant is understandable. Although very useful, concerns over donor site morbidity, chondrocyte viability, disease transmission from allogeneic tissue, and lack of integration with the margins of a transplant are understandable.

The procedure most related to human tissue engineering is ACI. First described in rabbits by Grande and later in humans by Brittberg et al. to treat knee chondral lesions, ACI uses arthroscopically harvested chondrocytes that are subsequently cultured in monolayer (so-called ‘passaged chondrocytes’). The chondrocyte suspension is then implanted into the defect and sutured under a watertight periosteal patch. This treatment, which requires two operations spaced six to 8 weeks apart, was originally indicated in patients with focal lesions 2–10 cm² in size. Randomized clinical trials have yielded mixed results on the ability of ACI-like procedures to produce enhanced structural repair over microfracture, with minimal clinical differences at 5 years. While clinical results are generally favorable, risks include periosteal hypertrophy, delamination of the graft and arthrofibrosis. In addition, the ability of ACI to reliably produce hyaline-like cartilage has been challenged, with some animal models suggesting that some healing is stimulated by the ingrowth of progenitor cells from breached subchondral bone or from the periosteal patch. Furthermore, cultivating chondrocytes in monolayer culture to increase cell numbers, known as passaged chondrocytes, results in a decreased capacity to produce hyaline-like matrix due to chondrocyte de-differentiation.

A variant of ACI is found in procedures utilizing particulated articular cartilage. Animal and subsequent clinical studies have demonstrated minced cartilage without bone or cell culture can provide a cell source for cartilage repair. Chondrocytes from minced cartilage display a standard chondrocyte phenotype and are thought to migrate from the graft ECM, multiply and form hyaline-like cartilage integrated with native tissue. Available commercial products include deNovo NT and CartiDede, which utilizes autogenous cartilage tissue harvested intra-operatively and distributed on a polycaprolactone/polyglycolic acid scaffold secured under a polydioxane mesh, while deNovo FT utilizes particulated viable autologous allograft hyaline cartilage pieces that are secured into a defect with fibrin glue. Both products and have found promising short-term results.

The application of tissue engineering principles has resulted in the progressive refinement of the ACI-like procedures to address some of the above shortcomings. For example, we have shown that passaged chondrocytes that have adapted a fibroblast-like morphology can undergo redifferentiation when co-cultured with non-passaged (or primary) chondrocytes and reacquire the ability to form hyaline cartilage. The mechanism underlying this redifferentiation is unclear, but may be related to direct cell–cell communication, ECM microenvironment produced by chondrocytes, or paracrine signaling. Regardless, these cells could then be used to redifferentiate other passaged chondrocytes, thus forming a stable phenotype that could be utilized in ACI procedures. Additional studies are required to evaluate the efficacy of our co-culture method in vivo.

The evolution of ACI has resulted in four described ‘generations’ that have been expanded upon in other reviews. We propose the following divisions between ACI generations in Table II, with each generation using more advanced tissue engineering technologies. Clinically, each generation is thought to represent a move towards less patient morbidity (i.e., arthroscopic instead of open procedures; or one-stage operations) or the enhanced production of hyaline cartilage.

MSCs

MSCs are multipotent cells capable of differentiation into osteocyte, adipocyte and chondrocyte lineages under the appropriate conditions. Defined by their expression of certain cell surface molecules (i.e., CD73, CD105, CD90) and their ability to grow as adherent fibroblast-like cells in vitro, MSCs are referred to as stromal cells instead of previously named stem cells as they are ultimately restricted in the type of cells into which they can differentiate.

The process of collecting, isolating and growing MSCs from various sources is beyond the scope of this article (see review by Archer et al.). In brief, cells are obtained via bone marrow aspiration or tissue enzymatic degradation and expanded in culture.

