TISSUE ENGINEERING: Part B
Volume 20, Number 6, 2014

DOI: 10.1089/ten.teb.2014.0034

Enhancing Chondrogenic Phenotype for Cartilage Tissue Engineering: Monoculture and Coculture of Articular Chondrocytes and Mesenchymal Stem Cells

Kelsea M. Hubka, BS, Rebecca L. Dahlin, PhD, Ville V. Meretoja, PhD, F. Kurtis Kasper, PhD, and Antonios G. Mikos, PhD

Articular cartilage exhibits an inherently low rate of regeneration. Consequently, damage to articular cartilage often requires surgical intervention. However, existing treatments generally result in the formation of fibrocartilage tissue, which is inferior to native articular cartilage. As a result, cartilage engineering strategies seek to repair or replace damaged cartilage with an engineered tissue that restores full functionality to the impaired joint. These strategies often involve the use of chondrocytes, yet *in vitro* expansion and culture can lead to undesirable changes in chondrocyte phenotype. This review focuses on the use of articular chondrocytes and mesenchymal stem cells (MSCs) in either monoculture or coculture for the enhancement of chondrogenesis. Coculture strategies increasingly outperform their monoculture counterparts with regard to chondrogenesis and present unique opportunities to attain chondrocyte phenotype stability *in vitro*. Methods to prevent chondrocyte dedifferentiation and promote chondrocyte redifferentiation as well as to promote the chondrogenic differentiation of MSCs while preventing MSC hypertrophy are discussed.

Introduction

Articular cartilage is an avascular and aneural tissue that functions to resist compression, to prevent adjacent bone contact, and to provide a low-friction surface for joint articulation.[1] Composed primarily of specialized extracellular matrix (ECM) components with high water content, cartilage is viscoelastic and, as such, can withstand high compressive loading.[2] Cartilaginous ECM contains collagen type II, noncollagenous proteins, and proteoglycans, such as aggrecan, that have sulfated glycosaminoglycans (GAGs) that absorb water. Chondrocytes are responsible for the generation and maintenance of this ECM and are the sole, differentiated cellular resident of articular cartilage.[1]

As a result of its low cellularity and lack of vasculature, cartilage retains a low rate of regeneration, and focal or degenerative lesions, caused by trauma or disorders, can lead to osteoarthritis.[3] As such, significant damage to cartilage tissue of articulating joints, such as the knee, can result in persistent pain and impaired motility. For lesions requiring operative treatment, many surgical options exist, but none of these options result in ideal cartilage tissue repair.[3] Surgical options that involve a cellular component such as autologous chondrocyte implantation require tissue biopsy, *in vitro* cell expansion, and cell implantation.[3] Clinical research has investigated the use of biomaterial scaffolds to facilitate the placement and retention of cells within a defect site.[4,5] However, *in-vitro*-expanded chondrocytes undergo extensive dedifferentiation[6,7] and produce repair tissue that is inferior to hyaline cartilage for the intended purposes of load bearing and low-friction joint movement. Many of these treatments are suggested to be most suitable for younger, active patient populations.[3] An increasing rate of osteoarthritis is observed in the aging population[3] due to wear and tear of the articulating joints and the inverse relationship between age and regeneration capacity. Clinicians and researchers alike recognize a need for improved cartilage repair and regeneration, and cartilage tissue engineering strategies have been investigated in response to these issues.

Cartilage engineering seeks to repair or replace damaged tissue with an engineered construct that restores full functionality to the impaired joint. Such strategies embrace the combination of scaffold, cells, and bioactive molecules for tissue regeneration.[8] Scaffolds of natural or synthetic materials are often designed to mimic native cartilage ECM. Articular chondrocytes (ACs) and mesenchymal stem cells (MSCs) are often employed as the cellular component since

Department of Bioengineering, Rice University, Houston, Texas.

chondrocytes are the native, differentiated cell type, and MSCs are the precursor or progenitor cells that possess the ability to differentiate into functional chondrocytes.[1] Growth factors, such as transforming growth factor-β (TGF-β) and insulin-like growth factor (IGF), are typically used to induce chondrogenesis of the cells.[9] Still, even with growth factor signaling and culture on biomaterial scaffolds, maintaining the chondrogenic phenotype of chondrocytes and MSCs remains a significant challenge in cartilage tissue engineering.

Cellular phenotype changes in culture

Current cartilage repair or replacement options that utilize implanted cells are limited by the number of cells available for isolation and by the uncontrolled phenotypic changes in those cells. As such, chondrocytes and MSCs have been investigated as cell sources for cartilage engineering due to their well-established ability to generate cartilage-like ECM under the appropriate culture conditions.[10,11]

These cells can be isolated from many different tissue sources in the body that contribute to detectable phenotypic differences among chondrocytes or MSCs.[12,13] Nonload-bearing regions of the cartilage can be harvested for chondrocyte isolation, but tissue availability is limited and often results in donor site morbidity.[14] For articular cartilage regeneration, ACs isolated from cartilage of articulating joints produce ECM with properties most similar to that of hyaline cartilage of the knee versus the ECM produced by auricular or costal chondrocytes.[15] MSCs are typically isolated from bone marrow or adipose tissue sources.[16,17] MSCs of bone marrow require less chondrogenic stimulation to achieve chondrogenic differentiation than MSCs of adipose tissue.[18–20] Still, even the utilization of ACs and bone-marrow-derived MSC populations presents issues like the loss of the chondrogenic phenotype of chondrocytes and hypertrophy of chondrogenically induced MSCs.[10,11,21]

The loss of chondrocyte phenotype upon proliferation *in vitro* was first documented in 1960 and termed dedifferentiation.[6] Phenotypically, dedifferentiation involves a decrease in expression of chondrocyte markers, such as collagen type II, aggrecan, and the transcription factor SOX9, and an increase in expression of fibroblastic markers, such as collagen type I and versican.[22–25] This is of critical importance for cartilage tissue engineering since dedifferentiated chondrocytes become fibroblastic and do not produce hyaline cartilage, which is necessary for the proper function of articulating joints. Alarmingly, dedifferentiation has been reported as early as the first passage in monolayer culture.[25,26] Extensive evidence exists demonstrating rapid chondrocyte dedifferentiation during culture expansion on polystyrene. Thus, the loss of chondrocyte phenotype prior to utilization in experimentation or implantation is of huge concern for the tissue engineering field.

The other cell population, the adult MSCs, possesses the ability to proliferate *ex vivo* and differentiate into multiple specialized cell types, including chondrocytes upon chondrogenic induction and osteoblasts upon osteogenic induction.[27] The widespread use of MSCs in tissue engineering strategies goes beyond their multipotent differentiation ability, and includes trophic and immunomodulatory effects.[28–31] Bone-marrow-derived MSCs are typically obtained via iliac crest marrow aspiration, which has a lower rate of donor site morbidity compared with cartilage harvest required for chondrocyte isolation. Unlike chondrocytes, MSCs can be expanded *in vitro* without the risk of losing their phenotype. However, MSCs tend to simultaneously obtain both chondrogenic and hypertrophic properties upon persistent exposure to chondrogenic stimuli, such as TGF-β and dexamethasone, prevalent in current culture methods.[32]

MSC hypertrophy is characterized by an increase in cell volume; an upregulation in the expression of collagen type X, matrix metalloproteinases, and vascular endothelial growth factor; and an increase in alkaline phosphatase (ALP) activity, which are all targets of the Runx2 transcription factor, a marker typically associated with osteogenic differentiation.[33] Hypertrophy of MSCs has even been harnessed for bone tissue engineering applications, attempting to mimic the progression of endochondral ossification to form osseous tissue.[34,35] This process might be beneficial for full-thickness osteochondral defect repair targeting specifically the subchondral bone layer,[36] but it is contraindicated to repair frictionless articulating joint surfaces. In addition, MSCs with hypertrophic phenotype express and produce collagen type I preferentially over collagen type II,[34] indicative of fibrocartilage formation instead of hyaline cartilage. Therefore, promoting chondrogenic differentiation while preventing hypertrophy of chondrogenic MSCs is crucial in the application of MSCs for the generation of articular cartilage-like matrix.

