Functional biomaterials for cartilage regeneration

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Abstract: The injury and degeneration of articular cartilage and associated arthritis are leading causes of disability worldwide. Cartilage tissue engineering as a treatment modality for cartilage defects has been investigated for over 20 years. Various scaffold materials have been developed for this purpose, but has yet to achieve feasibility and effectiveness for widespread clinical use. Currently, the regeneration of articular cartilage remains a formidable challenge, due to the complex physiology of cartilage tissue and its poor healing capacity. Although intensive research has been focused on the developmental biology and regeneration of cartilage tissue and a diverse plethora of biomaterials have been developed for this purpose, cartilage regeneration is still suboptimal, such as lacking a layered structure, mechanical mismatch with native cartilage and inadequate integration between native tissue and implanted scaffold. The ideal scaffold material should have versatile properties that actively contribute to cartilage regeneration. Functional scaffold materials may overcome the various challenges faced in cartilage tissue engineering by providing essential biological, mechanical, and physical/chemical signaling cues through innovative design. This review thus focuses on the complex structure of native articular cartilage, the critical properties of scaffolds required for cartilage regeneration, present strategies for scaffold design, and future directions for cartilage regeneration with functional scaffold materials.

Key Words: cartilage, hyaline cartilage, regeneration, functional materials


INTRODUCTION
Articular cartilage, particularly of the diarthrodial joint, is of great importance for physiological mobility. However, the structure and function of this tissue are frequently disrupted or damaged upon physical trauma or in degenerative joint diseases such as osteoarthritis, which is the leading cause of disability in older persons, afflicting an estimated 27 million people in the United States alone.1 Cartilage tissue lacks neural connections and vascularization, as well as a latent pool of stem cells/chondro-progenitors, which limits its self-reparative capacity through endogenous healing mechanisms. Lesions left unrepaired or which have undergone improper repair subsequently form tissues of inferior biochemical and mechanical strength, thereby rendering the cartilage susceptible to osteoarthritic development. In addition, mechanical forces encountered at the site of cartilage defects often impede potential de novo cartilage regeneration. Autologous chondrocyte implantation (ACI) is currently the only FDA-approved cell-based therapeutic strategy for repairing focal cartilage defects of younger patients, while arthroscopic lavage, abrasion arthroplasty, subchondral drilling, and microfracture all lead to suboptimal healing and
regeneration of cartilage defects. Although the recent development of tissue engineered cartilage is a promising treatment modality for the repair of damaged or defective tissue, we are currently unable to generate cartilaginous tissues with properties similar to that of native articular cartilage due to the complex hierarchical structure of natural cartilage. Overall, the long-term clinical prognosis is not good.2

Articular cartilage is a thick and highly organized tissue that lines the articulating ends of diarthrodial joints, which is crucial for efficient functioning of the joint. This tissue has very exquisite organization at both the macro and microscale that results in its unique biochemical and biomechanical properties (Fig. 1). It can be regarded as a composite, organic solid matrix that is saturated with water and mobile ions.3 Within native cartilage, stiff and elastic cross-linked collagen fibrils/fiber bundles help cartilage to resist lateral expansion on axial compression by maintaining a rigid framework. The articular cartilage specific proteoglycan (PG), aggrecan, with its highly sulfated glycosaminoglycan (GAG) chains attached to collagen fibrils, sequesters large amounts of ions and water via negative charges.4 Upon compression, some water molecules are forced out, causing reversible deformation of cartilage and temporarily increasing the contact area, while most water molecules remain under hydrostatic pressure at their original location through GAG, contributing to the compression stiffness and lubrication properties of cartilage.

