Review Article

Cartilage Regeneration

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Abstract

Cartilage damaged by trauma has a limited capacity to regenerate. Current methods of managing small chondral defects include palliative treatment with arthroscopic débridement and lavage, reparative treatment with marrow-stimulation techniques (eg, microfracture), and restorative treatment, including osteochondral grafting and autologous chondrocyte implantation. Larger defects are managed with osteochondral allograft or total joint arthroplasty. However, the future of managing cartilage defects lies in providing biologic solutions through cartilage regeneration. Laboratory and clinical studies have examined the management of larger lesions using tissue-engineered cartilage. Regenerated cartilage can be derived from various cell types, including chondrocytes, pluripotent stem cells, and mesenchymal stem cells. Common scaffolding materials include proteins, carbohydrates, synthetic materials, and composite polymers. Scaffolds may be woven, spun into nanofibers, or configured as hydrogels. Chondrogenesis may be enhanced with the application of chondroinductive growth factors. Bioreactors are being developed to enhance nutrient delivery and provide mechanical stimulation to tissue-engineered cartilage ex vivo. The multidisciplinary approaches currently being developed to produce cartilage promise to bring to fruition the desire for cartilage regeneration in clinical use.

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Osteoarthritis (OA), which is characterized by cartilage destruction, affects approximately 27 million adults in the United States.[1] Treatment options are limited. Cartilage has limited capacity for self-repair because of its limited vascularity, which results in poor replicative capacity of chondrocytes, the main cell type in cartilage.

Current treatment methods for well-defined osteochondral defects include drilling, autologous chondrocyte implantation (ACI), and osteochondral allograft. These options result in the formation of fibrocartilage containing collagen types I and II, which has less strength and resilience than does cartilage. Fibrocartilage scar tissue has a higher coefficient of friction than does cartilage, which can hinder motion and lead to earlier degeneration. Degenerated joints with larger cartilage defects or lesions, as seen in OA, are often managed ultimately with total joint arthroplasty. Although these current treatment methods reduce pain and increase mobility, there is a growing need for options that restore the native biologic properties of cartilage.

The limited capacity of damaged cartilage to regenerate and the potential morbidity associated with implanting or transferring bone and cartilage make cartilage regeneration an attractive alternative. The field of cartilage tissue engineering is being

Table 1

Cell Types Used in Cartilage Tissue Engineering

Type	Cells	Advantages	Disadvantages
Differentiated	Adult chondrocytes	Autologous tissue Differentiated cells	Donor site morbidity Limited cell availability Dedifferentiation and loss of chondrocytic phenotype
	Neonatal/fetal chondrocytes	Higher rate of cartilage matrix synthesis than adult chondrocytes Low immunogenicity	Limited cell availability
Pluripotent stem cells	Embryonic stem cells	Indefinite self-renewal Ability to differentiate into multiple cell and tissue lineages	Ethical concerns Difficulty in controlling and directing specific differentiation Tumorigenicity
	Induced pluripotent stem cells	Autologous origin Indefinite self-renewal No ethical concerns	Safety concerns Difficulty in controlling and directing specific differentiation Tumorigenicity
Mesenchymal stem cells	Bone marrow stem cells	Autologous tissue source High level of collagen type II production	May undergo hypertrophy upon extended culture or after implantation More invasive harvesting
	Adipose-derived stem cells	Autologous tissue source Abundant Minimally invasive harvesting	Lower chondrogenic capacity Lower level of collagen type II production
	Synovial-derived stem cells	Autologous tissue source Highest chondrogenic capacity	Retains some fibroblastic characteristics

advanced to create biologically compatible, synthetic cartilage constructs. These constructs are composed of appropriate cell types seeded within biomaterial scaffolds to produce a durable tissue repair system that potentially can be implanted in a single step. Many different components are necessary to construct tissue-engineered cartilage, including the various constituent cell types, biomimetic scaffolds, inductive bioactive factors, gene therapy, and the use of bioreactors for ex vivo cartilage tissue engineering.

Cell Types

Chondrocytes

Initially, cell-based therapy for repairing cartilage lesions used chondrocytes, the principal cell type found in cartilage. The technique of ACI was first described by Brittberg et al.[2] Chondrocytes are harvested from a non–weight-bearing area of the joint and are expanded ex vivo. In a separate surgical procedure, an autologous periosteal flap is harvested and sewn over the chondral defect, and chondrocytes are injected in a collagen-containing suspension into the defect and sealed with fibrin glue. Although this procedure reduces pain and swelling for small lesions, associated donor site morbidity exists from harvesting the periosteum and chondrocytes.[3] The second-generation ACI technique, also known as collagen-covered ACI, uses a collagen membrane rather than periosteum to cover the lesion, which thereby prevents compromise to other regions of bone or cartilage. The third-generation technique, matrix-assisted ACI, further improved the process with placement of chondrocytes onto biomaterial scaffolds that were then placed into lesions. This technique has the advantage of maintaining chondrocytes within the matrix instead of injecting them within the lesion (Table 1).