Numerous methods to address chondrocyte dedifferentiation and redifferentiation and MSC chondrogenic differentiation and hypertrophy are discussed in this review. Separate sections are devoted to cocultures of ACs and MSCs, as they have recently shown great promise in avoiding some of the common problems seen in the corresponding monocultures.

Preventing AC Dedifferentiation

Use of chondrocyte monoculture to prevent dedifferentiation

Monoculture is the classical method of culturing a single cell type, as opposed to multiple cell populations, that is useful for distinguishing effects of isolated compounds or culture conditions on a particular cell type. Several parameters, including cell seeding density, media components, culture substrates, and oxygen tension, have been varied in the culture of ACs to determine effects on chondrocyte phenotype and to mitigate dedifferentiation (Table 1).

Chondrocyte seeding density was investigated after observing expansional dedifferentiation *in vitro*. High chondrocyte seeding density resulted in less dedifferentiation compared with low seeding density cultures, as determined by collagen type II production.[22,24] The high seeding density inhibited cell spreading as compared with the low seeding density.[24] Expansional dedifferentiation could be a direct result of cell spreading, which is a function of seeding density and causes morphological and cytoskeletal changes to the naturally spherical chondrocytes.[24] As later reported, decreased chondrocyte spreading, which decreased actin stress fiber formation, led to increased chondrocyte marker expression such as SOX9,[37] promoting the spherical differentiated chondrocyte phenotype. In one interesting study,

Table 1. Articular Chondrocyte Monoculture: Methods to Prevent Dedifferentiation

Dominant factor	*Specific factors*	*Species and cell source*	*Ref. No.*
Seeding density	High seeding density (decreased cell spreading)	Porcine ACs	24
Growth factor	BMP-2	Human ACs	39
	FGF-2/TGF-β1	Human ACs	40
Substrate coating	Collagen I	Rabbit ACs	45
	Collagen II	Murine ACs	46
	ECM	Porcine ACs	47
Hypoxia	1.5%	Bovine ACs	50
	5%	Bovine ACs	49
Hypoxia and 3D perfusion	5%	Bovine ACs	41

ACs, articular chondrocytes; BMP-2, bone morphogenetic protein-2; ECM, extracellular matrix; FGF-2, fibroblast growth factor-2; TGF, transforming growth factor; 3D, three dimensional.

a continuously expanding culture surface provided room for AC expansion over 10 days while benefitting from an initial high cell seeding density, demonstrating decreased AC dedifferentiation.[38]

Beyond seeding density, culture media components have also been altered and investigated. Growth factors have been used extensively as media supplements to promote a differentiated phenotype. Supplementation of medium with bone morphogenetic protein-2 (BMP-2) demonstrated the ability to reduce dedifferentiation of serially passaged chondrocytes, as revealed by gene expression analysis.[39] However, supplementation of serum-containing or serum-free medium with combinations of epidermal growth factor, platelet-derived growth factor-bb, fibroblast growth factor-2 (FGF-2), and TGF-β1 during monolayer expansion actually enhanced AC proliferation and dedifferentiation, as indicated by gene expression for collagen types I, II, and X; aggrecan; and versican.[40] Thus, it was concluded that FGF-2/TGF-β supplementation in expansion was ideal to accelerate proliferation and even the subsequent redifferentiation of these cells in a 3D substrate when exposed to TGF-β1 and dexamethasone postexpansion.[40] Rapid proliferation, while ideal for clinical applications, is correlated with dedifferentiation of ACs.[41–44] Therefore, while growth factors may aid in increasing proliferation and delaying chondrocyte dedifferentiation, they do not fully prevent phenotypic change and are considerably expensive, so a balance must be achieved regarding the advantages and disadvantages of growth factor supplementation.

While media components act as soluble signals, the substrate on which cells are cultured can be used to provide insoluble signals or act as a physical stimulus to effect change in cells. Used as a substrate or coating, many cartilage-related proteins have been tested to prevent dedifferentiation during cellular proliferation and expansion. Protein coatings are postulated to prevent morphological changes in the chondrocytes, thereby inhibiting dedifferentiation. A decrease in dedifferentiation was reported when chondrocytes were cultured on collagen-type-I-coated surfaces compared to culture on polystyrene plastic.[45] Further, chondrocytes cultured in 3D collagen type I gels after primary cell isolation or expansion on a collagen type I substrate expressed similar levels of collagen type II and GAGs, suggesting that prior expansion on collagen type I may suppress dedifferentiation.[45] It is important to clarify that collagen type I, while most prevalent in the human body, is not the primary collagen in cartilage, and when produced by chondrocytes indicates dedifferentiation, the precise mechanism we seek to prevent. Therefore, its use to avoid chondrocyte dedifferentiation should be carefully considered. Collagen type II, the most abundant collagen found in cartilage, has also been used as a coating.[46] Collagen-type-II-coated surfaces were found to maintain chondrocyte phenotype as compared to uncoated plastic for expansion.[46] Similarly, plastic coated with ECM derived from synovial stem cells reportedly enhanced expansion and delayed dedifferentiation of chondrocytes in comparison to chondrocytes cultured on uncoated plastic.[47] Still, in direct conflict with these reports, evidence exists demonstrating that chondrocytes dedifferentiated during expansion even on protein coatings of collagen type I, collagen type II, and fibronectin.[48] While these studies utilized different species for AC isolation, such opposing results suggest the need for more rigorous testing of cellular responses to coatings to prevent dedifferentiation, including examining gene expression levels throughout expansion on protein substrates. If optimal chondrocyte expression levels and clinically relevant cell numbers are achieved during expansion on a particular coating, then chondrocytes could be passaged at that time to prevent further dedifferentiation.

Native cartilage lacks vasculature, and as a result, has a low oxygen tension. To mimic this property of cartilage, researchers have explored hypoxic chondrocyte expansion as a means to delay or prevent dedifferentiation. Chondrocyte expansion in hypoxic culture conditions with 1.5–5% oxygen enhanced production of cartilaginous matrix and increased chondrogenic gene expression.[49,50] Low oxygen levels inhibit collagen type I expression, thereby suppressing the fibroblastic, dedifferentiated phenotype.[49] Further, even reports that demonstrate no benefit of hypoxia during AC expansion showed that exposure to hypoxia after expansion improves chondrocyte redifferentiation potential.[51,52] It is important to note that hypoxic cultures often suffer from the technical difficulty of maintaining strict control over the oxygen tension during culture medium changes, and it is unclear how these fluctuations may affect the cells. Still, hypoxic culture remains a promising method to delay dedifferentiation during expansion. Hypoxia, protein coatings, and growth factors should all be tested together to determine combinatorial effects of these culture conditions, as none of them is sufficient alone to maintain the original chondrocyte phenotype.