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**Table II**

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<tr>
<th>Generation</th>
<th>Description</th>
<th>Defining features</th>
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<tbody>
<tr>
<td>First</td>
<td>Autograft chondrocytes are obtained via arthroscopy, expanded in culture, and re-implanted under a periosteal or collagen patch during a second operation.</td>
<td>Periosteal/collagen patch used AND no scaffolds</td>
</tr>
<tr>
<td>Second</td>
<td>Autograft chondrocytes are obtained via arthroscopy, chondrocytes are expanded on a scaffold, and the chondrocyte/scaffold complex is inserted into the knee at a later operation without a periosteal/collagen patch.</td>
<td>Basic scaffolds AND no periosteal patch</td>
</tr>
<tr>
<td>Third</td>
<td>Introduces either chondro-conductive or -inductive scaffolds, xenogenic cells, biphase graft constructs, or mechanically conditioned chondrocytes during the culturing process.</td>
<td>Utilizes all three components of tissue engineering (introduces growth factors/mechanical conditioning) OR introduces non-self cell types OR attempts to reproduce zonal architecture of mature cartilage</td>
</tr>
<tr>
<td>Fourth</td>
<td>Utilizes stromal cells, stem cells, or gene therapy to produce chondrocytes.</td>
<td>Stem cells/gene therapy for chondrogenesis</td>
</tr>
</tbody>
</table>

The application of tissue engineering research has lead to a gradual refinement in ACI-Like techniques. Each generation is thought to allow for a less invasive procedure (thus decreasing patient morbidity), increase the reliability of hyaline cartilage formation, improve graft uptake or decrease the number of surgical procedures required. Note: Matrix-Induced Autologous Chondrocyte Implantation (MACI) procedures refer to second-generation or older ACI procedures depending on the type of matrix utilized.
Flow cytometry may be used to select cells expressing known MSC surface markers. Culture conditions are then optimized to induce differentiation into the desired cell line. In this case, chondrocytes were generated.

Bone marrow represents the main source of MSCs (so-called bmMSCs), although umbilical cord, adipose tissue, synovial membrane and articular cartilage represent alternate sources. It should be noted that MSCs obtained from varying cellular sources express differing densities and types of cell surface proteins/markers. For example, CD34+ is identified only on adipose derived MSCs, Tissue Non-Specific Alkaline Phosphatase (TNAP) is exclusively found on bmMSCs, and Stage Specific Embryonic Antigen 4 (SSEA-4) is expressed by placenta derived MSCs. These differences may reflect differences in chondrogenesis noted amongst MSC cell lines in some studies. For example, a comparison of bone marrow, adipose derived, muscle derived or synovial derived stromal cells obtained from the same individual revealed synovial derived cells had a superior potential for chondrogenesis and produced larger aggregates over time when compared with bmMSCs. The clinical utility of this finding is unclear, as synovium-derived MSCs have yet to be used in humans, and as previously mentioned the cellular composition and presence of environmental stimuli may be as important as the origin of the stromal cell. Cellular responses to growth factors or scaffolds may differ not only between different sources of MSCs but also within them. For example, Battula et al. utilized monoclonal antibodies to identify antigens associated with rapidly growing bmMSCs: CD271 and CD56. Cells expressing both antigens proliferated more than 30 times faster than an unsorted pool of bmMSCs. The results of this study also suggest that cells expressing CD271, CD56 and TNAP recovery, many other cell types have been induced to acquire ESC-like properties, including potential for teratoma formation. Since this discovery, many other cell types have been induced to acquire ESC-like phenotype. Recently, induced pluripotent stem cells (iPSCs) are an alternate method of creating cells with ESC-like properties. As originally described by Takahashi and Yamanaka, mouse fibroblasts can be transduced with the transcription factors Oct3/4, Sox2, Klf4 and c-Myc, transforming them into cells with ESC-like pluripotency. iPSC express ESC cell marker genes and demonstrate ESC-like growth capabilities, including potential for teratoma formation. The use of such cell lines in the treatment of osteoarthritis involves potential for ESCs that have differentiated to chondrocytes to undergo de-differentiation into other cell lineages (i.e., skeletal muscle), and to date no one has produced sufficient hyaline cartilage tissue from ESCs suitable for joint resurfacing. Safety concerns are paramount, as undifferentiated residual ESCs are known to be tumorogenic. Recent animal studies have suggested that injection of ESCs into a joint cavity results in teratoma formation, while localized injection into osteochondral defects does not. Additionally, joint immobilization may encourage tumor formation while joint mobility encourages chondrogenesis. While showing promise, additional work is required to better understand the forces involved in producing a clinically suitable, homogenous chondrocyte population from hESCs. Indeed, no trial in humans has yet been published, although animal studies have been reported.

