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REVIEW

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Biomaterial-Mediated Delivery of Microenvironmental Cues for Repair and Regeneration of Articular Cartilage

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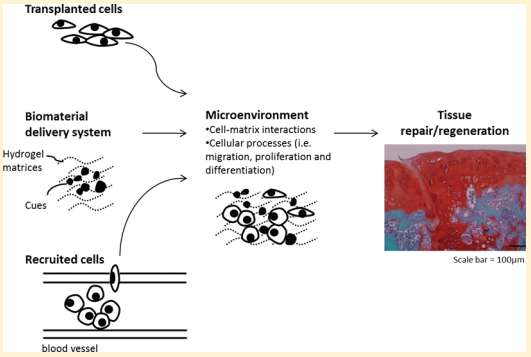
ABSTRACT: Articular cartilage injuries are one of the most challenging problems in musculoskeletal medicine due to the poor intrinsic regenerative capacity of this tissue. The lack of efficient treatment modalities motivates research into tissue engineering: combining cells, biomaterials mimicking extracellular matrix (scaffolds) and microenvironmental signaling cues. The aim of this review is to focus on the use of biomaterials as delivery systems for microenvironmental cues in relation to their applications for treatment of cartilage defects. The latest advances in cartilage tissue engineering and regeneration are critically reviewed to demonstrate an outline of challenges toward biomaterial-based approaches of cartilage regeneration.

KEYWORDS: biomaterials, delivery systems, cartilage, microenvironment, repair, regenerative medicine, tissue engineering



INTRODUCTION

Articular cartilage injuries, often caused by trauma, have limited ability to heal, which over time may lead to osteoarthritis.[1,2] Osteoarthritis is the most common form of arthritis, affecting 27 million people in United States, and the number of cases is increasing due to aging of the population and an obesity epidemic.[3,4] Many treatment modalities have been evaluated for their clinical efficacy to relieve patients from pain and to restore their movements. While there are some promising results, most current cartilage repair techniques eventually lead to fibrocartilage formation and cartilage degeneration after a temporary relief of symptoms.[5,6]

Due to its limited regenerative capacity, cartilage is an ideal candidate for tissue engineering. Tissue engineering represents an integrative strategy to reconstruct or reconstitute a tissue both structurally and functionally by the use of biomaterials as scaffolds, in combination with cells and signaling cues/regulators incorporated within to modulate cellular processes including proliferation, differentiation and tissue morphogenesis.[7,8]

This review aims to provide an overview of emerging trends in cartilage tissue engineering and regenerative medicine. In particular, we will focus on the use of biomaterials as delivery systems for microenvironmental signaling cues and regulators in relation to their applications for cartilage repair. Recent advances in cartilage tissue engineering and regeneration are also critically reviewed to demonstrate an outline of challenges toward biomaterial-based approaches of cartilage regeneration. For a more comprehensive review of the diverse types of biomaterials in cartilage tissue engineering and repair, the reader is encouraged to refer to several recent reviews.[9−11]

ARTICULAR CARTILAGE STRUCTURE AND REGENERATION

Articular cartilage is a unique avascular, aneural and alymphatic load-bearing tissue which is supported by the underlying subchondral bone. The extracellular matrix (ECM) is composed of a hydrated network of type II collagen fibrils, which are specifically arranged architecturally, and water-retaining proteoglycan (aggrecan) molecules linked to hyaluronic acid. The fibrillar type II collagen structure is stabilized by types IX and XI collagens, and the basement membrane molecules, laminin, type IV collagen and perlican can be found in the pericellular ECM. This combination of molecules gives the articular cartilage its unique ability to withstand the repetitive compressive loading in daily activities.[12]