Furthermore, the solid matrix of articular cartilage has a highly specific ultrastructural arrangement consisting of different zones varying with depth from the articular surface, forming a hierarchical structure that is typically divided into three zones: superficial zone (surface to 10–20% of thickness), middle zone (20–70%), and deep zone (70–100%). Each zone has distinct ECM composition and organization, cell morphology, and metabolic activity.5 Collagen fibrils in the superficial zone are oriented parallel to the articular surface and impart high tensile strength to withstand the tensile stress encountered under joint loads.6 The relatively small amount of PG attached onto the collagenous membrane could act as a barrier of high resistance against fluid flow when cartilage is compressed.7 In the middle zone, the larger collagen fibers are randomly oriented, while aggrecan content reaches its maximal level.8,9 In the deep zone, the collagen fibers form bundles that are oriented perpendicular to and attached to the calcified cartilage and subchondral bone. Collagen content per wet weight does not change significantly with depth, but depth-dependent increases in hydroxylysine and hydroxylysyl pyridinoline cross-links exist, and together with the presence of other minor collagens isoforms such as type IX and type XI,10 play a critical
role in the regulation of fibril size, interfibril cross-linking, and interactions with cartilage proteoglycans, thereby contributing to the mechanical properties of the tissue.\textsuperscript{11,12} The concentration of these PG aggregates increases from the articular surface to the middle zone, contributing to the high swelling pressure and water content, thus increasing the compressive modulus of the tissue.\textsuperscript{13,14} Chondrocytes at the different zones of articular cartilage have distinct morphologies and organization, and express zone-specific markers. Chondrocytes in superficial zone are flattened and clustered in a horizontal fashion at a relatively high density. Chondrocytes in the middle zone are more spherical and randomly oriented, while they are larger and organized in vertical columns in the deep zone.\textsuperscript{15} Cartilage can be regarded as a biphasic material with complex mechanical properties such as anisotropy, nonlinearity and viscoelasticity.

Cartilage was initially proposed to be one of the first tissues to be successfully tissue-engineered through a simple combination of scaffolds, cells, growth factors, and proper mechanical loading. However, the neo-cartilage developed by conventional tissue engineering strategies has a number of drawbacks such as lacking a layered structure, mechanics mismatching with native cartilage and inadequate adhesion between native tissue and implanted scaffold.\textsuperscript{16,17} Functional cartilage regeneration can only be achieved through a well-orchestrated interplay of biomechanical properties, unique hierarchical structures, extracellular matrix (ECM), and bioactive factors that are conducive for the differentiation and proliferation of chondrocytes within scaffolding biomaterials.\textsuperscript{19} The cells seeded into the scaffolding materials are responsible for the synthesis and metabolism of ECM; while the scaffolds should provide the optimal environmental conditions such as three-dimensionality, proper mechanical loading, low oxygen supply, spatiotemporally orchestrated ECM cues, morphogens and growth factors, to coax tissue development.

A plethora of biomaterials have been fabricated and evaluated for cartilage regeneration, in the form of sponges, hydrogels, electrospun fibers, and microparticles, each of which could provide some unique properties for chondrogenesis.\textsuperscript{15} While some of these biomaterials show promising prospects, few of them prove to promote quick and functional cartilage regeneration. Natural biomaterials, derived from either polymer (agarose, alginate, chitosan, and hyaluronate) or protein (collagen, gelatin, fibrin, and silk) are biocompatible but have poor mechanical strength and relatively high degradation rate in most cases.\textsuperscript{20} Synthetic biodegradable polymers offer some important advantages such as controllable degradation rate, high reproducibility, high mechanical strength, and easy manipulation into specific shapes, however, the cell recognition signals are usually missing in such scaffolds.\textsuperscript{15,21} Increasingly, surface or bulk modification of the scaffolding biomaterial have been explored to fabricate composite scaffolds that combine the advantages of synthetic polymeric material with that of natural materials to create biomimetic scaffolds.\textsuperscript{21,22} Although a few of these scaffolds are in the market and some are in clinical application,\textsuperscript{23} the materials used for patient cartilage defect healing are still primarily based on natural materials such as collagen I/III (Carticele\textsuperscript{(R)}, Chondro-Gide\textsuperscript{(R)}), or fibrin glue (Tisseel\textsuperscript{(R)}).

Functional biomaterials are materials that can provide specific bioactive signals to control the biological environment for cell recruitment and differentiation of stem/progenitor cells as well as maturation of the secreted ECM, and/or are able to respond to changes in their environment during the process of tissue formation.\textsuperscript{24} Functional biomaterials through innovative design can provide specific bioactive signals that should overcome the various challenges faced, such as the inadequate chondrogenic differentiation, lack of ECM maturation, lack of natural anisotropic structures, and inadequate spatiotemporal cell–cell and ECM–cell interaction. Functional biomaterials should ideally possess one or more properties beyond normal biomaterials\textsuperscript{25} and should be designed to respond to changes in their environment during the process of tissue formation.\textsuperscript{24} This review will focus on several high-priority objectives in designing biomaterials used for cartilage regeneration, including biomimetic hierarchical structures, mechanocompatible biomaterials, biosignals to promote chondrogenic differentiation, and integration with host tissues. Critical properties required for functional cartilage regeneration will be discussed; and the essential properties of functional biomaterials to fulfill these goals will be addressed (Table 1). Recent progress in this field will be summarized and future directions for the development of functional biomaterials are proposed.