Although these techniques have been shown to be effective for improving patient function, these harvested chondrocytes are grown in vitro, which can lead to dedifferentiation or loss of phenotype, thereby rendering them useless for the regeneration of hyaline cartilage.[4] A more viable option is neonatal or fetal chondrocytes (eg, DeNovo NT Natural Tissue Graft; Zimmer), which grow significantly faster than adult chondrocytes and more closely resemble cells from native cartilage, with higher proteoglycan and collagen types II and IX content.[5] However, as with adult chondrocytes, the availability of juvenile chondrocytes is limited.

Pluripotent Stem Cells

Given the limitations of chondrocytes, researchers have investigated the use of other cell types that can differentiate into chondrocytes. Pluripotent stem cells, such as embryonic stem cells (ESCs), are attractive options for tissue regeneration because of their potential for indefinite self-renewal and their ability to differentiate into multiple tissue types. However, ESCs are derived from the inner cell mass of blastocyst-stage embryos,[6] and their derivation raises ethical concerns. An alternative is another pluripotent cell type, induced pluripotent stem cells (iPSCs). These ESC-like stem cells are developed from a patient's own skin or blood cells by means of gene transduction using ESC-specific transcription factors; in principle, these can be used in multiple tissue applications.[7] Although these pluripotent cells have multiple capabilities, their undifferentiated nature and tendency to grow without restraint may lead to the development of tumor; teratoma formation in vivo is well recognized.[8]

Clinical studies using ESCs are currently underway for implantation in the setting of spinal cord regeneration and for managing Stargardt macular degeneration. Although ESCs and iPSCs may some day be viable treatment options for these conditions, their direct differentiation into chondrocytes is at an early stage of investigation,[9] and there are no current clinical studies examining the use of pluripotent stem cells to manage cartilage damage.

Mesenchymal Stem Cells

Another cell alternative for regenerating cartilage that has minimal tumorigenic capacity is mesenchymal stem cells (MSCs). These adult tissue–derived cells have a high proliferative capacity and have the potential for multipotent differentiation. MSCs have the ability to differentiate along various cell lineages, including chondrocytes, adipocytes, osteoblasts, and myocytes.[10] MSCs are an ideal option for cartilage regeneration because they represent a readily available and accessible supply of cells, and they have the capacity for considerable expansion and differentiation. Growth factors such as transforming growth factor-β (TGF-β) and bone morphogenetic protein (BMP) are used to induce chondrogenesis in MSCs. MSCs can also migrate and incorporate into musculoskeletal tissue and exert effects on tissue microenvironment; additionally, they have anti-inflammatory and immunosuppressive properties that may be useful in managing OA and rheumatoid arthritis.[11] A porcine study demonstrated that an intra-articular injection of MSCs with hyaluronic acid could facilitate cartilage regeneration after induced injury.[12]

Of the multiple sources of MSCs, bone marrow stem cells (BMSCs) are the most commonly used. An in vitro model demonstrated that osteochondral defects in rabbits could be marginally repaired with injected BMSCs and fully repaired with BMSCs embedded in a synthetic extracellular matrix (ECM).[13] In an equine study, full-thickness osteochondral defects created in the femoral trochlear ridge were treated either with microfracture combined with concentrated bone marrow aspirate containing MSCs or with microfracture alone.[14] Improved macroscopic filling of the lesion and higher collagen type II content were demonstrated in the combined treatment group.

Although multiple animal studies have examined the capacity of BMSCs to repair cartilage, few clinical studies have been conducted. Initial case reports using BMSCs embedded in a collagen gel implanted within an autologous periosteum cover to manage patellar articular cartilage defects demonstrated pain-free walking for at least 4 years after the index procedure.[15] Another study compared patients who did and did not undergo BMSC transplantation for medial femoral condyle osteochondral defects.[16] Although clinical outcomes were similar, patients treated with BMSC transplantation demonstrated improved arthroscopic and histologic articular cartilage growth 42 weeks postoperatively.