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Depending on the depth of lesion, cartilage defects can be partial thickness (chondral) or full thickness (osteochondral). Chondral defects do not penetrate the subchondral bone and therefore cannot be accessed by host blood supply, macrophages and mesenchymal stem cells (MSCs) originating from the bone marrow. The repair relies on the limited mitotic activities of the resident chondrocytes and is rarely effective.[13] Conversely, the osteochondral defects are lesions penetrating the subchondral bone, and in such cases, the bone marrow provides vascularization and MSCs to help in repair.[14] However, the reparative tissue resulting is usually fibrocartilaginous and prone to possible functional deterioration in the long term.[14−16] Despite the poor outcome of natural repair, it is still the basis of several current orthopedic procedures such as microfracture and drilling. Other treatment options include debridement/chondroplasty; osteochondral autografting/allografting; and cell-based therapies using cultured autologous chondrocytes.[17] Among these procedures, the cell-based therapy involving autologous chondrocyte implantation (ACI) has been shown to have the most promising clinical outcomes for cartilage repair. There are, however, several problems associated with ACI including donor site morbidity, loss of chondrocyte phenotype upon *ex vivo* expansion and healing with inferior fibrocartilage formation at the defect site.[18] Recent efforts have been directed at exploring the use of stem cells for cartilage tissue engineering and repair applications.[19,20]

BIOMATERIAL-ASSISTED CELL-BASED THERAPIES IN CLINICAL USE

When transplanting cells for cartilage repair, two general approaches are adopted. The first approach involves cell isolation and direct injection of these cells, with minimal manipulations, into the defect site.[21] The second approach involves extensive *in vitro* manipulations where cells may be seeded into a biomaterial scaffold, allowed to proliferate and differentiate, and subsequently injected or implanted into the defect site. In this instance, the biomaterial scaffold serves to localize the cells in the defect site at the time of implantation.[22]

Cell-based therapy-ACI for cartilage repair was first developed in the late 1980s by Grande et al.,[23] and later brought into clinical application by Brittberg et al. in 1994.[24] This first generation approach involved isolation of chondrocytes from a cartilage biopsy, expansion *in vitro* to sufficient numbers, and injection into the defect covered with a periosteal flap.[24] Two further generations of ACI have been developed with refinements in the implantation method. The second generation made use of an off-the-shelf collagen membrane, instead of the autologous periosteum, to suture over the cartilage defect, prior to cell injection. The third generation, also known as matrix-induced ACI (MACI), was an innovation based on biomaterials as scaffolds. In MACI, chondrocytes were seeded within the porous surface of the collagen membrane in the operating room, just prior to implantation into the defect. Other commonly used biomaterial scaffolds include the collagen gel[25] and hyaluronan polymer.[26] This approach using biomaterials as scaffolds for chondrocytes allows the ease of implantation into the defects without the need of periosteal flap, and may ultimately be performed arthroscopically and without sutures for fixation.[27,28]

This tissue engineering paradigm clearly demonstrates the advancement of cell-based therapy to biomaterial-assisted cell therapy, and strongly motivates the field of biomaterials research and cartilage tissue engineering. The latest strategies rely on biomaterial-based therapies, which are currently in the developmental stages, and involve the use of biomaterials as a central delivery system for microenvironmental cues and regulators to manipulate transplanted cells and to orchestrate host cell response *in vivo*. Furthermore, it is envisaged that ease of clinical application and functional improvements in cartilage repair and regeneration would be achieved through development of innovative biomaterial systems (e.g., injectable biomaterials).[29,30]

BIOMATERIAL DESIGN CONSIDERATIONS FOR CARTILAGE TISSUE ENGINEERING AND REGENERATION

Numerous scaffolding biomaterials, both natural and synthetic, have been tested for cartilage repair, either by themselves or in concert with the delivery of cells and/or bioactive cues and regulators. Some of these materials, such as collagen,[25] hyaluronan[26] and fibrin,[31] are already clinically used for cartilage repair.

Ideally, scaffolds serve as a framework (1) to structurally reinforce the defect and prevent the collapse of surrounding tissue; (2) to orchestrate endogenous host cell response, including major cellular processes (i.e., migration, proliferation and differentiation); (3) to degrade in a timely manner, in order to induce tissue ingrowth and proper orientation of the ECM; and (4) to meet the needs of the surgical procedures involving either minimally invasive techniques using injectable matrices for chondral defects or preformed and stiff matrix for osteochondral defects. Each of these criteria in the design of biomaterials is important in guiding cell attachment, migration, proliferation and differentiation to regenerate the tissue, but it appears difficult to combine all of them successfully. For example, hydrogel systems that mimic the native cartilage microenvironment including high water retention and spherical morphology of encapsulated cells may not be suitable for *in situ* repair of large osteochondral defects due to the lack of mechanical strength.