Chondrocyte dedifferentiation occurs so rapidly in expansion that approaches intended to prevent full dedifferentiation are not as commonly pursued as methods aimed at redifferentiating chondrocytes after the fact. Further, chondrocyte expansion of at least 20-fold (>4 population doublings) is prerequisite to clinical or experimental use due to both the limited availability of primary tissue from which cells are isolated and the inherent low cellularity of adult articular cartilage.[53,54] While current methods have successfully delayed chondrocyte dedifferentiation, no methods have achieved complete success in preventing the process. Delaying dedifferentiation during expansion allows for additional passages of chondrocytes with sufficient phenotype stability prior to experimentation or implantation, resulting in greater chondrocyte numbers at the end of expansion. This increase in cell number prior to utilization is needed for any application that requires large numbers of cells. However, since proliferation is correlated with dedifferentiation, preventing dedifferentiation is a practical concern. Still, a balance must be met between methods to increase proliferation and to prevent dedifferentiation. While most methods aim to delay or prevent dedifferentiation until the point of clinical application or *in vitro* 3D culture, it should be noted that dedifferentiation has shown to continue in expanded ACs even in 3D culture on porous scaffolds after the expansion phase.[41,55]

Use of cocultures to prevent AC dedifferentiation

Direct and indirect cocultures of ACs and MSCs in a number of different configurations have been employed to determine the effects of these two cell types on one another. Specifically, cocultures of ACs with MSCs have been analyzed for the ability to promote a stable AC phenotype (Table 2). As an added benefit, AC and MSC cocultures would require fewer chondrocytes than AC monocultures, and therefore, fewer passages of chondrocytes would be needed to reach a clinically or experimentally relevant number of cells. As cocultures reduce the number of chondrocytes needed, expansion of chondrocytes would be decreased or made progressively unnecessary.[56,57]

Primary ACs and MSCs in pellet coculture have been shown to maintain the differentiated AC phenotype. It has been demonstrated that these results were not mediated by soluble factors alone by comparing transwell indirect coculture to direct cell pellet coculture.[58] MSCs provided trophic factors that accelerated AC proliferation in coculture.[58] Further, in AC–MSC coculture (2:1 cell ratio), primary ACs expressed greater levels of SOX9, collagen type II, and aggrecan when cocultured with MSCs in direct contact than noncontact cocultures or monocultures of these cells alone, suggesting that direct contact with MSCs prevents dedifferentiation of primary ACs.[59] Additional experiments that seek to increase the expansion of ACs without dedifferentiation should include MSCs in direct-contact coculture. However, much more rigorous testing should be applied to determine optimal culture conditions over time to maintain a differentiated chondrocyte phenotype during expansion.

Coculturing primary ACs with MSCs demonstrated increased chondrogenesis compared with monoculture AC control groups of equal total cell seeding density, requiring less chondrocytes for coculture groups than for monoculture groups with improved results.[55] This particular study utilized a cell ratio of 30:70 ACs to MSCs, suggesting a 70% reduction in the number of chondrocytes necessary in this culture system. Less chondrocytes would be needed in cocultures of ACs and MSCs than monocultures of ACs alone, so the potential for minimizing dedifferentiation due to expansion of ACs via decreased expansion remains promising in cocultures of ACs and MSCs. Although dedifferentiation is extensively investigated, it continues to elude researchers. Consequently, methods of redifferentiating dedifferentiated chondrocytes are needed.

Promoting AC Redifferentiation

Despite attempts to avoid dedifferentiation, current expansion methods still do not maintain the chondrocyte phenotype. Thus, approaches to redifferentiate ACs after dedifferentiation have been investigated and have achieved promising results. Chondrocytes undergo *in vitro* expansional dedifferentiation or loss of phenotype as modulated by a number of cellular pathways, which have previously been elucidated.[60,61] While noted that dedifferentiation typically occurs in monolayer culture with cells assuming a flat spindle-like morphology, redifferentiation is largely conducted in 3D culture to encourage a spherical morphology similar to the natural shape of chondrocytes *in vivo*.[23,62] Many of the same methods are used both to prevent dedifferentiation and to redifferentiate the expanded ACs: growth factors, substrate composition, hypoxia, mechanical stimulation, and coculture with MSCs.

Use of chondrocyte monocultures to promote redifferentiation

Media components, 3D culture systems, oxygen tension, and mechanical stimulation have been varied in the culture of expanded ACs to determine effects on chondrocyte

Table 2. Coculture: Methods to Prevent Articular Chondrocyte Dedifferentiation

Direct/indirect coculture	*Percentage of ACs in coculture*	*Species and cell source*	*Ref. No.*
Direct, 3D pellet	25	Human MSCs and Bovine and human ACs	58
Direct, 3D porous scaffold	30	Rabbit MSCs and Bovine ACs	55
Direct, 2D coculture	66	Rat MSCs and Rat ACs	59

MSCs, mesenchymal stem cells.

phenotype, as summarized in Table 3. Similar to attempts to prevent dedifferentiation, media supplementation with growth factors and hormones, such as dexamethasone, has been used to induce chondrogenic redifferentiation of ACs. In one study, chondrocytes expanded in the presence of serum-containing medium supplemented with FGF-2 and TGF-β1 then subsequently redifferentiated in serum-free medium, supplemented with TGF-β1 and dexamethasone, showed the greatest upregulation of chondrogenic gene expression and increase in production of cartilage-like ECM compared with any other growth-factor-supplemented media formulation.[40] Further, it has been demonstrated that even transient growth factor signaling with TGF-β3 can be used to improve biomechanical and biochemical properties of the resultant tissue-engineered construct to be comparable to properties of native cartilage tissue.[63] Consequently, growth factors play a major role during expansion of chondrocytes and even influence the ability of the chondrocytes to be redifferentiated in 3D culture after expansion.

As previously stated, 3D culture systems are most commonly used to promote redifferentiation as they support the spherical shape of chondrocytes. Both natural and synthetic scaffolds have been tested experimentally for their ability to promote AC redifferentiation. Natural scaffolds, such as hydrogels made from hyaluronan, a naturally occurring GAG in articular cartilage, have demonstrated the ability to support redifferentiation of chondrocytes after dedifferentiation, indicated by an increase in collagen type II and aggrecan expression levels.[64,65] Further, a chondrocyte-seeded hyaluronan-based hydrogel system has demonstrated safety and efficacy in clinical application for the treatment of articular cartilage lesions.[66] Other natural materials, such as agarose and alginate not native to articular cartilage, are also frequently employed to support redifferentiation of chondrocytes. Chondrocytes cultured in agarose hydrogels with TGF-β1-supplemented medium demonstrated restored levels of some microRNA expression comparable to the differentiated chondrocyte phenotype.[25] Further, redifferentiation of ACs cultured in agarose gels without TGF-β supplementation has been demonstrated via increased collagen type II and proteoglycan production.[23] Another natural material, alginate, has been used in several 3D formats, including beads or hydrogels, and has shown to support redifferentiation of chondrocytes and promote phenotype stability.[60,67–70] Cells passaged one to four times were able to redifferentiate in alginate beads, but only limited redifferentiation was observed in passages five through eight.[71] Yet, it was reported that AC monoculture at a lower cell seeding density in 3D alginate beads could not fully redifferentiate chondrocytes that had undergone expansional dedifferentiation through four passages.[26] Synthetic scaffolds have also been used to promote chondrogenic phenotype maintenance, including a thermo-reversible hydrogel construct embedded with chondrocytes that demonstrated the ability to promote chondrogenesis.[72] Although synthetic porous scaffolds allow for the advantageous infiltration of tissue and ECM, these scaffolds tend to enable cell spreading more than hydrogels and such spreading promotes dedifferentiation[24]; however, composite porous scaffolds of natural proteins and synthetic materials could mitigate dedifferentiation and enhance redifferentiation due to the presence of proteins while porosity supports tissue infiltration. Still, due to incongruent evidence, improvements must be made to scaffold materials and designs to promote chondrocyte redifferentiation in order to reestablish chondrocyte phenotype before clinical use.