Adipose-derived stem cells are less chondrogenic than BMSCs but are more plentiful and easily accessible.[17] These cells can produce cartilage with a high total collagen content but lower levels of collagen type II. Previous studies comparing chondrogenic capacity between MSCs derived from different sites demonstrated that synovial-derived stem cells were superior to bone marrow, periosteum, skeletal muscle, and adipose tissue.[18] However, synovial-derived stem cells may retain some fibroblastic capacity after implantation, which makes this cell type less effective as a cartilage substitute.

Scaffolds

For tissue engineering, cells must be seeded on a temporary structure to establish a three-dimensional structure that retains the seeded cells and provides mechanical support to aid in the development of cartilage over time. Thus, scaffold biomaterials must be biodegradable, noncytotoxic, mechanically competent (ie, similar to surrounding tissue), and able to regulate cell activity; must have appropriate surface chemistry; and must have the capacity to be shaped into different sizes and forms. The four main groups of scaffolding that may be applied in cartilage tissue engineering are protein-based polymers, carbohydrate-based poly-

Table 2

Commonly Used Scaffold Biomaterials in Cartilage Tissue Engineering

Scaffold Class	Scaffold Material	Advantages	Disadvantages	Examples
Protein-based polymers	Fibrin	Naturally occurring material, low toxicity Decreased cost Promotes cell adhesion and migration Biodegradable	Poor mechanical strength	—
	Collagen	Enhances cell adhesion Multiple, well-established processing technologies Biodegradable	Variable physical chemical properties Variable degradation properties Difficult to handle	NeoCart (Histogenics)
Carbohydrate-based polymers	Hyaluronic acid	Naturally occurring material, low toxicity Supports mesenchymal stem cell and epithelial cell migration Can fill irregular defects	Poor mechanical strength	Hyalograft C autograft (Anika Therapeutics)
	Alginate	Abundantly available Naturally occurring Biodegradable	Slow degradation Poor mechanical strength Cannot be used as long-term implant	—
Synthetic polymers	Polylactic acid (PLA)	High tensile strength High modulus (able to bear loads) Can be made into different forms	Chain depolymerization due to monomer formation with excessive heating of PLA Local acidosis upon biodegradation	Cartilage Repair Device (Kensey Nash)
	Polyglycolic acid (PGA)	Good mechanical strength High modulus Natural degradation product (glycolic acid)	Rapid degradation Degradation product, glycolic acid, may cause local tissue acidosis	—
	Polycaprolactone	Good osteoinductive potential Nontoxic degradation products Good mechanical properties	Releases acid upon degradation	—
	Polylactic-co-glycolic acid	Enhanced mechanical strength compared with PLA or PGA alone Biodegradable and biocompatible Resistance to hydrolysis	Amorphous	TruFit Plug (Smith & Nephew)
Bioceramics	Hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$)	Bioactive material Forms a rapid and strong bond to bone	Difficult to shape scaffolds Stiff and brittle material	MaioRegen (Fin-Ceramica Faenza SpA)

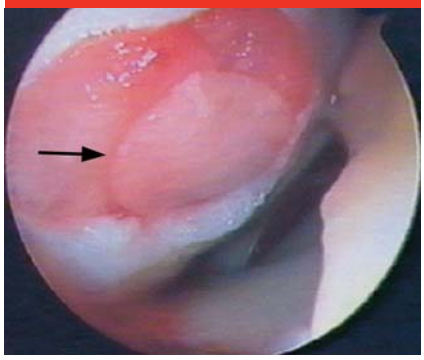
mers, synthetic polymers, and composite polymers, which contain combinations of biomaterials from the first three groups[19] (Table 2).

Protein-based Polymers

Fibrin, gelatin, and collagen are examples of protein-based polymers used in bioengineered scaffolds. Fibrin, a protein matrix derived from fibrinogen, is a key component of blood clots. Gelatin is formed from denatured collagen and can bind growth factors, proteins, and peptides, as well as allow for cell adhesions. Collagen is the major structural component of the ECM, and its use as a scaffolding material allows cells to retain their phenotypes.[20]

NeoCart (Histogenics), a collagen type I scaffold seeded with autologous chondrocytes, was implanted in 21 patients with grade III chondral defects of the distal femur.[21] These patients were randomly compared with nine patients who received microfracture treatment of the same type of lesion. At 2-year follow-up, patients treated with the scaffold material had significantly lower pain

Figure 1



Arthroscopic image demonstrating cartilage integration (arrow) of the hyaluronic acid–based scaffold Hyalograft C autograft (Anika Therapeutics), which was used to manage cartilage defects. The patient demonstrated significantly improved function postoperatively. (Reproduced with permission from Marcacci M, Kon E, Zaffagnini S, Iacono F, Filardo G, Delcogliano M: Autologous chondrocytes in a hyaluronic acid scaffold. *Operative Techniques in Orthopaedics* 2006;16[4]:266-270.)

scores ($P < 0.05$) than before surgery, as well as improved function and increased motion, compared with the microfracture group.