Biological Criteria in Biomaterial Design: Cellular Microenvironment. Biomaterials for cartilage tissue engineering applications have been traditionally evaluated for their physical (e.g., porosity and mechanical compressive strength) and chemical (e.g., degradation) properties.[32] In recent decades, design specifications for these biomaterials have been extended to the biological criteria that include a consideration of signals which cells receive from interacting with the biomaterial and the bioactive cues incorporated within the synthetic microenvironment (i.e., microenvironmental cues).[33,34] Previously, investigators found that chondrocytes could maintain their phenotype or redifferentiate after serial passaging by culturing in 3-dimensional (3D) systems utilizing the use of hydrogel-based biomaterials such as agarose[35] and alginate.[36] Since then, several 3D culture systems utilizing scaffold-based culture and bioreactor stimulation have been created for culture of chondrocytes as well as for chondrogenic differentiation of adult and embryonic stem cells.[37,38] In this instance, an attractive approach to develop an understanding of the interactions between the target cells and the biomaterial with its microenvironmental cues could be based on such *in vitro* 3D biomaterial-based culture model systems to mimic the *in vivo* cartilage microenvironment.[39] Notably, the ability of stem cell or progenitor populations, as contrasted to differentiated cell population (i.e., chondrocytes), to participate in cartilage regeneration has been found in many studies to be dependent on the provision of a more complex microenvironment, such as the incorporation of specific differentiation cues in

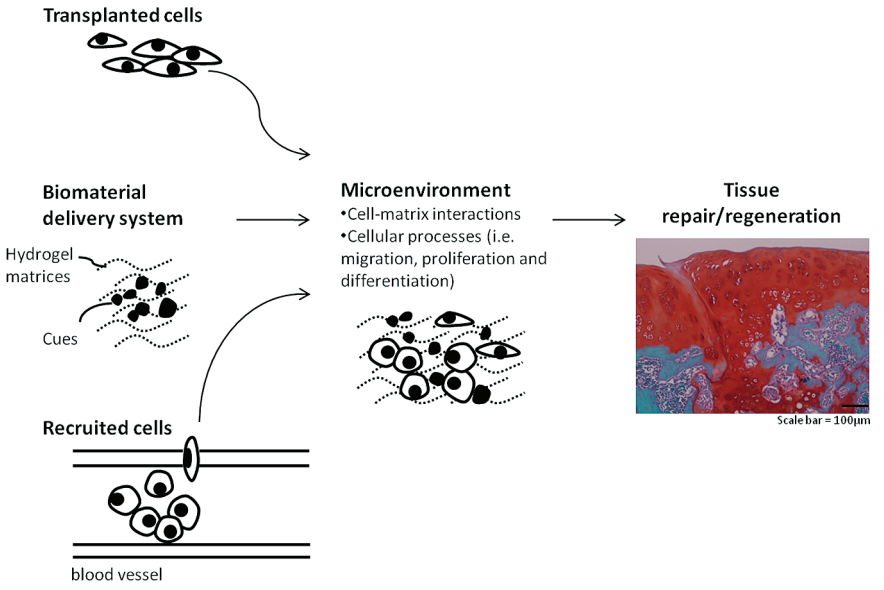

Figure 1. Appropriate biomaterial systems (e.g., injectable hydrogel matrices) can be used to either transplant donor or recruit host cell populations. In either case, the biomaterial delivery system can be designed to incorporate the appropriate cues to modulate the cellular microenvironment toward tissue repair and regeneration.

a timely manner to direct differentiation to the chondrogenic lineage, and to maintain the stable cartilage phenotype.[40,41]

Despite tissue engineering and modeling systems demonstrating some success *in vitro*, new challenges are faced when a strategy is translated *in vivo*. In the course of surgical intervention, it is inevitable that there will be trauma to the tissue being treated and its immediate surroundings, resulting in a rather disrupted cellular microenvironment. As a result, the outcome of any regenerative strategy or repair process depends on several factors in the cellular microenvironment including the level of oxygenation and vascularization, the presence of inflammatory triggers, the composition of endogenous cell population(s), and the complexity of biochemical and biophysical cues and their signaling. In addition, remodeling and restoration of the reparative tissue to its native state of zonal organization[42] and resistance to angiogenesis also appear uniquely critical for long-term normal tissue function of the cartilage.[43]