While used to prevent dedifferentiation, hypoxia has also been investigated for its ability to influence chondrogenic redifferentiation of chondrocytes, thereby mimicking the microenvironment of native cartilage. Oxygen tension levels of 1.5–5%, in combination with 3D culture, have caused significant redifferentiation of expanded chondrocytes and phenotype stability.[67,73] Hypoxic-cultured chondrocytes encapsulated in alginate demonstrated significant upregulation of chondrogenic markers, including collagen type II, aggrecan, SOX9, and GAGs, compared with chondrocytes cultured in higher oxygen levels and had expression levels comparable to primary chondrocytes.[70,74] To further explore the mechanism by which hypoxia enhanced cartilage matrix synthesis by ACs, it was revealed that hypoxia-inducible

Table 3. Articular Chondrocyte Monoculture: Methods to Promote Redifferentiation

Dominant factor	*Specific factors*	*Species and cell source*	*Ref. No.*
Growth factor	TGF-β1 and dexamethasone	Human ACs	40
	TGF-β3	Bovine ACs	63
Biomaterial	Hyaluronan hydrogel	Human ACs	64
	Hyaluronan-methacrylate hydrogel	Human ACs	65
	Agarose hydrogel	Rabbit ACs	23
	Agarose hydrogel	Bovine ACs	25
	Alginate beads	Rabbit ACs	60
	Alginate beads	Bovine ACs	68
	Alginate beads	Human ACs	69,71
	Poly(N-isopropylacrylamide-co-acrylic acid) hydrogel	Rabbit ACs	72
Hypoxia and 3D culture	Alginate beads and hypoxia (5% O_2)	Bovine ACs	67,70
	Alginate beads and hypoxia (5% O_2)	Human ACs	74
	MPEG-PLGA scaffold and hypoxia (1%, 5% O_2)	Human ACs	73
Mechanical stimulation	Flow perfusion (0.05 mL/min flow rate)	Rabbit ACs	77
	Dynamic laminar flow; rotating-wall vessel	Human ACs	78
	Dynamic laminar flow; rotating-wall vessel	Bovine ACs	79

MPEG-PLGA, methoxy-poly(ethylene glycol)-block-poly(lactic-co-glycolic acid).

factor-2α (HIF-2α) is at least partly responsible for the hypoxic induction of SOX9 that is directly involved in the upregulation of several matrix genes.[75] Biosynthetic activity and collagen type II production has been shown to increase in hypoxia as well.[67] Incorporation of hypoxia into culture systems for the redifferentiation of chondrocytes should be further explored with consideration for oxygen gradients that may be created within 3D constructs.[76] Such gradients should be fully elucidated with the potential to be harnessed for the generation of organized cartilage tissue.

Mechanical stimulation in the form of compression, hydrostatic pressure, tension, or shear stress has also been explored to enhance chondrogenic phenotype. Rabbit ACs seeded onto poly(glycolic acid) scaffolds and subjected to flow perfusion in a closed bioreactor system produced collagen type II but not type I, as determined via immunohistochemical staining and western blot, suggesting a redifferentiation of expanded chondrocytes.[77] Expanded human ACs cultured in a rotating bioreactor for 12 weeks aggregated and expressed collagen type II in culture, demonstrating redifferentiation.[78] While many bioreactor systems have been detailed in the literature, such as those utilizing dynamic laminar flow[79] and dynamic compression[80,81] to increase GAG and collagen production by ACs, a detailed analysis of chondrocyte gene expression and phenotype requires further elucidation. Beyond the ability to redifferentiate expanded chondrocytes, a bioreactor should be further optimized to redifferentiate these cells as rapidly as possible *in vitro* prior to clinical use, and further effort should be made to monitor the changes in phenotype in culture over time. Additionally, methods to redifferentiate ACs should be combinatorial, involving multiple approaches detailed previously.

Use of cocultures for the redifferentiation of ACs

Coculturing ACs with other cell types has been used as a method to promote chondrocyte redifferentiation *in vitro* (Table 4). Typical proportion of ACs in these cocultures has been 20–35%, demonstrating the decreased need for AC expansion in cocultures as previously discussed. When cultured with primary chondrocytes, expanded dedifferentiated chondrocytes underwent redifferentiation, as indicated by upregulation of aggrecan, collagen type II, and SOX9 gene expression accompanied by a decrease in collagen type I expression.[82] Further, cocultures of these cells, primary and expanded ACs, demonstrated that even small numbers of primary cells could rescue the phenotype of the expanded, dedifferentiated ACs.[82] Even a xenogeneic coculture proved successful in enhancing cartilage tissue formation compared with monocultures as measured by GAG content, in the coculture of expanded human chondrocytes with primary bovine chondrocytes.[83] While the cocultures of primary and expanded chondrocytes enhanced chondrocyte redifferentiation and only require the isolation of one cell type, this coculture method employs chondrocytes only, but still needs relatively high numbers of harvested chondrocytes. Alternatively, cocultures of chondrocytes with MSCs can be used to decrease the number of chondrocytes needed for experimentation or implantation.[56]

Bone marrow MSCs and expanded ACs have been cocultured to promote AC redifferentiation. It has been shown that expanded chondrocytes cocultured with MSCs at the end of culture were similar to primary chondrocytes as measured by collagen type I, collagen type II, and aggrecan expression as well as GAG content when cultured on porous poly(ε-caprolactone) (PCL) scaffolds.[56] MSCs generally are credited with providing significant paracrine or trophic effects that upregulate the AC chondrogenic phenotype in coculture.[84–86] Interestingly, MSCs treated with TGF-β3 and BMP-6 were reported to increase in chondrogenic gene expression of TGF-β3, BMP-2, IGF-1, and FGF-2,[87] and the production of these molecules has been suggested to contribute to AC redifferentiation when cocultured with ACs.[84] Thus, the exposure of MSCs to growth factors could greatly impact the effect of the coculture. While trophic factors generated by MSCs have been suggested to play a role in redifferentiation of ACs, the retention of MSCs in culture is disputed. Several reports observed a gradual loss of MSCs in coculture.[56,86] Even when MSCs remained in the coculture for the duration of the culture period, the chondrocytes were responsible for the cartilaginous matrix production.[84] Further, cocultures still require growth factor supplementation like monocultures, making the primary benefit of coculturing a decreased need for chondrocyte expansion.[55] Nonetheless, even transient exposure of cocultures to TGF-β3 enhanced the chondrogenic phenotype of cell types compared with cultures not exposed to growth factor, and showed a stabilized phenotype of cocultures compared with

Table 4. Coculture: Methods to Promote Articular Chondrocyte Redifferentiation

Direct/indirect coculture	*Percentage of ACs in coculture*	*Species and cell source*	*Ref. No.*
Direct, 3D porous scaffold	25	Bovine and rabbit MSCs and Bovine ACs	56
Direct, 3D porous scaffold	30	Rabbit MSCs and Bovine ACs	55
Direct, 3D porous scaffold	25	Rabbit MSCs and Bovine ACs	88
Direct, 3D pellet	33	Human MSCs and Bovine ACs	84
Direct, 3D pellet	20	Human MSCs and Human and bovine ACs	85,86
Direct, 3D porous scaffold	20 (primary ACs)	Bovine primary and expanded ACs	82
Direct, 3D pellet	20 (primary ACs)	Bovine primary ACs and Human expanded ACs	83

either cell type alone.[88] Further, the cocultures required lower concentrations of growth factor in comparison to the monocultures. Cocultures of MSCs and ACs have proven beneficial over their monoculture counterparts for the reduction in initial chondrocyte number and increase in cartilaginous ECM expression.

Whether in direct-contact pellet coculture or on porous scaffolds, reports suggest that MSC trophic factors promote chondrocyte proliferation and cartilaginous matrix production in coculture, which would indicate a stable chondrocyte phenotype.[85,86] Yet an opposing report exists suggesting that MSCs in a noncontact coculture system with expanded ACs downregulate chondrocytic differentiation of ACs by a decrease in GAG and collagen type II production.[89] However, direct-contact coculture could mitigate this reported result and should be directly compared with this particular noncontact coculture system in the future. While more research must be done to determine cellular interactions, a majority of published data support that direct-contact coculture of ACs and MSCs promotes AC redifferentiation, thereby decreasing the need for large numbers of chondrocytes that would decrease total passaging of chondrocytes required to reach clinically relevant numbers. MSCs could help alleviate this need by promoting chondrocyte proliferation or undergoing chondrogenic differentiation in culture to establish a greater population of chondrocytes.

Chondrogenic Differentiation of MSCs and Preventing MSC Hypertrophy

While treatment of ACs with chondrogenic stimuli can lead to a stable phenotype, MSCs, on the other hand, tend to undergo chondrogenic differentiation and hypertrophy. Hypertrophic MSCs cannot produce articular cartilage, so prevention of such a phenotype remains the goal for articular cartilage engineering that utilizes MSCs.