Carbohydrate-based Polymers

Carbohydrates, such as hyaluronan, alginate, chitosan, agarose, and polyethylene glycol, also have been used in hydrogel scaffolds. These scaffolds are comprised of cross-linked polymers that absorb a great deal of water, which is similar to the properties of cartilage ECM. They are also efficient in cell encapsulation, which allows chondrocytes to maintain their spherical morphology within the scaffold.[22] Hydrogel scaffolds may be modified by their mechanism of gelation, the inclusion of synthetic materials, and the addition of growth factors to enhance chondrogenesis.

One study demonstrated improved functional scores at 2-year follow-up in patients treated with the hyaluronic acid–based scaffold Hyalograft C autograft (Anika Therapeutics).[23] At 7-year follow-up, 62 patients who were treated with this scaffold for cartilage defects with an average size of 2.5 cm^2 underwent clinical and radiographic evaluation.[24] Significant improvement in function and pain was seen in study patients, and postoperative MRI evaluation showed complete filling of the cartilage defect in 57% of the lesions and complete integration of the scaffold in 62%. The Hyalograft C autograft is not yet available in the United States (Figure 1).

In one study, alginate was used in scaffolds seeded with adult allogenic chondrocytes and implanted in 21 patients.[25] At a mean follow-up of 6.3 years, clinical scores improved (ie, Western Ontario and McMaster Universities Osteoarthritis Index, visual analog scale). At a mean follow-up of 6.1 years, MRI remained stable. The four failures reported consisted of periosteal flap loosening, delamination of repair tissue, decline of clinical function, and thinning of the repair tissue as visualized on MRI. Despite these failures, the development of carbohydrate-based polymers as scaffolding material for cartilage holds promise.

Synthetic Polymers

Synthetic polymer–based scaffolds using polylactic acid, polyglycolic acid, polycaprolactone, and polylactic-co-glycolic acid (PLGA) are the most common, and the materials may be woven or made into electrospun nanofibers.[26] A synthetic scaffold containing PLGA, polyglycolic acid, and calcium sulfate was implanted in patients with patellofemoral cartilage defects, and patients were followed postoperatively for up to 2 years.[27] This study demonstrated improved short-term results; however, subchondral bone was not restored even with the formation of hyaline cartilage. PLGA also has been combined with calcium sulfate in the commercially available TruFit Plug (Smith & Nephew), a synthetic resorbable biphasic implant that encourages the growth of cartilage and bone.[28] Polylactic acid serves as the scaffold for the Cartilage Repair Device (Kensey Nash), which contains β-tricalcium phosphate to stimulate bone growth and a collagen type I matrix to stimulate the growth of cartilage.[29] This device was approved for use in Europe in 2010 and was made available in the United States in 2012.

Other synthetic scaffolding materials include polybutyric acid, carbon fiber, Dacron, and Teflon. Ceramics, such as hydroxyapatite, tricalcium phosphate, and bioactive glass, are also considered when developing scaffolds for cartilage replantation because these materials promote the growth of a bone-like apatite layer to anchor the overlying cartilage scaffold to the existing bed of the osteochondral defect.

The authors of a recent study evaluated the management of knee chondral or osteochondral defects using a three-dimensional scaffold (MaioRegen, FinCeramica Faenza SpA) with layered collagen type I fibrils and hydroxyapatite nanoparticles to form a synthetic cartilage-and-bone scaffold.[30] The size of the treated lesions ranged from 1.5 to 6.0 cm^2. At 2-year follow-up, patient clinical scores had improved, especially in active patients. MRI demonstrated complete graft integration in 70% of patients. A large, multicenter clinical trial is currently underway in Europe to further study the use of this scaffold in managing osteochondral defects.