**RESEARCH PROGRESS ON FUNCTIONAL BIOMATERIALS FOR CARTILAGE REGENERATION**

**Biomimetic hierarchical structures**

The first challenge to make functional biomaterials is to mimic the highly organized zonal architecture of articular cartilage: specifically its spatiomechanical properties and ECM composition,\textsuperscript{36} as well as to engineer a conducive microenvironment for inducing stem/progenitor cells to differentiate into specific chondrocyte phenotype and guide ECM maturation.\textsuperscript{37} There are two generic strategies to achieve this target, scaffold-based and cell-based approaches.

The scaffold-based approach relies on biomaterials to provide structural and mechanical strength, and spatiotemporal organization for cartilage regeneration with biomimetic zones. The physical properties, together with the biological and chemical cues of the scaffolds can coax embedded cells to specific phenotypes.\textsuperscript{38,39} Different combination of materials and morphogens can facilitate chondrogenesis or osteogenesis in continual but distinct portions of the scaffolds,\textsuperscript{40} while small chemical groups could induce mesenchymal stem cells to differentiate into different lineages.\textsuperscript{39} Hydrogels with collagen gradient enhances cartilage regeneration by recruiting more mesenchymal stem cells (MSCs), compared with hydrogels constituted of exactly the same materials but without collagen gradients.\textsuperscript{41} Agarose gels with depth-dependent mechanical properties were
adapted to fabricate nonhomogenous layered cartilage. Nevertheless, differences in Young’s Modulus of different layers became indistinct with extended in vitro culture.42

The cell-based approach aims to seed chondrocytes or differentiate stem/progenitor cells to chondrocytes in different layers, relying on the cell-directed generation of zonal phenotypic specificity. Multilayered photopolymerizing hydrogel encapsulating chondrocytes harvested from superficial, middle, and deep zones, respectively, were cultured in vitro to make hierarchical cartilage layers similar to their native counterparts.27 It has been shown that chondrocyte phenotypes are under dual regulation by internal genetic factors and the external environment.22 ECM composition in the microenvironment has a crucial effect in directing progenitor cells to differentiate to chondrogenic cells. Studies with the incorporation of ECM components, gelatin/chondroitin/HA in poly-(lactic-caprolactone) have significantly augmented the proliferation of MSCs and GAG synthesis.26 Various research groups including ours have shown that incorporation of a biomimetic surface to the scaffold could dramatically alter the differentiation outcomes of MSCs. Coating of the biomaterial with chondroitin sulfate (CS), a major GAG found in cartilage, resulted in enhanced formation of hyaline cartilage.43–46 Though provision of some ECM components has been shown to improve the chondrogenic differentiation of MSC, with enhancement in ECM deposition in general and formation of more hyaline cartilage, individual ECM components have different effects. For example, collagen type II has the tendency to induce hypertrophic chondrogenic phenotypes, while chondroitin sulfate helps to maintain pre-hypertrophic phenotypes.44,47–49 By incorporating a unique combination of ECM components (CS, HA, and metalloproteinase sensitive peptides) into polyethylene glycol (PEG) hydrogel, MSC can be differentiated in the presence of a distinct ratio of PG to collagen, forming tissue of different compressive modulus, corresponding to the mechanical strength of zonal cartilage.50 These results indicate the potential for a composite scaffold with specific biomaterial compositions that can direct progenitor stem cells to give rise to zonal cartilage.

Hybrid heterogenous scaffolds based on prototyped biomaterials and specific cell populations can be used to develop zonal cartilage. Cross-linkable hydrogels such as agarose, gelatin, or PEG 51,52 or synthetic biomaterials such as poly(e-caprolactone) are mixed with the cell suspension before being printed using a predesigned program to fabricate three-dimensional scaffolds. Biologically active molecules such as laminin, fibronectin, and glycosaminoglycan can be added to the bio-inks to promote zonal cartilage formation.29 Heterogeneous geometries and high cell viability can be achieved through these methods, but whether or not the cells and matrix deposition in the native orientation can facilitate biological properties and functions have to be investigated further.