Use of monocultures to promote MSC chondrogenesis and prevent hypertrophy

MSCs have been subjected to different culture configurations, growth factor supplementation, biomaterials, oxygen tensions, dynamic culture, and mechanical stimulation to support chondrogenic differentiation and prevent hypertrophy, as summarized in Table 5. These culture conditions are similar to those used to enhance the chondrogenic phenotype of ACs detailed previously.

MSCs subjected to TGF-β1 supplementation and cultured on composite PCL/ECM or PCL scaffolds underwent greater chondrogenic differentiation than MSCs cultured without supplementation regardless of scaffold, demonstrating the need for growth factor supplementation even in the presence of ECM.[90] MSCs encapsulated in agarose hydrogels underwent chondrogenic differentiation when exposed to TGF-β3-supplemented medium.[91] Even transient exposure of MSCs to TGF-β3 led to an increase in mechanical properties of the resultant construct over time.[92] While growth factors induce chondrogenic differentiation of bone marrow MSCs, the selection of culture supplements may need to be tailored to the cell source such as those derived from bone marrow or adipose tissue. Further, media supplementation is often observed beneficial in combination with other parameters in culture systems and should be continually investigated for additive or combinatorial effects in future culture designs.

Natural and synthetic scaffolds present unique advantages and disadvantages in MSC monoculture. Natural collagen-containing scaffolds have been used widely to mimic the natural ECM found within the body.[27,93,94] Collagen type I has been mixed with GAGs to form a scaffold that supported chondrogenic differentiation of MSCs in combination with TGF-β1 supplementation.[27] Another study demonstrated that *in vivo* implantation of collagen type II scaffolds seeded with MSCs that had no prior induction with TGF-β sufficiently repaired the cartilage defect site.[93] Many composite scaffolds have been tested to increase mechanical strength via synthetic materials while increasing biocompatibility with natural ECM components. The combination of the synthetic polymer, a porous poly(lactic acid) (PLA), with natural materials perlecan domain I, collagen type II fibril, and BMP-2 complexes resulted in greater GAG accumulation when MSCs were grown within this scaffold over any other combination of these factors in PLA or alone, indicative of chondrogenic differentiation.[94] Fibrin-polyurethane (fibrin-PU) composite hydrogel scaffolds have also been used in a culture system to chondrogenically differentiate MSCs *in vitro*.[95] Electrospun PCL scaffolds coated with

Table 5. MSC Monoculture: Methods to Promote Differentiation and Prevent Hypertrophy

Dominant factor	*Specific factors*	*Species and cell source*	*Ref. No.*
Growth factor	TGF-β1	Rabbit MSCs	90
	TGF-β3	Porcine MSCs	91
	TGF-β3 and PTHrP (*in vivo*)	Human MSCs implanted in nude mice	96
Biomaterial	Collagen type I–GAG scaffold	Rat MSCs	27
	Collagen type II scaffold (*in vivo*)	Rabbit MSCs implanted in rabbits	93
	Poly(lactic acid) with perlecan domain I, collagen type II fibril, BMP-2 complexes	Mouse MSCs	94
Hypoxia and 3D culture	Agarose hydrogel and hypoxia (5% O_2)	Porcine MSCs	98
	Pellet culture and hypoxia (1% O_2)	Human MSCs	99
Mechanical stimulation	Dynamic loading of fibrin-polyurethane scaffolds	Human MSCs	95,100
	Cyclic compressive loading	Rabbit MSCs	101,102
	Dynamic loading with TGF-β1 or IGF-1	Bovine MSCs	103

GAG, glycosaminoglycan; IGF, insulin-like growth factor; PTHrP, parathyroid hormone-related protein.

chondrocyte-derived ECM demonstrated the ability to support chondrogenic differentiation of MSCs after induction with TGF-β1.[96] In one study, hyaluronic acid hydrogels loaded with TGF-β3 encapsulated in alginate microspheres were implanted subcutaneously in nude mice, but significant calcification was observed after 8 weeks.[96] In response, the combined delivery of both TGF-β3 and parathyroid hormone-related protein (PTHrP) was used and demonstrated reduced calcification in the same animal model,[96] representing a step toward promoting chondrogenesis and preventing hypertrophy of MSCs. MSCs still tend to form fibrocartilage *in vivo*, so promotion of a chondrogenic phenotype using 3D scaffolds of natural and synthetic materials as guides for tissue engineering remains the goal.

Hypoxia has been shown to promote chondrogenesis of MSCs in monolayer culture via HIF-1α.[97] Hypoxia of 5% oxygen was even found to be a greater promoter of chondrogenesis than mechanical stimulation for MSCs treated with TGF-β3.[98] Further, hypoxia of 1% oxygen was able to both induce chondrogenesis and inhibit hypertrophy and apoptosis of chondrogenically differentiating MSCs exposed to TGF-β1.[99] Hypoxia should be included in future studies to better mimic the native cartilage microenvironment *in vitro*.

The dynamic culture of MSCs using bioreactors has also been shown to be advantageous for chondrogenesis. Dynamic loading of fibrin-PU hydrogel scaffolds seeded with *SOX9*-transduced MSCs had higher GAG synthesis and lubricin expression even without exogenous growth factor supplementation.[100] In another study, MSCs cultured in fibrin-PU scaffolds without growth factor supplementation deposited greater GAG in all loaded conditions over the nonloaded control, resulting in greater chondrogenic-to-hypertrophic gene expression ratios when subjected to shear and compressive loads.[95] Cyclic compressive loading of MSCs in agarose hydrogels resulted in greater expression of the chondrogenic markers TGF-β1, collagen type II, and aggrecan.[101,102] In another culture system, with agarose hydrogel and TGF-β3 supplementation, initiating compressive loading at 21 days instead of at the start of culture yielded better results.[91] Cartilage-like matrix production increased when subjected to the combination of both dynamic compressive loading and growth factor supplementation with TGF-β1 or IGF-1, demonstrating the use of multiple influences to enhance chondrocyte phenotype.[103] Mechanical stimulation can be used to more closely mimic the forces sustained by native cartilage *in vivo*; however, the type, duration, and magnitude of the force are of great importance to regenerate cartilage tissue.

MSC monoculture conditions were altered experimentally to promote chondrogenesis and prevent hypertrophy but fell short of either goal. Cocultures of MSCs and ACs have been used as a means to improve upon the successes of monoculture of MSCs with more promising results.

Use of cocultures to promote MSC chondrogenesis and prevent hypertrophy

Direct- and indirect-contact cocultures of ACs and MSCs have demonstrated the ability to stimulate chondrogenesis and prevent hypertrophy of MSCs. These cell types have been cocultured in the form of cell pellets, in hydrogels, and on porous polymers (Table 6).

Cell pellets of ACs and MSCs have resulted in decreased MSC hypertrophy, indicated by a decrease in collagen type X expression, and the maintenance of the differentiated AC phenotype *in vitro* established by direct-contact coculture, not mediated by soluble factors from an indirect coculture.[58] Numerous reports propose that coculture of cells in close proximity [104,105] or direct cell-to-cell contact is preferential for MSC chondrogenesis and prevention of hypertrophy.[106,107] Although another report suggests that ACs could not prevent chondrogenic MSC hypertrophy or promote full chondrogenesis in micromass pellet culture, without chondrogenic stimuli the cocultures still produced hyaline-like cartilage in as little as a 1:3 cell ratio of ACs to MSCs.[108] Additionally, evidence exists demonstrating that growth factor supplementation is not consistent or necessary for chondrogenesis in pellet coculture.[109]

MSCs and ACs in direct-contact pellet coculture expressed higher levels of chondrogenic genes and produced a greater distribution of ECM than either cell type in monoculture.[109] Further, with increasing AC percentage in pellet coculture ranging from 5% to 25% ACs, collagen type II expression increased and collagen type X decreased.[106] Besides micromass pellet culture, hydrogels have been used to coculture these cell types *in vitro*.[72,110] To distinguish between AC and MSC gene expression in coculture sample analysis, green-fluorescent-protein-transfected MSCs[95] and cells from different species[56,32,111] have been used.