Growth Factors

In contrast to cells and scaffolds, which provide the network by which

Table 3

Common Growth Factors Used in Cartilage Regeneration

Growth Factor	General Effects on Chondrocytes/Cartilage
BMP-2	Stimulates ECM production Increases ECM turnover Increases aggrecan degradation
BMP-7	Stimulates ECM production Inhibits cartilage degradation by decreasing ILs and MMPs
FGF-2	Increases aggrecan degradation Inhibits proteoglycan synthesis Upregulates MMPs
FGF-18	Stimulates ECM production in injured joints Increases chondrocyte proliferation
IGF-1	Stimulates ECM production Decreases ECM catabolism
PDGF	Chemotactic factor for mesenchymal cells Suppresses IL-1–induced cartilage degradation
PRP	Biologic cocktail of multiple growth factors and cytokines
TGF-β1	Stimulates ECM production Inhibits cartilage degradation by decreasing ILs and MMPs

BMP = bone morphogenetic protein, ECM = extracellular matrix, FGF = fibroblast growth factor, IGF = insulin-like growth factor, IL = interleukin, MMP = matrix metalloproteinase, PDGF = platelet-derived growth factor, PRP = platelet-rich plasma, TGF = transforming growth factor

cartilage is regenerated, growth factors are biologically active polypeptides that are endogenous molecules. Growth factors can be applied to stimulate cell growth, enhance chondrogenesis, and augment the management of cartilage defects (Table 3).

Transforming Growth Factor-β Superfamily

Members of the TGF-β superfamily, such as TGF-β1, BMP-2, BMP-7, TGF-β3, and cartilage-derived morphogenetic proteins-1 and -2, are used to stimulate cartilage repair by inducing chondrogenic differentiation and stimulating production of cartilage ECM.[31] The most common growth factor used to stimulate chondrogenesis is TGF-β, which stimulates ECM synthesis, chondrogenesis in the synovial lining, and BMSCs, while decreasing the catabolic activity of interleukin-1 (IL-1). BMP-2 has been used in other orthopaedic applications to stimulate bone growth, either in the setting of fracture healing or the formation of a fusion mass, but it has the potential to stimulate matrix synthesis and reverse chondrocyte dedifferentiation. BMP-7 helps to stimulate cartilage matrix synthesis, acts synergistically with other anabolic growth factors, and inhibits catabolic factors, such as matrix metalloproteinase (MMP)-1, MMP-13, IL-1, IL-6, and IL-8.

Fibroblast Growth Factor Family

Members of the fibroblast growth factor (FGF) family, specifically FGF-2 (ie, basic FGF [bFGF]) and FGF-18, act by binding to cell surface receptors, promoting anabolic pathways, and decreasing the activity of the catabolic enzyme aggrecanase. In a murine model, subcutaneous administration of FGF-2 reduced OA, whereas FGF-2 knockout mice were found to have accelerated OA.[32] However, caution is warranted when using FGF-2 because higher doses may promote increased inflammation by antagonizing insulin-like growth factor (IGF)-1 and upregulating MMPs.

Insulin-like Growth Factor

IGF-1 helps maintain articular cartilage integrity and induces anabolic effects for cartilage repair while decreasing catabolic effects. IGF-1 works better in combination with other growth factors, such as TGF-β and BMP-7. Mice with chronic IGF-1 deficiency are more likely to develop articular cartilage lesions, and increased IGF-1 results in increased protection of the synovial membrane.[33]

Platelet-derived Growth Factor

Platelet-derived growth factor is a chemotactic factor for mesenchymal cells. It has been shown to stimulate wound healing and promote the formation of cartilage with increased proteoglycan production and cell proliferation.[34] Platelet-derived growth factor also has been shown to suppress IL-1β–induced cartilage degradation by downregulating nuclear factor-κB signaling.

Platelet-rich Plasma

Platelet-rich plasma (PRP) is considered to be a potential source of growth factors, given its role in wound healing and in the management of other musculoskeletal diseases. Clinical studies have been conducted evaluating the role of intra-articular injections of PRP in the management of OA.

In a study of patients with knee OA, Spaková et al[35] demonstrated that patients treated with three intra-articular injections of PRP had better clinical function and less pain than did patients treated with three intra-articular injections of hyaluronic acid. A different study demonstrated

that patients with hip OA that was managed with ultrasonography-guided injection of PRP into the affected hip demonstrated improved patient assessment scores (ie, Western Ontario and McMaster Universities Osteoarthritis Index, Harris hip) and decreased pain at 6-month follow-up.[36]

Gene Therapy

The concept of using gene therapy to manage musculoskeletal conditions was first proposed for the treatment of rheumatoid arthritis.[37] Biologic factors applied to suppress cytokines, such as tumor necrosis factor-α and IL-1β, have been integral in managing rheumatoid arthritis. The search for therapeutic targets that could be used to treat cartilage degradation through viral or nonviral vectors by in vivo or ex vivo means is currently being investigated for clinical application.