Most importantly, these processes have serious impact on cell survival, biosynthetic activity, and organization. Early studies to assess the donor cell fate of transplanted allogeneic chondrocytes, implanted in polylactic acid scaffolds[44] or alginate hydrogel matrices[45] into rabbit osteochondral defects, showed that >85% of cells were lost over 4 weeks post-transplantation. Similarly, our group performed a study transplanting human embryonic stem cell (hESC)-derived chondrogenic cells in hyaluronan hydrogel matrices into an immunosuppressed rat osteochondral defect model. By 12 weeks when the remodeling process had largely been completed, human cells accounted only for 4% of all cells in the regenerated cartilage layer, while none could be detected in the regenerated subchondral bone.[46]

Hence, a significant challenge in cartilage regenerative medicine is to apply effective strategies to modulate these processes in the *in vivo* cellular microenvironment and to orchestrate tissue formation toward functional cartilage regeneration. Unraveling the complexities of the cellular microenvironment and cell-biomaterial interactions will most likely improve the success of a tissue engineering strategy in cartilage repair.

Inspired by nature, tremendous efforts have focused on development of biomaterials as delivery systems for microenvironmental cues to provide an instructive microenvironment to regulate transplanted and host cell populations toward cartilage regeneration, as schematically represented in Figure 1 and discussed below.

■ BIOMATERIAL DELIVERY SYSTEMS FOR MICROENVIRONMENTAL CUES AND REGULATORS

Microenvironmental cues may be inherent in the biomaterial itself or conferred by functionalization routes unique to the biomaterials. These cues serve to "functionalize" the biomaterials in efforts to mimic the cellular microenvironment, to enhance its biodegradability and biocompatibility, to regulate host cell response, and in a cell delivery approach, to guide the transplanted cell population(s) toward cartilage integration and functional regeneration.

Precise control over presentation of microenvironmental cues allows one to regulate major cellular processes such as migration, proliferation and differentiation.[47] This is particularly important as both the types and quantities of the cues provided by the biomaterial are likely to play a crucial role in determining cell fate, as illustrated below with specific examples.

1. Scaffold Architecture and Geometric Cues. Scaffold architecture and geometry play a major role in dictating cellular behavior.[48] To date, a wide range of biomaterials in the forms of sponges, meshes and hydrogels have been used for both 3D cell culture and for cartilage repair.[49] Among all of these, hydrogels are biomaterials that could potentially be injected transcutaneously into joints, and therefore allow minimally invasive cell transplantation. Hydrogels are hydrated, water-insoluble polymeric networks cross-linked by water-soluble precursors, composed of either natural polysaccharides and proteins or synthetic polymers and their derivatives.[50] Hydrogels mimic the native cartilage microenvironment in many aspects. Besides having a high water content similar to the native cartilage, hydrogels promote