MSCs are commonly utilized in tissue engineering, with bmMSCs being the most common cell source utilized clinically in humans. For example, in an observational cohort study by Nejadnik et al., ACI was compared with a group that received a similar treatment using autologous bmMSCs instead of chondrocytes. The authors concluded there was no difference in clinical outcome between groups at 24 months after surgery. Additionally, Wakedani et al. utilized culture expanded autologous bmMSCs embedded on a collagen sheet for the treatment of patellofemoral joint chondral defects in a small case series. The bmMSCs were transplanted into the defect and secured with a periosteal graft or synovium (similar to first generation ACI techniques), with symptomatic improvement noted for as long as 27 months. Longer-term follow-up studies have confirmed this to be a safe procedure without development of tumor or infections in a group of 40 patients over 11 years. These results suggest at the very least equivalence in clinical outcome between implantation of chondrocytes or bmMSCs in ACI-type procedures in terms of short-term symptomatic relief. While biopsies obtained during second look arthroscopies suggest the presence of hyaline-like cartilage in both the bmMSCs and ACI groups in one trial, this is based on a small subset of the original study population requiring arthroscopy for symptomatic knees. Thus, true superiority of bmMSCs over earlier generation ACI techniques remains unproven.

Scaffolds

Scaffolds are three-dimensional chondro-condusive biomaterials which facilitate chondrocyte number expansion and/or organization while also providing a mechanically stable support for human chondrocyte implantation. Of note, ‘chondro-conductive’ substances support chondrocyte growth whereas ‘chondro-inductive’ substances induce the differentiation to, and maintenance of, the chondrogenic cellular phenotype. Safran et al. listed the requirements for the ideal scaffold including: biocompatible, biodegradable, permeable, noncytotoxic, mechanically stable, able to support chondrocyte growth, versatile, readily available and easy to manufacture. Additionally, appropriate porosity is considered another characteristic, with pore sizes between 100 μm and 300 μm thought to best optimize cellular seeding and differentiation while facilitating waste/nutrient dispersion. Scaffolds currently represent a key component in chondrogenic differentiation of the aforementioned cell lineages as the three-dimensional environment is believed to facilitate the cellular and cell–matrix interactions encouraging chondrogenesis. For that reason, the scaffolds have been used to augment microfracture or ACI-type procedures by facilitating chondrocyte transfer and speeding graft incorporation with the ultimate hope of increasing the proportion of ‘hyaline’ or ‘hyaline-like’ cartilage.

Available scaffolds fall into one of four broad categories: protein, carbohydrate, synthetic, and composite. Protein-based scaffolds include collagen, gelatin and fibrin; carbohydrate polymers include hyaluronan, alginate, alginate, and polyactic/polyglycolic acids; and synthetic scaffolds include Teflon, carbon fiber, Dacron, and hydroxyapatite.
Clinical use of scaffolds in cartilage regeneration

One of the most commonly used scaffolds is collagen, a natural scaffold which also contains sites for cellular adhesion and has been shown to influence chondrocyte differentiation. Collagen scaffolds have also been utilized to enhance marrow stimulation techniques, with scaffolds being inserted post-microfracture as a one-step procedure. One commercial example is Chondro-Gide (Geistlich Pharma AG, Wolhusen, Switzerland), a I/III collagen scaffold used in a technique termed Autologous Matrix-Induced Chondrogenesis (AMIC) with promising non-comparative 2-year results but a paucity of data suggesting structural superiority over microfracture alone. Collagen scaffolds have also been used instead of periosteal patches to secure cultured chondrocytes to cartilage defects in ACI procedures (see NeoCart results but a paucity of data suggesting structural superiority over theoretical benefits).

Ethylene oxide and poly propylene oxide. There are many materials investigated for this property. Gelatin, and the injectable nature that facilitates arthroscopic insertion, is one hydrogel in clinical use. Gelrin C (Anika Therapeutics, Bedford, MA) have also been utilized in cartilage regeneration. These carbohydrate-based polymers have the proposed benefit of being fully resorbed in 3 months after being degraded to hyaluronic acid and are thought to encourage chondrogenic differentiation as evident by an increased presence of type II collagen and aggrecan with a decrease in type I collagen. While published clinical trials suggest good outcomes with hyaluronic acid-based grafts at 3 years after implantation, with a high percentage displaying hyaline or “hyaline-like” cartilage 18 months post implantation, this product is not available in North America and was recently withdrawn from the European market by the manufacturer.