Therapeutic Targets

In the case of OA, five gene therapeutic targets that enhance chondrogenesis have been extensively studied: growth factors, including TGF-β, BMP, FGF, IGF-1, and epidermal growth factor; transcription factors (eg, SOX9); signal transduction molecules (eg, SMADs); proinflammatory cytokine inhibition (ie, TNF-α, IL-1β); and apoptosis or senescence inhibition (B cell lymphoma-2, -XL; inducible nitric oxide synthase) (Table 4). Of these molecules, only TGF-β1 has been studied in the clinical setting. Phase I has been completed of a clinical trial examining the management of knee arthritis using TissueGene-C (TissueGene), a cell-mediated gene therapy system in which allogenic chondrocytes express TGF-β1.[38] The safety of the product was established, with only minor local reactions from administration of the injection. Further studies are under way to determine functional improvements, clinical results, and radiographic parameters used to evaluate the management of OA.

Vectors

The vectors used to deliver gene therapy include nonviral and viral constructs. Nonviral delivery methods such as naked DNA, DNA liposomes, and complexed DNA have the advantage of being noninfectious; however, they are transient. Viral vectors, including adenovirus, adeno-associated virus, herpes simplex virus, foamy virus, and lentivirus, are beneficial because they allow for stable gene expression through insertion of the DNA into the host chromosome. However, altering the host DNA has the potential for insertional mutagenesis as well as host immune reaction to viral proteins.

Delivery

Gene delivery is conducted in vivo or ex vivo, depending on the location of delivery. Gene therapy delivery to synovium is better than delivery to cartilage because synovium has a larger surface area, with a thin lining of synoviocytes. Ex vivo delivery to both tissues is beneficial in that gene transfer can be used to augment cartilage repair. However, in vivo approaches are less labor intensive and costly than cell culture and maintenance ex vivo. Cartilage formation can be enhanced with use of gene constructs of appropriate chondrogenesis-enhancing factors to facilitate the delivery of gene therapy to chondrocytes and MSCs.[39]

Table 4

Gene Delivery Targets Used in Cartilage Regeneration

Category	Gene Target
Growth factors	TGF-β, BMP, FGF, IGF-1β, EGF
Transcription factors	SOX9
Signal transduction molecules	SMADs
Proinflammatory cytokine inhibition	TNF-α, IL-1
Apoptosis	Bcl-2, Bcl-XL, iNOS

Bcl = B cell lymphoma, BMP = bone morphogenetic protein, EGF = epidermal growth factor, FGF = fibroblast growth factor, IGF = insulin-like growth factor, IL = interleukin, iNOS = inducible nitric oxide synthase, SOX = Sry-related HMG-box, TGF = transforming growth factor, TNF = tumor necrosis factor

Bioreactors

The engineered cartilage tissue construct, consisting of cells, scaffold, and growth factors, must be cultured in a controlled manner that facilitates nutrient supply, metabolite exchange, and generation of a three-dimensional construct within a contained environment that mimics physiologic conditions. This is generally accomplished by using automated bioreactors that are capable of delivering mechanobiologic activation to cell-loaded scaffolds that are used to develop ex vivo cartilage tissue. Automated processing using bioreactors also increases reproducibility and decreases contamination.[40] The three main types of bioreactors that have been used for cartilage tissue constructs are hydrostatic, dynamic loading, and hydrodynamic.

Hydrostatic bioreactors are medium-filled chambers that can administer hydrostatic pressure to enhance chondrogenesis of MSCs and to condition engineered tissue constructs by mimicking the hydrostatic load in joints.

Dynamic-loading bioreactors are motorized to generate mechanical loading to cells or tissue constructs, in either confined or unconfined conformations, at specific frequencies and magnitudes of strain. This type

of dynamic loading also mimics certain aspects of physiologic weight bearing and has been found to improve MSC chondrogenesis and mechanical properties of engineered cartilage.

In general, hydrodynamic bioreactors consist of instrumentations that rotate or agitate to enhance nutrient transport, gas exchange, and metabolite removal to engineered constructs that are either suspended in medium or fixed in place. Use of hydrodynamic bioreactors on chondrogenic constructs has been reported to enhance matrix proteoglycan production, resulting in constructs with compressive properties more similar to native cartilage.[4]

Summary

The field of cartilage tissue engineering has advanced quickly in the past decade, and many novel approaches have been developed. However, although early results have been promising, engineered cartilage with properties identical to those of native cartilage is currently unavailable.