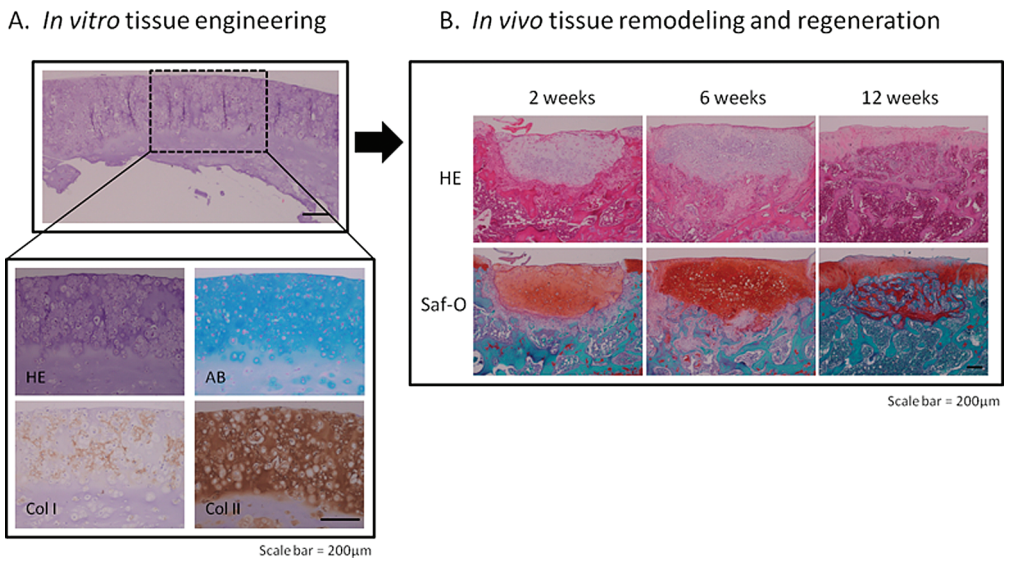

Figure 2. A wide variety of biomaterial systems are under development for delivery of therapeutic cells and cues, and these biomaterials must typically support tissue formation both *in vitro* and *in vivo*. (A) *In vitro* tissue engineering. In this example, hESC-derived chondrogenic cells were encapsulated in hyaluronan-based cross-linked hydrogel system.[46] Cells maintained their spherical morphology and cell−matrix interactions. ECM synthesis and deposition characteristic of hyaline-like neocartilage was promoted by cotreatment of TGF-β1 and BMP-7. (B) *In vivo* tissue remodeling and regeneration. Cell−hydrogel constructs implanted into critical sized osteochondral defects in rats demonstrated orderly remodeling and regeneration into osteochondral tissue, with characteristic architecture features including a hyaline-like neocartilage layer and a regenerated subchondral bone. (HE, Hematoxylin and Eosin; AB, Alcian Blue; Saf-O, Safranin-O; Col I, type I collagen; Col II, type II collagen; scale bar = 200 μm).

cell−cell and cell−matrix interactions, entrapment of secreted ECM and maintenance of spherical cellular morphology (Figure 2). In addition, this cell−scaffold architecture will likely offer unique advantages with regard to cell−matrix signaling and cell-mediated scaffold biodegradability.[51] Despite these advantages of hydrogels, porous scaffolds are still commonly employed as they can provide mechanical properties that more closely parallel that of the native cartilage tissue.

Controlling the geometric cues presented by the porous scaffolds has also dramatically influenced cellular processes and tissue regeneration. The porosity and pore size of collagen-based scaffolds controlled by the density of cross-linking, for example, have been shown in several studies to influence the cell adhesion and morphology, cell−cell and cell−matrix interactions and cellular condensation and chondrogenesis.[52,53] More recently, biphasic scaffolds have been created for the repair of osteochondral defects, in view that cartilage restoration depends largely on underlying subchondral bone regeneration.[54] Two different materials make up the biphasic architecture of the scaffold: the upper one is deemed to be conducive to chondrogenesis and is destined for the cartilaginous compartment of osteochondral defects, while the lower ceramic plug forms the underlying subchondral bone compartment.[55] Given the flexibility in varying the material, structural and mechanical properties of the cartilage and bone phases, biphasic design of the scaffold architecture represents an appealing strategy to many investigators.[56,57] This design concept has been demonstrated in several studies and observed improvements in the overall cartilage and bone regeneration, although integration of the reparative tissue with the host cartilage is still a problem, and one that has not yet been fully overcome.[58]

On a separate note, in the delivery of soluble cues to induce cartilage regeneration, the resultant effects can in part be regulated in concert with the appropriate geometric cues provided by the scaffold (e.g., shape, porosity and pore size). For example, in bone morphogenetic protein-2 (BMP-2) delivery to induce ectopic osteogenesis and chondrogenesis, scaffolds of a smaller pore size may favor cartilage formation by limiting vascular invasion in the microenvironment.[59]

Therefore, geometric cues delivered by the scaffolds can be designed, either by themselves or in concert with other cues to orchestrate the host cell response, in addition to regulating the transplanted cell population(s), to impact on the overall cartilage regeneration.