Interestingly, even when cocultured with osteoarthritic ACs, MSCs were able to undergo chondrogenic differentiation

Table 6. Coculture: Methods to Promote Differentiation and Prevent Hypertrophy

Direct/indirect coculture	*Percentage of ACs in coculture*	*Species and cell source*	*Ref. No.*
Direct, 3D pellet and scaffold	25	Human MSCs and ACs	106
Direct, 3D bilaminar cell pellet	25	Human MSCs and ACs	107
Direct, 3D pellet	25	Human MSCs and ACs	108
Direct, 3D pellet	50	Equine MSCs and ACs	109
Direct, 3D pellet	50	Human MSCs and ACs	112
Direct, 3D porous scaffold	30	Rabbit MSCs and Bovine ACs	32
Direct, 2D coculture	66	Rabbit MSCs and ACs	114
Indirect, 3D porous scaffold	50	Rabbit MSCs and Bovine ACs	116
Indirect, 3D hydrogel	50	Rabbit MSCs and ACs	110
Indirect, 3D hydrogel	20	Human MSCs and ACs	104

without hypertrophy presenting a unique advantage to coculture systems over monoculture systems.[105] Further, in an *in vitro* study that intended to mimic an injured joint microenvironment via hypoxia and inflammatory conditions, the cocultured MSC/AC group produced significantly more proteoglycans than either cell type alone.[107] One proposed mechanism for coculture-induced prevention of hypertrophy is that ACs secrete PTHrP in coculture with MSCs, which inhibits MSC hypertrophy, as indicated by a decrease in ALP and collagen type X expression.[112] ACs have also been shown to support chondrogenesis of MSCs in coculture.[113] However, cocultures of immortalized MSCs and ACs in a 3D alginate hydrogel showed both increased chondrogenesis of ACs and osteogenesis of MSCs after 28 days at a 2:1 ratio of MSCs:ACs but not for cell ratios 1:1 or 1:2; therefore, there may be limitations with cell ratios in certain culture conditions that would promote a hypertrophic phenotype of MSCs.[111]

Cocultures of ACs and MSCs have been shown to increase production of cartilaginous ECM as compared with monocultures[114] even without prior chondrogenic induction.[115] As such, direct and indirect cocultures have been utilized to generate ECM-coated porous scaffolds.[57,116,117] The increase in cartilaginous ECM components suggests that coculture promotes the chondrogenic phenotype of the cultured cells. However, future work must be done to more clearly elucidate the cell type that is responsible for observed results, as current understanding of the issue is somewhat vague as discussed previously.[56,58] Regardless, cocultures have demonstrated advantages over monoculture counterparts and should be further explored.

Cocultures for cartilage tissue engineering have employed other cell types as well. MSCs have been cocultured with chondrons, homogenized cartilage tissue, and demonstrated *in vivo* that engineered tissues of chondrons with MSCs repaired cartilage focal defects better than microfracture surgery to repair cartilage defects.[118] Additionally, MSC/chondrocyte cocultures were then compared with MSC/chondron cocultures in which the MSC/chondron coculture produced greater GAG to DNA weight-to-weight ratios *in vitro* than MSC/chondrocyte cocultures, suggesting a benefit of utilizing homogenized tissue over isolated cells.[118] Future work should focus on clearly defining the phenotype of cells used throughout culture, so as to clearly demarcate the effects of particular factors alone or in combination with other factors over time to determine duration of cell exposure to *in vitro* culture prior to clinical application for enhanced cell phenotype.

Future Directions

ACs and bone-marrow-derived MSCs have been investigated as possible cell sources for cartilage regeneration strategies. However, ACs and MSCs exhibit unstable phenotypes as a result of common expansion or culture methods, so research has focused on altering or combining these methods in order to achieve an improved chondrogenic phenotype of these cells for possible use in clinical application. These cell types have been exposed to external stimuli in an effort to attain a resultant cell population with a phenotype similar to that of native ACs. In both monoculture and coculture strategies, hypoxia and growth factors have shown usefulness in delaying dedifferentiation of ACs in expansion, promoting redifferentiation of ACs in post-expansion culture, preventing hypertrophy of MSCs, and promoting chondrogenic differentiation of MSCs. As expected, the use of just one stimulus has not proven to be successful in enhancing chondrocyte phenotype, but combinations of stimuli, such as hypoxia, chondrogenic growth factors, and protein substrates, have shown benefits. Most interestingly, many of the issues encountered during monoculture of either ACs or MSCs alone are resolved via coculture of ACs and MSCs together, such as MSC hypertrophy and difficulty of redifferentiating expanded chondrocytes. However, the prevention of full AC dedifferentiation remains an issue for both monoculture and coculture strategies that must be addressed. Maintaining the chondrocyte phenotype by preventing dedifferentiation entirely would eliminate the need for redifferentiation and allow for increased expansion of chondrocytes for clinical application.

Cocultured ACs and MSCs act to enhance the chondrogenic phenotype of neighboring cells within the coculture system via trophic factors. Coculturing these two cell types synergistically delays dedifferentiation and promotes subsequent redifferentiation of ACs and increases the chondrogenic differentiation while preventing the hypertrophic phenotype of MSCs *in vitro*. A number of approaches to enhance chondrocyte phenotype for cartilage tissue engineering have been reviewed, but full chondrocyte phenotype characterization still remains an issue for the field. Most currently used chondrogenic markers are associated with ECM molecules like collagen type II and aggrecan, emphasizing the significance of matrix composition for cartilage function. However, this approach to identify chondrocytes primarily by select matrix markers is perhaps too simplistic. Large-scale expression profiling has revealed potential new markers by comparing changes in gene expression of primary, dedifferentiated, and redifferentiated ACs.[39,119] Yet, this pathway analysis needs to be validated before these markers can be considered new indicators of chondrogenic differentiation. Further, markers of dedifferentiation and redifferentiation should be standardized so as to increase the reliability of results aimed at characterizing and preventing the dedifferentiation and redifferentiation of chondrocytes. Relatively few chondrogenic markers are used to distinguish between differentiated and dedifferentiated chondrocytes and include proteins and transcription factors. Further, only a few markers of hypertrophic MSCs are used to distinguish between chondrogenic and hypertrophic MSCs. While time and cost associated with full characterization of these cell phenotypes must be considered, the field would benefit from the use of a standardized array of markers for efforts geared toward phenotype stabilization.

Future areas of interest should include the investigation of organized cartilage tissue within cocultured constructs for cartilage regeneration efforts that may more closely mimic the native cartilage structure over monoculture counterparts. AC and MSC cocultures should be further evaluated in *in vivo* models of cartilage defects in order to determine optimal culture conditions prior to implantation. Additionally, cocultures could be leveraged for their multiple cell types and investigated for their ability to repair osteochondral defects *in vivo* as coculturing ACs with MSCs may improve the

regeneration of both the cartilaginous and osseous layers of osteochondral tissue and potentially improve integration with surrounding tissue. Lastly, implantation of ACs and MSCs into a cartilage defect site has the potential to provide trophic effects to host and implanted cell populations for improved cartilage regeneration. The coculture of ACs with MSCs presents exciting progress for the field of cartilage tissue engineering.

Acknowledgment

The authors gratefully acknowledge support from the U.S. National Institutes of Health (R01 AR057083 and R01 AR048756).

Disclosure Statement

No competing financial interests exist.