Exciting potential exists for hydrogel scaffolds. Hydrogels are a liquid polymer that can be stimulated to undergo cross-linking to form a water-insoluble gel. Materials investigated for this property include: alginate, fibrin, and synthetic polymers of polyethylene oxide and poly propylene oxide. There are many theoretical benefits to this technology: the ability of growth factors to diffuse through the gel, water content that mimics native cartilage, and the injectable nature that facilitates arthroscopic insertion. One hydrogel in clinical use is Gelrin C (Regentis, Haifa, Israel). Gelrin C is a biodegradable polymer used as a hydrogel of polyethylene glycol diacrylate bound to fibrinogen and degrades within 6–12 months. It is injected into a previously microfractured defect as a gel that polymerizes in situ. In vitro, Gelrin C exhibits innate chondrogenic and osteoconductive potential, is nonimmunogenic, and in an in vivo model demonstrated type II collagen and proteoglycan synthesis in treated vs untreated defects. This product is being investigated in an ongoing multicenter clinical trial.

While chitosan/glycerol copolymer marketed as BST-Cargel (Piramal Healthcare Ltd, Vikhroli West, Mumbai), has shown promise in previous studies and is being investigated in phase III clinical trials, few other types of scaffolds have been clinically investigated in humans.

Growth factors

As chondrocyte or stromal cell growth is regulated by an interplay between mechanical and chemical stimuli, guided utilization of growth factors is advantageous when engineering cartilage. Growth factors are considered to be chondro-inductive substances and the factors commonly associated with cartilage growth and maturation fall into one of three broad categories: the Transforming Growth Factor-β (TGF-β) family, Insulin-like Growth Factors (IGFs) and Fibroblast Growth Factor (FGF). The known actions of major chondrogenic growth factors are summarized in Table III. Broadly, these factors are thought to be involved in maintaining chondrocyte phenotype during monolayer passage, encouraging chondrogenic differentiation of stem cells or stromal cells, maintaining chondrocyte phenotype, and encouraging collagen type II synthesis. For example, human ESCs and mesodermal cells undergo chondrogenic differentiation in the presence of growth factors such as TGF-β and BMP-2. Additionally, cell lines can be genetically engineered to express proteins whose goal is to enhance chondrogenic matrix production, such as type II collagen. For example, Umr-3TM genetically engineered MDSCs to express BMP-4 and found type II collagen was expressed as early as 4 weeks in rats. Cell lineage also impacts growth factor activity. For example, aggrecan upregulation occurs when bmMSCs are exposed to TGF-β1, while BMP-6 is needed to redifferentiate passaged chondrocytes.

This table explores our evolving knowledge of major growth factors in chondrocyte regeneration.

<table>
<thead>
<tr>
<th>Table III</th>
<th>Known actions of growth factors in chondrogenesis</th>
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<tbody>
<tr>
<td>Transforming Growth Factor B (TGF-β)</td>
<td>Superfamily</td>
</tr>
<tr>
<td>Anabolic effect on chondrocytes</td>
<td>Maintains chondrocyte phenotype</td>
</tr>
<tr>
<td>Redifferentiates passaged chondrocytes</td>
<td>TGF-β1 promotes cell proliferation and inhibits matrix metalloproteinases</td>
</tr>
<tr>
<td>GDF-5 upregulates GAG and type II collagen production by hMSC derived chondrocytes</td>
<td></td>
</tr>
<tr>
<td>Bone Morphogenic Proteins (BMPs; Member of TGF-β Superfamily)</td>
<td></td>
</tr>
<tr>
<td>Encourage undifferentiated mesenchymal cells towards chondrocyte phenotype</td>
<td></td>
</tr>
<tr>
<td>BMP-2 enhances redifferentiation of passaged chondrocytes</td>
<td></td>
</tr>
<tr>
<td>BMP-4 and BMP-6 increase type II collagen and the accumulation of proteoglycans while decreasing type I collagen synthesis</td>
<td></td>
</tr>
<tr>
<td>BMPs-2, -12 (GDF-7) and -13 enhanced collagen, GAG and cellular growth of cultured chondrocytes</td>
<td></td>
</tr>
<tr>
<td>IGFs</td>
<td></td>
</tr>
<tr>
<td>IGF-1 encourages chondrocyte proliferation</td>
<td></td>
</tr>
<tr>
<td>IGF-1 (with TGF-β1) promotes proteoglycan accumulation, type II collagen synthesis and maintenance of chondrocyte phenotype</td>
<td></td>
</tr>
<tr>
<td>IGF-1 binds to proteoglycans via IGF-binding protein, thus being release to counter-act cartilage breakdown during periods of catabolism</td>
<td></td>
</tr>
<tr>
<td>Insulin promotes chondrogenesis</td>
<td></td>
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Another way to administer growth factors in a less directed manner is Platelet-Rich Plasma (PRP), a growth factor-rich concentrate that can be easily acquired through centrifugation of patient’s own blood creating an autologous preparation of serum with high concentrations of platelets, cytokines, and growth factors. The application of PRP acts to amplify the concentration of chemical mediators in the microenvironment of the injured area such as TGF-β, IGF-1, Vascular Endothelial Growth Factor (VEGF), and Platelet-Derived Growth Factor (PDGF), among others. In vitro studies maintaining chondrocytes in the presence of PRP instead of fetal bovine serum have shown increased proliferation of human chondrocytes. Additionally, MSC’s cultured in the presence of PRP can demonstrate differentiation towards chondrogenic and osteogenic lineages. This technique may be used in the future as a way to deliver autologous growth factors to chondrocytes expanded in vitro prior to in vivo use.
Clinical impact