2. Mechanical Cues. With advancements in material science and engineering, biomaterial scaffolds can now not only serve as simple carriers for biomedical applications but also act as directive substrates for stem cells, and mediate major cellular processes including differentiation to the desired lineage. In a landmark study, Engler and colleagues demonstrated that MSC differentiation can be modulated by varying the elasticity of the substrate.[60] They showed in a 2D system that a soft substrate promotes differentiation of MSCs into neuronal-like cells, moderate elasticity favors myogenic differentiation, and a stiff substrate that mimics the mineralized collagen matrix of bone favors osteogenesis. These findings highlight the mechanical aspect of the cellular microenvironment in stem cell lineage specification. A recent study by Liu and colleagues demonstrated that chondrogenic differentiation of MSCs in 3D polyethylene glycol (PEG) hydrogel environment is influenced in part by the matrix elasticity, where softer matrix induced a greater degree of chondrogenic differentiation.[61] It remains unclear, however, if these findings are the result of active induction of differentiation or permissive selection of a subset of cell population for differentiation. Nevertheless, the optimal elasticity of the matrix for chondrogenic differentiation of stem cells may be obtained from the native cartilage tissue, but this remains to be demonstrated.

3. Adhesive Cues. Adhesion to substrate is required to allow transplanted cell survival, and manipulating the presentation of the adhesion cues, spatially and temporally, is likely to influence major cellular processes (i.e., migration, proliferation and differentiation) of the transplanted and host cell populations as they concomitantly participate in tissue repair.[62]

A wide variety of natural ECM-based biopolymers (e.g., collagen and fibrin) are currently used for cartilage repair due to their intrinsic cell-binding abilities, as are synthetic polymers to which adhesion is regulated by adsorbed proteins such as RGD (Arg-Gly-Asp)[63,64] motifs and collagen mimetic peptides (CMP).[65] These peptides have been shown in several studies to impact on the differentiation and proliferation of cells, and their ability to form cartilaginous tissue.[61,63−65] Similarly, synthetic peptides which mediate adhesion can also be presented in the form of injectable self-assembling hydrogels, coupled as side chains to the polymer backbones, or as components of synthetic proteins that provide "designer" combinations of bioactive domains to serve as binding sites for growth factors [e.g., transforming growth factor-β1 (TGF-β1)] to influence local cellular processes,[66] or as enzymatic degradation sites for matrix metalloproteinases and aggrecanases to control scaffold biodegradability and ECM remodeling.[67] For instance, Shah and colleagues recently reported an injectable coassembly system of peptide amphiphile (PA) molecules designed to form nanofibers for cartilage regeneration by displaying a high density of TGF-β1 binding epitopes (peptide sequence-HSNGLPL), and demonstrated the utility of the system in the repair of chondral defects pretreated by microfracture in a rabbit model, without the need of exogenous source of the growth factor.[66]

4. Soluble Cues and Regulators. Controlled presentation of soluble cues (e.g., cytokines, growth factors and small molecules) from biomaterial delivery systems can be utilized to regulate the fate of transplanted cells and host cell populations to participate in tissue repair. Major growth factors that have regulatory effects on chondrogenesis and cartilage formation include members of TGF-β superfamily, insulin-like growth factor (IGF) and fibroblast growth factor (FGF).[68,69]

In the simplest approach, morphogens, cytokines and growth factors that regulate proliferation, differentiation and biosynthetic activity may be physically encapsulated, covalently coupled, or associated via secondary bonding with the biomaterial system.[70] Additional delivery systems in the forms of liposomes[71] or microparticles[72] may also be involved to regulate the release of growth factors. Multiple factors can be delivered simultaneously or sequentially to facilitate multiple stages of cell differentiation and to orchestrate tissue formation.[72,73] The latest innovations involve cell-free biomaterial-based approaches to deliver soluble cues to induce cartilage regeneration by means of *in vivo* tissue engineering[74] or inducing endogenous cell homing and repair.[75]