References

1. Adolphe, M. ed. Biological Regulations of the Chondrocytes. Boca Raton: CRC Press, 1992.
2. Mak, A.F. The apparent viscoelastic behavior of articular cartilage—the contributions from the intrinsic matrix viscoelasticity and interstitial fluid flows. J Biomech Eng **108,** 123, 1986.
3. Falah, M., Nierenberg, G., Soudry, M., Hayden, M., and Volpin, G. Treatment of articular cartilage lesions of the knee. Int Orthop **34,** 621, 2010.
4. Basad, E., Ishaque, B., Bachmann, G., Stürz, H., and Steinmeyer, J. Matrix-induced autologous chondrocyte implantation versus microfracture in the treatment of cartilage defects of the knee: a 2-year randomised study. Knee Surg Sports Traumatol Arthrosc **18,** 519, 2010.
5. Ebert, J.R., Robertson, W.B., Woodhouse, J., Fallon, M., Zheng, M.H., Ackland, T., and Wood, D.J. Clinical and magnetic resonance imaging-based outcomes to 5 years after matrix-induced autologous chondrocyte implantation to address articular cartilage defects in the knee. Am J Sports Med **39,** 753, 2011.
6. Holtzer, H., Abbott, J., Lash, J., and Holtzer, S. The loss of phenotypic traits by differentiated cells *in vitro*, I. Dedifferentiation of cartilage cells. Proc Natl Acad Sci U S A **46,** 1533, 1960.
7. Albrecht, C., Tichy, B., Nurnberger, S., Hosiner, S., Zak, L., Aldrian, S., and Marlovits, S. Gene expression and cell differentiation in matrix-associated chondrocyte transplantation grafts: a comparative study. Osteoarthritis Cartilage **19,** 1219, 2009.
8. Shin, H., Seongbong, J., and Mikos, A.G. Biomimetic materials for tissue engineering. Biomaterials **24,** 4353, 2003.
9. Blunk, T., Sieminski, A.L., Gooch, K.J., Courter, D.L., Hollander, A.P., Nahir, A.M., Langer, R., Vunjak-Novakovic, G., and Freed, L.E. Differential effects of growth factors on tissue-engineered cartilage. Tissue Eng **8,** 73, 2002.
10. Freyria, A.M., and Mallein-Gerin, F. Chondrocytes or adult stem cells for cartilage repair: the indisputable role of growth factors. Injury **43,** 259, 2012.
11. Huey, D.J., Hu, J.C., and Athanasiou, K.A. Unlike bone, cartilage regeneration remains elusive. Science **338,** 917, 2012.
12. Puetzer, J.L., Petitte, J.N., and Loboa, E.G. Comparative review of growth factors for induction of three-dimensional *in vitro* chondrogenesis in human mesenchymal stem cells isolated from bone marrow and adipose tissue. Tissue Eng Part B Rev **16,** 435, 2010.
13. Schulze-Tanzil, G. Activation and dedifferentiation of chondrocytes: implications in cartilage injury and repair. Ann Anat **191,** 325, 2009.
14. Berninger, M.T., Wexel, G., Rummeny, E.J., Imhoff, A.B., Anton, M., Henning, T.D., and Vogt, S. Treatment of osteochondral defects in the rabbit's knee joint by implantation of allogeneic mesenchymal stem cells in fibrin clots. J Vis Exp **75,** e4422, 2013.
15. Isogai, N., Kusuhara, H., Ikada, Y., Ohtani, H., Jacquet, R., Hillyer, J., Lowder, E., and Landis, W.J. Comparison of different chondrocytes for use in tissue engineering of cartilage model structures. Tissue Eng **12,** 691, 2006.
16. Hildner, F., Albrecht, C., Gabriel, C., Redl, H., and 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 **5,** e36, 2011.
17. O'Sullivan, J., D'Arcy, S., Barry, F.P., Murphy, J.M., and Coleman, C.M. Mesenchymal chondroprogenitor cell origin and therapeutic potential. Stem Cell Res Ther **2,** 8, 2011.
18. Vidal, M.A., Robinson, S.O., Lopez, M.J., Paulsen, D.B., Borkhsenious, O., Johnson, J.R., Moore, R.M., and Gimble, J.M. Comparison of chondrogenic potential in equine mesenchymal stromal cells derived from adipose tissue and bone marrow. Vet Surg **37,** 713, 2008.
19. Im, G.I., Shin, Y.W., and Lee, K.B. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? Osteoarthritis Cartilage **13,** 845, 2005.
20. Afizah, H., Yang, Z., Hui, J.H., Ouyang, H.W., and Lee, E.H. A comparison between the chondrogenic potential of human bone marrow stem cells (BMSCs) and adipose-derived stem cells (ADSCs) taken from the same donors. Tissue Eng **13,** 659, 2007.
21. Gardner, O.F., Archer, C.W., Alini, M., and Stoddart, M.J. Chondrogenesis of mesenchymal stem cells for cartilage tissue engineering. Histol Histopathol **28,** 23, 2013.
22. Von der Mark, K., Gauss, V., von der Mark, H., and Müller, P. Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture. Nature **267,** 531, 1977.
23. Benya, P.D., and Shaffer, J.D. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell **30,** 215, 1982.
24. Watt, F.M. Effect of seeding density on stability of the differentiated phenotype of pig articular chondrocytes in culture. J Cell Sci **89,** 373, 1988.
25. Hong, E., and Reddi, A.H. Dedifferentiation and redifferentiation of articular chondrocytes from surface and middle zones: changes in microRNAs-221/-222, -140, and -143/145 expression. Tissue Eng Part A **19,** 1015, 2013.
26. Darling, E.M., and Athanasiou, K.A. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. J Orthop Res **23,** 425, 2005.
27. Farrell, E., O'Brien, F.J., Doyle, P., Fischer, J., Yannas, I., Harley, B.A., O'Connell, B., Prendergast, P.J., and Campbell, V.A. A collagen-glycosaminoglycan scaffold supports adult rat mesenchymal stem cell differentiation