It is hoped that advances in the three components of tissue engineering will lead to improved patient outcomes. Unfortunately, most developments in cartilage tissue engineering have yet to translate into measurable clinical gains or have yet to be applied to human populations due to novelty and/or safety concerns. The main orthopedic utilization of tissue engineering has focused on enhancing either marrow stimulation (i.e., microfracture) or ACI-type techniques.

Many recent systematic reviews on ACI have been published, most often comparing ACI to stimulatory (i.e., microfracture) techniques. Vasiliadis et al. conducted a systematic review of randomized trials comparing various ACI treatments to other available treatment options (e.g., microfracture, mosaicplasty). They identified nine trials and found no superiority of ACI over other treatments. Nonetheless, they concluded that the evidence was of poor quality and too heterogeneous to make any definitive clinical recommendations. A similar review of nine studies was conducted by Vykken et al. In contrast to the previous review, their data suggested that among high-quality trials, ACI resulted in better clinical outcomes and tissue quality when compared to osteochondral grafts. However, the authors noted that the differences between groups were small and may not reflect clinical significance, and ultimately concluded that additional research is required.

Harris et al. elaborated on differences between studies in their review of level I and II evidence. Of seven studies comparing microfracture to ACI, they found three trials showed better clinical results with ACI after 1–3 years follow-up, one study reporting better results after microfracture at 2 years, and three trials reporting no difference after 1–5 years. They found a defect size of >4 cm² predicted better outcomes with ACI when compared to other treatments. There was no apparent difference between open or arthroscopic procedures, or first and second-generation ACI techniques.

Clinicians must also consider the complications of a procedure before making a recommendation. Harris et al. reviewed all failures and complications from ACI therapies published in 82 studies. An overall failure rate of 5.8% was noted for ACI procedures, with a mean time to failure at 22 months. Techniques utilizing periosteal patches had the highest failure rates (7.7%), with lower failure rates for all arthroscopic procedures (3.3%) or those using second-generation ACI techniques (0.83%). ACI techniques using periosteal patches were associated with an unplanned re-operation rate of 27%, which decreased to 5% in second-generation ACI, and 1.4% in all arthroscopic second-generation ACI techniques. Taken along with the preceding reviews, the available literature has yet to identify a functional benefit to evolving ACI techniques but the overall complication rates and need for re-operation has decreased in all arthroscopic and second-generation techniques.