**Mechanocompatible biomaterials**

Mechanical stimulation is not only essential for embryonic development of articular joint,28,30 but is also critical for in vitro chondrogenesis as well as functional cartilage regeneration in adults through up-regulation of genes, activation of signaling pathways and maturation of ECM.53,54 Viscoelasticity and extra-low lubricant coefficient even under 5 MPa physiological load,55 and proper Young’s Modulus (0.4–0.8 MPa) is particularly important for neocartilage development.31,56,57 With the development of cartilage tissue engineering, the mechanical environment of cells and their microenvironments has aroused much interest, and many mechanoactive scaffolding biomaterials have been developed.

Mechanical compatibility of scaffolding materials with native cartilage is critical for functionality of regenerated cartilage at both the macroscopic and microscopic levels, at least for relatively large chondro-defects, as physiological loads cannot be effectively compensated by surrounding tissues (Fig 2).58,59 An anisotropic three-dimensional woven polyglycolic acid structure has been fabricated with mechanical properties similar to native cartilage. When incorporated with hydrogels, it could provide good permeability for chondrocytes to attach and proliferate.31 Elastomeric scaffolds such as poly1, 8-octanediol citrate (POC)60 and poly-(l-lactide-co-ε-caprolactone) that could deliver mechanical stimulation to cells or tissues and promote cartilage formation have been developed.61 We have attempted to fabricate scaffolds with mechanical properties similar to articular cartilage. Cartilage tissue is regarded as biphasic, being predominantly composed of collagen fibers and proteoglycan.

### TABLE I. Key Properties of Biomaterials Essential for Functional Articular Cartilage Tissue Engineering

<table>
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<tr>
<th>Key Properties of Biomaterials</th>
<th>Examples</th>
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<tr>
<td>Hierarchical structures</td>
<td>Scaffold-based</td>
<td>Physical or chemical gradients in scaffolds to produce zonal cartilage</td>
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<td></td>
<td>Cell-based</td>
<td>Multilayered photopolymerizing hydrogels</td>
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<td>Hybrid scaffolds</td>
<td>Proto-typed biomaterials and specific cell population to develop zonal cartilage</td>
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<td>Mechano-compatible</td>
<td>Young’s Modulus, viscoelasticity, and extra-low lubricant coefficient</td>
<td>Woven Poly-glycolic acid with hydrogel</td>
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<td>Mechanical sensitivity</td>
<td>Mechanical signals</td>
<td>PLCL scaffold</td>
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<td>Cell aggregation</td>
<td>Cell to cell aggregation</td>
<td>Topography of materials induced cell aggregation collagen</td>
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<tr>
<td></td>
<td>Cell to matrix aggregation</td>
<td>Functionalized chondroitin sulphate</td>
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<tr>
<td>Integration with native cartilage</td>
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Based on this understanding, poly-lactide-co-caprolactone (PLCL) was adopted as a mimic to collagen fibers for providing a relative rigid frame structure, while chitosan was immobilized on PLCL as a substitute for GAG. Although the chitosan-PLCL scaffolds had a similar viscoelastic property to native cartilage, their Young's Modulus was one magnitude less than native cartilage. Several drawbacks could be attributed to this inferiority in mechanical properties, such as inferior mechanical properties of PLCL compared with collagen, inappropriate PLCL/chitosan ratio, difference in amount of charge density between chitosan and GAG, and lack of other functional molecules (fibronectin, hyaluronic acid, decorin, etc.) to fortify the scaffolds. Integration of graphene and carbon nanotube could effectively enhance mechanical properties of polymeric scaffold, however, their nondegradation properties hinder clinical application to some extent.