Significant obstacles remain, and the future of cartilage engineering lies in addressing issues such as ensuring optimal and stable chondrogenic cellular phenotype and cartilage matrix production, preventing matrix and cellular degradation, promoting appropriate cartilage integration, and delivering antioxidant and anti-inflammatory factors to provide durable cartilage constructs.

Challenges include regulatory hurdles, as well as safety, viability, and potential immunogenicity of the engineered tissue. The variety and depth of emerging technologies have the potential to revolutionize the field of cartilage regeneration, which is expected to develop and flourish in the next decade.

References

Evidence-based Medicine: Levels of evidence are described in the table of contents. In this article, reference 21 is a level I study. References 2, 16, 35, 36, and 38 are level II studies. References 1 and 31 are level III studies. References 3, 23-25, 27, and 30 are level IV studies. References 4, 6, 8, 10, 11, 15, 19, 20, 22, 28, 34, 37, 39, and 40 are level V expert opinion.

References printed in **bold type** are those published within the past 5 years.

1. Lawrence RC, Felson DT, Helmick CG, et al: Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part II. *Arthritis Rheum* 2008;58(1):26-35.
2. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L: Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994; 331(14):889-895.
3. Wood JJ, Malek MA, Frassica FJ, et al: Autologous cultured chondrocytes: Adverse events reported to the United States Food and Drug Administration. *J Bone Joint Surg Am* 2006;88(3):503-507.
4. Kuo CK, Li WJ, Mauck RL, Tuan RS: Cartilage tissue engineering: Its potential and uses. *Curr Opin Rheumatol* 2006; 18(1):64-73.
5. **Adkisson HD IV, Martin JA, Amendola RL, et al: The potential of human allogeneic juvenile chondrocytes for restoration of articular cartilage. *Am J Sports Med* 2010;38(7):1324-1333.**
6. Keller G: Embryonic stem cell differentiation: Emergence of a new era in biology and medicine. *Genes Dev* 2005;19(10):1129-1155.
7. **Loh YH, Agarwal S, Park IH, et al: Generation of induced pluripotent stem cells from human blood. *Blood* 2009; 113(22):5476-5479.**
8. **Toh WS, Lee EH, Cao T: Potential of human embryonic stem cells in cartilage tissue engineering and regenerative medicine. *Stem Cell Rev* 2011;7(3):544-559.**
9. **Koyama N, Miura M, Nakao K, et al: Human induced pluripotent stem cells differentiated into chondrogenic lineage via generation of mesenchymal progenitor cells. *Stem Cells Dev* 2013; 22(1):102-113.**
10. Tuan RS: Stemming cartilage degeneration: Adult mesenchymal stem cells as a cell source for articular cartilage tissue engineering. *Arthritis Rheum* 2006;54(10):3075-3078.
11. Chen FH, Tuan RS: Mesenchymal stem cells in arthritic diseases. *Arthritis Res Ther* 2008;10(5):223.
12. Lee KB, Hui JH, Song IC, Ardany L, Lee EH: Injectable mesenchymal stem cell therapy for large cartilage defects: A porcine model. *Stem Cells* 2007;25(11): 2964-2971.
13. Liu Y, Shu XZ, Prestwich GD: Osteochondral defect repair with autologous bone marrow-derived mesenchymal stem cells in an injectable, in situ, cross-linked synthetic extracellular matrix. *Tissue Eng* 2006; 12(12):3405-3416.
14. **Fortier LA, Potter HG, Rickey EJ, et al: Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. *J Bone Joint Surg Am* 2010;92(10):1927-1937.**
15. Wakitani S, Mitsuoka T, Nakamura N, Toritsuka Y, Nakamura Y, Horibe S: Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: Two case reports. *Cell Transplant* 2004;13(5):595-600.
16. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M: Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 2002;10(3):199-206.
17. **Guilak F, Estes BT, Diekman BO, Moutos FT, Gimble JM: 2010 Nicolas Andry Award: Multipotent adult stem cells from adipose tissue for musculoskeletal tissue engineering. *Clin Orthop Relat Res* 2010;468(9):2530-2540.**
18. Yoshimura H, Muneta T, Nimura A, Yokoyama A, Koga H, Sekiya I: Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. *Cell Tissue Res* 2007;327(3): 449-462.
19. Safran MR, Kim H, Zaffagnini S: The use of scaffolds in the management of articular cartilage injury. *J Am Acad Orthop Surg* 2008;16(6):306-311.
20. Ruoslahti E: RGD and other recognition sequences for integrins. *Annu Rev Cell Dev Biol* 1996;12:697-715.
21. **Crawford DC, DeBerardino TM, Williams RJ III: NeoCart, an autologous cartilage tissue implant, compared with microfracture for treatment of distal femoral cartilage lesions: An FDA**

phase-II prospective, randomized clinical trial after two years. *J Bone Joint Surg Am* 2012;94(11):979-989.