Available data on third generation techniques are mostly limited to prospective safety trials. Crawford et al. evaluated the safety of the third generation NeoCart procedure in a small prospective trial. The eight enrolled patients had improved pain, function, and range of motion at 2 years. MRI-measured defect fill was found to be 67–100% in six patients; 33–66% in one patient and less than 33% in one patient. No serious complications were associated with the implant. A small sample and lack of a comparison group ultimately limited the strength of this study. Cole et al. presented a randomized controlled trial (RCT) involving 29 patients to establish the safety of using the Cartilage Augmenting Implantation System (CAIS; DePuy Mitek, Inc., Raynham, MA) when compared to microfracture. CAIS utilizes minced autologous hyaline cartilage placed on an absorbable polyglycolic acid-polycaprolactone scaffold and affixed using absorbable polydioxonone staples. The authors found general improvement in clinical outcomes in both groups, although the CAIS group significant improvements in the clinical rating scales over the microfracture group at 24 months of follow-up. Radiographic evaluation of lesion fill and tissue integration was similar between groups. The microfracture group had significantly higher rates of intralesional osteophyte formation at 6 and 12 months. From this they concluded the CAIS is safe and effective but acknowledged their study was limited by small sample size, and may have been influenced by differences between study populations (more patients with acute onset of symptoms, more men and more full-time workers in the CAIS group). Neither of the above trials discussed the histological quality of the repair tissue (i.e., fibrocartilage vs hyaline-like cartilage).

Considering the high cost associated with engineering chondrocytes with equivilous clinical data, there has only been one study focusing on the cost-effectiveness of these therapies. While Clar et al. attempted a cost-comparison analysis in their systematic review of four RCTs, they were unable to generate conclusions due to limited evidence. They acknowledge that the QOL gain of ACI would need to be 70–100% greater than microfracture over 2 years, or alternatively 10–20% maintained over 10 years, to justify the use of ACI. While it is hoped that the theoretical benefit of ACI in the generation of durable hyaline cartilage may justify its use, clinical evidence demonstrating benefit of ACI over other techniques in this area is sparse. Furthermore, long-term studies are required to support the assertion that the hyaline cartilage (vs fibrocartilage) results in improved long-term biomechanical properties that delays or prevents the development of osteoarthritis. Thus, based on the above economic analysis and available long-term data, the cost-effectiveness analysis does not favor ACI over other less costly procedures that are potentially as efficacious.

Conclusions and future research

Current clinical research does not support a functional benefit of ACI techniques over older techniques like microfracture. There is, however, a trend towards less complications or need for re-operation in all arthroscopic and newer-generation ACI techniques. Histological support for ‘biomechanically superior’ hyaline cartilage filling the defect in ACI procedures is lacking, as is evidence that the presence of this tissue ultimately delays or halts the development of osteoarthritis. At this time many of the potential cell sources described above are still experimental, and may be decades away from clinical practice, if at all.

It is assumed that a multifactorial tissue engineering approach to cartilage regeneration is ideal — combining cells, and scaffold to create a biologically active graft. The role of growth factors is still controversial. There are many questions that still need to be answered if tissue engineering is to be utilized to repair a chondral defect with articular cartilage containing biologically active cells. For example, which cell when differentiated to a chondrocyte produces a matrix most similar to native hyaline cartilage and will this tissue decrease the risk of arthritis in those with osteochondral lesions? How will our evolving understanding of growth factors and scaffolds impact osteochondral repair? What conditions are necessary to encourage integration between native cartilage and the implanted graft? Are biphasic implants the best way to encourage stable integration of grafts? Do we need to recapitulate cartilage zonal organization with a deep calcified zone to facilitate integration and weight bearing?

The literature suggests that single staged procedures, all arthroscopic techniques, and avoiding periosteal patches appear to impact complication and revision rates, but there are many other
questions that arise when the above is to be applied clinically. Is there any functional benefit of newer-generation ACI techniques? How does scaffold-augmented microfracture compare to ACI procedures? What is the optimal post-operative rehabilitation process? Is there a long-term impact on rates of osteoarthritis? Is there a way to make the process more financially feasible?

As stated in this review: our understanding of the factors influencing optimization and application of cartilage tissue engineering is expanding. However, the clinical impact of this research has yet to be truly appreciated as these findings have yet to be translated into human use. As new techniques or products are introduced, decisions on their utility must be predicated on evidence based medicine and functional outcomes. Each new advance should also be scrutinized for ease of cell harvest and growth, quality of cartilage produced, and overall cost-effectiveness. Through all of these ongoing efforts and developments, cartilage tissue engineering should be a powerful tool for the treatment of chondral defects.

Contributions

All authors (BM, RK, JC & JT) were involved in the conception of this manuscript. One author (BM; email: brent.mollon@mail.utoronto.ca) drafted the article and takes responsibility for the integrity of the work as a whole. All other authors (RK, JC & JT) critically appraised the article for important intellectual content and revised it accordingly. All authors approved the final version of the manuscript.

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