Mechanical properties of degradable biomaterials are altered by degradation, as degradation gradually erodes the integral structures. On the other hand, mechanical properties of cell-laden biomaterials may improve with ECM deposition and maturity. These dual effects on mechanical properties have to be considered in scaffold design. The fundamental roles of biomaterials are not only to provide a temporary substrate on which transplanted cells can adhere but must also maintain mechanical integrity during the healing process, as well as deliver appropriate mechanical stimuli to the attached cells under normal physiological weight loading condition. Mechanical stiffness of tissue is not a static property. As tissues develop, their stiffness alters according to developmental changes in the extracellular matrix (ECM). Understanding the effect of time-dependent stiffening on cell maturation may be a critical design parameter for future material-based cell therapies, especially the use of progenitor cells. Biomaterials with time-dependent elastic modulus or stiffness mimicking tissue development, which provide fine-tuned support for cell differentiation and tissue maturation should be introduced in scaffold design for cartilage regeneration.

The fatigue property of the scaffolds is also of great importance for scaffold design in cartilage tissue engineering, especially when cartilage is subjected to repetitive weight bearing. To this end, nanoporous materials such as carbon nanotubes, with nonwetting liquid in an enclosed system might be used as an effective energy absorption and damping system. Under normal conditions, the liquid is not able to wet the nanopore. However, under external loading (i.e. compression or impact), the liquid will be pushed into nanopores, and, thus, the force from the external load will be transferred into the solid–liquid interfacial energy or dissipated as heat by the internal friction. Due to the super-high specific surface area, the nanoporous energy damping system can have a much higher energy damping efficiency than traditional damping materials. In addition, the nanoporous energy damping system can be reused to protect against the multiple external loads. When one external load is removed, the water inside the nanopore can flow out since the free energy of water molecules is higher inside the nanopore (nanostate) than outside (bulk state). The system will fully recover to the initial state to prepare for damping the next load. This property could potentially enhance the scaffolds’ ability to withstand high compression.
force during physiological high repetitive loading (5 MPa). Self-healing polymers or polymer composites which could recognize and repair cracks provide another strategy to make up for damages in biomaterials integrated with de novo cartilage anlagen.68

Mechanical signals are involved in cell proliferation and differentiation through membrane and integrin activation/internalization, as well as cytoskeleton and cell rigidity changes.69,70 However, the mechanotransduction process by which mechanical signals are sensed and transmitted to biochemical pathways and manifested as changes in cell behavior is not clearly understood. It is believed that ion channels, integrins, and the intracellular cytoskeleton are involved.71,72 Activation of ion channels in the plasma membranes of chondrocytes allows an influx of ions such as calcium into the cell that leads to the activation of intracellular signaling pathways. Transmembrane integrins physically anchor chondrocytes to the ECM, forming transmembrane connections to the cytoskeleton, thus enabling signal transduction. Initial activation through integrin adhesion to the matrix recruits other integrin molecules and cytoskeletal molecules, leading to activation of focal adhesion kinase (FAK) and protein (paxillin, b-catenin),73 remodeling of the actin cytoskeleton,74 as well as activation of the MEK–ERK–MAP kinase cascade.75,76 The response of chondrocytes to mechanical stimuli depends on the nature of the stimulus, and the presence of appropriate ECM composition to engage and to activate cell surface molecules. Understanding the role and mechanism of mechanical and electrochemical signals on chondrocyte function will enable future strategies to develop functional scaffolds.

Biomaterials to promote chondrogenic differentiation through cell–cell interaction

Articular cartilage composed of hyaline cartilage has distinct biochemical components and mechanical properties, compared with fibrocartilage. The predominant collagen type in hyaline cartilage is collagen type II, preferably more than 95%.77 Suboptimal differentiation of chondrocytes or progenitors leads to accumulation of collagen type I rather than collagen type II and results in fibrocartilage formation.78 Much effort have been made to derive the appropriate chondrogenic phenotype in differentiation development, including selection of proper materials, control of topography and mechanical properties of materials, regulating distribution of the cells through adjusting porosity and connection of the pores.79,80 Spherical morphologies of chondrocytes are closely related to their chondrogenic potential,82 on the other hand, a dedifferentiated fibroblastic morphology is related to adhesion of chondrocytes via integrin and subsequent actin stress fibre arrangement. This dedifferentiation of the chondrocytes can be reversed by seeding them in three-dimensional hydrogel83 or with cytochalasin D to artificially inhibit actin fiber arrangement.84 Proper cell aggregation could induce differentiation and appropriate phenotype of chondrocytes during embryonic development and cartilage regeneration.25 Chondrogenic differentiation of stem cells is initiated by cell aggre-