This concept of *in vivo* tissue engineering has been adopted in several studies to explore cartilage formation at ectopic sites in the body.[76,77] These *in vivo* engineered cartilage tissues can subsequently be used as autologous cartilage grafts for repair of cartilage defects.[77] Of note, periosteum, with its osteogenic and chondrogenic cambium cell layer, represents a promising ectopic site for inducing bone and cartilage formation, depending on the guiding biomaterial and its microenvironmental cues.[76−78] In one such study by Stevens and colleagues, calcium alginate was implanted subperiosteally, to induce neo-osteogenesis and functional bone formation.[79] This approach was later modified by Emans and colleagues for cartilage formation, where agarose or hyaluronan hydrogel loaded with soluble cues, TGF-β1 and suramin (angiogenesis inhibitor), was able to induce hyaline cartilage formation. Conversely, hyaluronan hydrogels without the soluble cues yielded only fibrous tissue in the study.[77] Clearly, there was a differential response by periosteum-derived progenitor cells to differentiate to either bone or cartilage, depending on the appropriate biomaterial and the supporting soluble cues.

As mentioned, microfracture is one of the current surgical treatment options that induces cartilage repair by perforating the subchondral bone. Although there is release of cytokines, growth factors and mesenchymal cells, along with a fibrin clot, into the defect site to induce cartilage repair, the repair tissue resulting from microfracture is predominantly fibrocartilage. The second approach leverages on the structural and chemical aspects of a biomaterial scaffold, together with the soluble cues, to provide a cellular microenvironment critical to induce endogenous cell homing and repair. Toward this end, soluble cues carried by the scaffold serve to create gradients to recruit host cells by chemotaxis into the defect site and induce subsequent cell differentiation and tissue development.[80] Potent inducers that have been incorporated as soluble cues into biomaterial delivery systems to facilitate mesenchymal cell migration and enrichment for cartilage repair include synovial fluid,[81] serum[82] and defined growth factors.[83,84]

In several studies including those as mentioned above, TGF-βs have been regarded as potent chondrogenic inducers.[85] More recently, there has also been interest in introducing antiangiogenic factors such as suramin,[77] Flt-1,[86] and endostatin,[87,88] in combination with the chondrogenic inducer, to induce better chondrogenesis and to help to restore the cartilage tissue to its native state of resistance to vascularization. However, at this time, there is still no clear beneficial effect of this approach in a cartilage defect model. It could be reasoned that early vascularization by microfacture is still needed to provide the fibrin clot (a provisional scaffold) and bone marrow-derived MSCs for tissue repair, particularly in the absence of exogenous cell transplantation.

The above studies clearly demonstrate that functional cartilage regeneration can be induced *in vivo* without the need for cell transplantation though judicious choice of a biomaterial scaffold and appropriate microenvironmental cues, as long as the necessary cell populations and signals can be recruited endogenously. At present, it appears that large defects (chondral and osteochondral) may not be restorable without cell delivery. In considering the potential of autologous cells for cartilage repair, however, one must take into consideration the fact that the cells are likely to suffer an age-related loss in their potential to proliferate and differentiate.[89−91] This is important as elderly people are particularly prone to osteoarthritic lesions of the articular cartilage. In this instance, assessment of one's intrinsic "regenerative capacity" might be helpful in devising an appropriate regenerative approach in a highly focused manner.

In summary, there is promise in future research involving the development of multifunctional biomaterial delivery systems that affect cartilage tissue regeneration on multiple levels including (1) delivering exogenous cells in a protective material system and enhancing cell survival *in vivo*; (2) displaying cues to enhance self-renewal and expansion of endogenous and transplanted stem cells in the microenvironment; and (3) delivering cues to mobilize endogenous stem cells and to stimulate their function, together with the transplanted cells, toward tissue-specific differentiation and functional cartilage regeneration.

CONCLUSION

Conventionally, cell-based therapies for cartilage repair have utilized simple cell injections, but there has been considerable progress recently in the development of biomaterial delivery systems, incorporating the critical microenvironmental cues, to guide the transplanted cells and to orchestrate the host cell response to participate in the tissue repair.[92] At present, there exist significant challenges in combining the various microenvironmental cues (e.g., geometric, mechanical, adhesive or soluble) within the design space of a biomaterial and applying them in a well-concerted and timely coordinated fashion to regulate cartilage formation *in vivo*. Through investigation in effective *in vitro* and *in vivo* systems, a deeper understanding of the cellular microenvironment and the cell−biomaterial interactions will likely contribute to the diversity of the microenvironmental cues that can be incorporated into future biomaterials to enhance functional cartilage regeneration.

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