22. Reddi AH, Becerra J, Andrades JA: Nanomaterials and hydrogel scaffolds for articular cartilage regeneration. *Tissue Eng Part B Rev* 2011;17(5):301-305.

23. Gobbi A, Kon E, Berruto M, Francisco R, Filardo G, Marcacci M: Patello-femoral full-thickness chondral defects treated with Hyalograft-C: A clinical, arthroscopic, and histologic review. *Am J Sports Med* 2006;34(11):1763-1773.

24. Filardo G, Kon E, Di Martino A, Iacono F, Marcacci M: Arthroscopic second-generation autologous chondrocyte implantation: A prospective 7-year follow-up study. *Am J Sports Med* 2011; 39(10):2153-2160.

25. Dhollander AA, Verdonk PC, Lambrecht S, et al: Midterm results of the treatment of cartilage defects in the knee using alginate beads containing human mature allogenic chondrocytes. *Am J Sports Med* 2012;40(1):75-82.

26. Li WJ, Tuli R, Okafor C, et al: A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. *Biomaterials* 2005;26(6):599-609.

27. Joshi N, Reverte-Vinaixa M, Díaz-Ferreiro EW, Domínguez-Oronoz R: Synthetic resorbable scaffolds for the treatment of isolated patellofemoral cartilage defects in young patients: Magnetic resonance imaging and clinical evaluation. *Am J Sports Med* 2012;40(6): 1289-1295.

28. Williams RJ, Gamradt SC: Articular cartilage repair using a resorbable matrix scaffold. *Instr Course Lect* 2008;57:563-571.

29. Elguizaoui S, Flanigan DC, Harris JD, Parsons E, Litsky AS, Siston RA: Proud osteochondral autograft versus synthetic plugs: Contact pressures with cyclical loading in a bovine knee model. *Knee* 2012;19(6):812-817.

30. Kon E, Delcogliano M, Filardo G, Busacca M, Di Martino A, Marcacci M: Novel nano-composite multilayered biomaterial for osteochondral regeneration: A pilot clinical trial. *Am J Sports Med* 2011;39(6):1180-1190.

31. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ: The role of growth factors in cartilage repair. *Clin Orthop Relat Res* 2011;469(10):2706-2715.

32. Chia SL, Sawaji Y, Burleigh A, et al: Fibroblast growth factor 2 is an intrinsic chondroprotective agent that suppresses ADAMTS-5 and delays cartilage degradation in murine osteoarthritis. *Arthritis Rheum* 2009;60(7):2019-2027.

33. Goodrich LR, Hidaka C, Robbins PD, Evans CH, Nixon AJ: Genetic modification of chondrocytes with insulin-like growth factor-1 enhances cartilage healing in an equine model. *J Bone Joint Surg Br* 2007;89(5):672-685.

34. Schmidt MB, Chen EH, Lynch SE: A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. *Osteoarthritis Cartilage* 2006;14(5):403-412.

35. Spaková T, Rosocha J, Lacko M, Harvanová D, Gharaibeh A: Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. *Am J Phys Med Rehabil* 2012;91(5):411-417.

36. Sánchez M, Guadilla J, Fiz N, Andia I: Ultrasound-guided platelet-rich plasma injections for the treatment of osteoarthritis of the hip. *Rheumatology (Oxford)* 2012;51(1):144-150.

37. Evans CH, Robbins PD, Ghivizzani SC, et al: Gene transfer to human joints: Progress toward a gene therapy of arthritis. *Proc Natl Acad Sci U S A* 2005; 102(24):8698-8703.

38. Ha CW, Noh MJ, Choi KB, Lee KH: Initial phase I safety of retrovirally transduced human chondrocytes expressing transforming growth factor-beta-1 in degenerative arthritis patients. *Cytotherapy* 2012;14(2):247-256.

39. Steinert AF, Nöth U, Tuan RS: Concepts in gene therapy for cartilage repair. *Injury* 2008;39(suppl 1):S97-S113.

40. Hildner F, Albrecht C, Gabriel C, Redl H, van Griensven M: State of the art and future perspectives of articular cartilage regeneration: A focus on adipose-derived stem cells and platelet-derived products. *J Tissue Eng Regen Med* 2011;5(4):e36-e51.