gation to undergo a condensation process with increased cell density and cell–cell interactions during embryonic development, which leads to distinct spherical cell morphology and expression of chondrogenic marker genes Sox9, Sox5, and Sox6.85 Correlation between stem cell differentiation lineage commitment and cell shape was reported. Cell shape change confers a switch between chondrogenic (round shape) and smooth muscle cell (spread and flattened) fates involving Rac1 signaling regulation of N-cadherin, underlying the tight coupling between lineage commitment and changes in cell shape and cell–cell adhesion that occur during morphogenesis (Fig 3).86,87 Temporal expression of N-cadherin,88 and cadherin-7,89 through intracellular interaction with catenin, links cadherins to the actin cytoskeleton, in which re-organization also involves Rho GTPases.90,91

Chondrocytes naturally aggregate into clusters of 6 to 10 cells in suspension, while surrounding pericellular matrix rich in collagen II and cartilage oligomeric protein (COMP) helps to maintain phenotype of chondrocytes.92 Direct cell–cell aggregation among chondrocytes might not be a normal occurrence in mature cartilage. Preaggregation of expanded chondrocytes before loading into a porous scaffold enhances the quality of the resulting tissues.72 It has been proposed that condensation and aggregation of chondrocytes during OA development protected them from apoptosis.34,93

Biomaterial-induced cell aggregation can be achieved through manipulation of the extracellular environment surrounding the cells. The design of grooved topographical structures that induce cell collision13 and chitosan-graft-lactose coated microcarriers,94 have been shown to promote cell aggregation. Inclusion of biodegradable moieties in three-dimensional hydrogel can enhance cell aggregation.95 The hydrophobicity of the extracellular matrix could play an important role in dictating cellular behaviors, through the development of tunable, synthetic matrices with control over their hydrophobicity without altering the chemical and mechanical properties of the matrix.95 MSCs adopt specific morphology, migration pattern and aggregation on these surfaces that results in specific lineage differentiation. Spatial-design of hydrophobicity within the scaffold structure could be employed to promote cellular aggregation that enhances cartilage tissue formation.

Integration of de novo cartilage with host tissues

When transplanting the cell-scaffold constructs into cartilage defects, the integration of biomaterials or implants with host tissues, including native cartilage and subchondral bone, is crucial for both immediate functionality and long-term performance of the tissue graft. If the integration of de novo cartilage with native cartilage and subchondral bone is unstable, the neo cartilage can easily break away from the native cartilage and cause severe damage again.25

Much attention has thus been focused on integration between neo cartilage and native cartilage. As relatively low turnover of natural cartilage impedes the integration, bio glues or adhesives have been used with the aim to enhance integration. Two types of adhesives are broadly used—
reactive and nonreactive adhesives, defined by whether or not the adhesive chemically reacts to the original materials. The strength of adhesion depends on many factors, including van der Waals forces between molecules, electrostatic forces, capillary forces, and chemical bonds between adhesive and substrate. Several nonreactive glues/adhesives have been commercialized and used clinically, including derivatives of cyanoacrylates (Superglue), Bioglue (glutar-aldehyde-albumin), and fibrin glue (Tisseal). Biomimetic topography has been developed by mimicking gecko feet with an aim to adhere in a dry environment without chemical reaction. However, the strength of the adhesive is usually too low to mechanically integrate neocartilage to surrounding cartilage and serves mainly to facilitate cell seeding. Reactive adhesives have been developed to enhance the integration. Tissue transglutaminase, naturally expressed in cartilage and other tissues, is a biocompatible and stronger adhesive than nonreactive adhesives. Biopolymer chondroitin sulphate (CS), one of the major components of cartilage extracellular matrix, modified chemically as a novel bioadhesive, not only integrates specific biomaterials with native cartilage, but also allows chondrocytes to migrate freely across the interface between the implant and native tissue. This in turn enhances mechanical stability of the hydrogel and tissue repair in cartilage defects in both the short term and long term.

As changes in mechanical and physiological properties of subchondral bone have been implicated in degradation of articular cartilage during development of osteoarthritis, it is reasonable to deduce that they are equally important for regeneration and functionality of de novo cartilage. Integration with subchondral bone is of great importance for de novo cartilage to transduce physiological loads, exchange nutrients and metabolic waste, as well as shaping internal structures. Many hybrid scaffolds have been made to regenerate cartilage and subchondral bone simultaneously. However, the integration of regenerated cartilage with subchondral bone have not been evaluated histologically and mechanically in vivo. Porous scaffolds with a specific design of intramedullary stem not only effectively regenerate articular cartilages, but also achieved firm integration with subchondral bone. Even with these recent progresses, some challenges remain: how to differentiate cells into the osteogenic lineage and chondrogenic lineage separately in vitro within a single culture system? Can hybrid scaffolds with continuous structures transduce mechanical forces? How to develop scaffolds with improved mechanical properties to further enhance cartilage regeneration?
stimulus properly from cartilage to subchondral bone in vivo?

Controlled delivery of biochemical factors

Controlled delivery of biochemical factors as a powerful tool to provide a more biomimetic microenvironment for seeded cells has been an ongoing research topic in tissue regeneration, as it can significantly enhance tissue formation. Ideally, the delivery vehicles should have quantitative control of target-binding affinities and drug-carrying capacities, therapeutic unloading response, and intrinsic properties for labeled or nonlabeled tracing. With regard to cartilage tissue engineering, increasing attention has been paid to spatiotemporal delivery of morphogenetic effectors such as growth factors. However, more comprehensive and in-depth understanding of the developmental and regenerative biology of articular cartilage, such as lineage differentiation and surface markers, is urgently needed, before this aim can be achieved.

Biochemical factors can be absorbed physically, cross-linked chemically, or embedded in degradable biomaterials, to provide a sustained bioactive factor release for cartilage regeneration. Micro/nano polymeric particles as potential carriers have been developed. Microparticles embedded with biochemical factors could be incorporated into the hydrogel as a beneficial carrier for sustained release of biochemical factors, especially in direct injectable systems to maintain the phenotype of chondrocytes. Specific targeting has been achieved through a combination of macromolecular therapeutics, or through local delivery such as transcutaneous delivery. The stimuli-responsive carrier could also be developed for the controlled release of bioactive factors and be used to enable on-demand controlled release profiles that may improve the cartilage tissue formation, therefore, environmental stimulus, such as mechanical stimulus, temperature sensitivity, change of pH value, electrical and optical signals, could be adopted to trigger the release of loaded biochemical factors. Microparticles with sophisticated internal structures could potentially release biochemical factors in response to multiple stimuli.

FUTURE DIRECTION

Effective cartilage tissue engineering, either by implantation of chondrocytes or progenitor cells, or migration of host cells from surrounding tissues, need to produce neocartilage tissue with physiological function. However, current tissue engineering protocols usually result in dysfunction of the implanted/recruited cells to some extent, with inadequate production of ECM, suboptimal ratio of individual components, and inferior ability to form mature ECM. At present, a detailed comprehensive understanding of the contribution of biological factors, architectural and mechanical properties and biochemical/material composition in neocartilage tissue formation is still elusive. Biomaterial research is able to contribute to these processes through spatiotemporal regulation of biological processes, facilitating ECM alignment and functionality, and providing research models to deepen our understanding of cartilage biology. Functional biomaterials, with the ability to control biological environments by providing appropriate physical and biochemical cues, can contribute significantly to conventional technologies, such as controlled delivery of biochemical factors, or by combining with other novel technologies, such as nanotechnology and other advanced techniques.

The development of nanotechnology and nanomaterials has provided powerful tools as well as deepened our understanding of regenerative biology at the molecular level. Nanomaterials can not only recreate extracellular microenvironment, but also compensate for scaffolds limitations, such as weak mechanical properties and inability of cells to self-assemble to three-dimensional tissues. Nanoparticles can be guided to specific targets to enhance efficacy and decrease drug dosage. It is reasonable to propose that synthetic materials can interact with host protein or other molecules at predetermined spatiotemporal locations, leading to specific responses that enhance host tissue regeneration.

Future strategies to fabricate biomaterials should be more objective based on available scientific data, instead of arbitrarily designing biomaterials based on experience. Detailed analysis of the engineered cartilage using traditional techniques such as histological, biochemical, and mechanical assays, can be coupled with sophisticated mapping analytical techniques, such as atomic force microscopy and micro-Raman spectroscopy. These can provide informative insight into the structure–function relationships of cartilage at both the micrometer and nanometer levels.

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