along osteogenic and chondrogenic routes. Tissue Eng **12,** 459, 2006.
28. Caplan, A.I. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol **213,** 341, 2007.
29. Caplan, A.I. Why are MSCs therapeutic? New data: new insight. J Pathol **217,** 318, 2009.
30. Koc, O.N., Gerson, S.L., Cooper, B.W., Dyhouse, S.M., Haynesworth, S.E., Caplan, A.I., and Lazarus, H.M. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol **18,** 307, 2000.
31. Aggarwal, S., and Pittenger, M.F. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood **105,** 1815, 2005.
32. Meretoja, V.V., Dahlin, R.L., Wright, S., Kasper, F.K., and Mikos, A.G. The effect of hypoxia on the chondrogenic differentiation of co-cultured articular chondrocytes and mesenchymal stem cells in scaffolds. Biomaterials **34,** 4266, 2013.
33. Studer, D., Millan, C., Öztürk, E., Maniura-Weber, K., and Zenobi-Wong, M. Molecular and biophysical mechanisms regulating hypertrophic differentiation in chondrocytes and mesenchymal stem cells. Eur Cell Mater **24,** 118, 2012.
34. Scotti, C., Tonnarelli, B., Papadimitropoulos, A., Scherberich, A., Schaeren, S., Schauerte, A., Lopez-Rios, J., Zeller, R., Barbero, A., and Martin, I. Recapitulation of endochondral bone formation using human adult mesenchymal stem cells as a paradigm for developmental engineering. Proc Natl Acad Sci U S A **107,** 7251, 2010.
35. Lau, T.T., Lee, L.Q., Vo, B.N., Su, K., and Wang, D.A. Inducing ossification in an engineered 3D scaffold-free living cartilage template. Biomaterials **33,** 8406, 2012.
36. Mackay, A.M., Beck, S.C., Murphy, J.M., Barry, F.P., Chichester, C.O., and Pittenger, M.F. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. Tissue Eng **4,** 415, 1998.
37. Tew, S.R., Murdoch, A.D., Rauchenberg, R.P., and Hardingham, T.E. Cellular methods in cartilage research: primary human chondrocytes in culture and chondrogenesis in human bone marrow stem cells. Methods **45,** 2, 2008.
38. Rosenzweig, D.H., Matmati, M., Khayat, G., Chaudhry, S., Hinz, B., and Quinn, T.M. Culture of primary bovine chondrocytes on a continuously expanding surface inhibits dedifferentiation. Tissue Eng Part A **18,** 2466, 2012.
39. Ma, B., Leijten, J.C., Wu, L., Kip, M., van Blitterswijk, C.A., Post, J.N., and Karperien, M. Gene expression profiling of dedifferentiated human articular chondrocytes in monolayer culture. Osteoarthritis Cartilage **21,** 599, 2013.
40. Jakob, M., Démarteau, O., Schäfer, D., Hintermann, B., Dick, W., Heberer, M., and Martin, I. Specific growth factors during the expansion and redifferentiation of adult human articular chondrocytes enhance chondrogenesis and cartilaginous tissue formation *in vitro*. J Cell Biochem **81,** 368, 2001.
41. Dahlin, R.L., Meretoja, V.V., Ni, M., Kasper, F.K., and Mikos, A.G. Hypoxia and flow perfusion modulate proliferation and gene expression of articular chondrocytes on porous scaffolds. AIChE J **59,** 3158, 2013.
42. Giovannini, S., Diaz-Romero, J., Aigner, T., Mainil-Varlet, P., and Nesic, D. Population doublings and percentage of S100-positive cells as predictors of *in vitro* chondrogenicity of expanded human articular chondrocytes. J Cell Physiol **222,** 411, 2010.
43. Dell'Accio, F., De Bari, C., and Luyten, F.P. Microenvironment and phenotypic stability specify tissue formation by human articular cartilage-derived cells *in vivo*. Exp Cell Res **287,** 16, 2003.
44. Temenoff, J.S., and Mikos, A.G. Review: tissue engineering for regeneration of articular cartilage. Biomaterials **21,** 431, 2000.
45. Kino-Oka, M., Yashiki, S., Ota, Y., Mushiaki, Y., Sugawara, K., Yamamoto, T., Takezawa, T., and Taya, M. Subculture of chondrocytes on a collagen type I-coated substrate with suppressed cellular dedifferentiation. Tissue Eng **11,** 597, 2005.
46. Shakibaei, M., De Souza, P., and Merker, H.J. Integrin expression and collagen type II implicated in maintenance of chondrocyte shape in monolayer culture: an immunomorphological study. Cell Biol Int **21,** 115, 1997.
47. Pei, M., and He, F. Extracellular matrix deposited by synovium-derived stem cells delays replicative senescent chondrocyte dedifferentiation and enhances redifferentiation. J Cell Physiol **227,** 2163, 2012.
48. Brodkin, K.R., García, A.J., and Levenston, M.E. Chondrocyte phenotypes on different extracellular matrix monolayers. Biomaterials **25,** 5929, 2004.
49. Das, R.H., van Osch, G.J., Kreukniet, M., Oostra, J., Weinans, H., and Jahr, H. Effects of individual control of pH and hypoxia in chondrocyte culture. J Orthop Res **28,** 537, 2010.
50. Egli, R.J., Bastian, J.D., Ganz, R., Hofstetter, W., and Leunig, M. Hypoxic expansion promotes the chondrogenic potential of articular chondrocytes. J Orthop Res **26,** 977, 2008.
51. Ströbel, S., Loparic, M., Wendt, D., Schenk, A.D., Candrian, C., Lindberg, R.L., Moldovan, F., Barbero, A., and Martin, I. Anabolic and catabolic responses of human articular chondrocytes to varying oxygen percentages. Arthritis Res Ther **12,** R34, 2010.
52. Schrobback, K., Klein, T.J., Crawford, R., Upton, Z., Malda, J., and Leavesley, D.I. Effects of oxygen and culture system on *in vitro* propagation and redifferentiation of osteoarthritic human articular chondrocytes. Cell Tissue Res **347,** 649, 2012.
53. Brittberg, M., Peterson, L., Sjogren-Jansson, E., Tallheden, T., and Lindahl, A. Articular cartilage engineering with autologous chondrocyte transplantation—a review of recent developments. J Bone Joint Surg Am **85(A Suppl 3),** 109, 2003.
54. Mandl, E.W., Van der Veen, S.W., Verhaar, J.A., and Van Osch, G.J. Multiplication of human chondrocytes with low seeding densities accelerates cell yield without losing redifferentiation capacity. Tissue Eng **10,** 109, 2004.
55. Meretoja, V.V., Dahlin, R.L., Wright, S., Kasper, F.K., and Mikos, A.G. Articular chondrocyte redifferentiation in 3D co-cultures with mesenchymal stem cells. Tissue Eng Part C **20,** 514, 2014.
56. Meretoja, V.V., Dahlin, R.L., Kasper, F.K., and Mikos, A.G. Enhanced chondrogenesis in co-cultures with articular chondrocytes and mesenchymal stem cells. Biomaterials **33,** 6362, 2012.
57. Levorson, E.J., Mountziaris, P.M., Hu, O., Kasper, F.K., and Mikos, A.G. Cell-derived polymer/extracellular matrix

composite scaffolds for cartilage regeneration, Part 1: investigation of cocultures and seeding densities for improved extracellular matrix deposition. Tissue Eng Part C **20,** 340, 2014.
58. Acharya, C., Adesida, A., Zajac, P., Mumme, M., Riesle, J., Martin, I., and Barbero, A. Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. J Cell Physiol **227,** 88, 2012.
59. Zuo, Q., Cui, W., Liu, F., Wang, Q., Chen, Z., and Fan, W. Co-cultivated mesenchymal stem cells support chondrocytic differentiation of articular chondrocytes. Int Orthop **37,** 747, 2013.
60. Yoon, Y.M., Kim, S.J., Oh, C.D., Ju, J.W., Song, W.K., Yoo, Y.J., Huh, T.L., and Chun, J.S. Maintenance of differentiated phenotype of articular chondrocytes by protein kinase C and extracellular signal-regulated protein kinase. J Biol Chem **277,** 8412, 2002.
61. Hwang, S.G., Yu, S.S., Lee, S.W., and Chun, J.S. Wnt-3a regulates chondrocyte differentiation via c-Jun/AP-1 pathway. FEBS Lett **579,** 4837, 2005.
62. Nehrer, S., Breinan, H.A., Ramappa, A., Young, G., Shortkroff, S., Louie, L.K., Sledge, C.B., Yannas, I.V., and Spector, M. Matrix collagen type and pore size influence behaviour of seeded canine chondrocytes. Biomaterials **18,** 769, 1997.
63. Byers, B.A., Mauck, R.L., Chiang, I.E., and Tuan, R.S. Transient exposure to transforming growth factor beta 3 under serum-free conditions enhances the biomechanical and biochemical maturation of tissue-engineered cartilage. Tissue Eng Part A **14,** 1821, 2008.
64. Grigolo, B., Lisignoli, G., Piacentini, A., Fiorini, M., Gobbi, P., Mazzotti, G., Duca, M., Pavesio, A., and Facchini, A. Evidence for redifferentiation of human chondrocytes grown on a hyaluronan-based biomaterial (HYAFF® 11): molecular, immunohistochemical and ultrastructural analysis. Biomaterials **23,** 1187, 2002.
65. Levett, P.A., Melchels, F.P., Schrobback, K., Hutmacher, D.W., Malda, J., and Klein, T.J. Chondrocyte redifferentiation and construct mechanical property development in single-component photocrosslinkable hydrogels. J Biomed Mater Res A 2013 [Epub ahead of print]; DOI: 10.1002/jbm.a.34924.
66. Marcacci, M, Berruto, M., Brocchetta, D., Delcogliano, A., Ghinelli, D., Gobbi, A., Kon, E., Pederzini, L., Rosa, D., Sacchetti, G.L., Stefani, G., and Zanasi, S. Articular cartilage engineering with hyalograft® C: 3-year clinical results. Clin Orthop Relat Res **435,** 96, 2005.
67. Domm, C., Schünke, M., Christesen, K., and Kurz, B. Redifferentiation of dedifferentiated bovine articular chondrocytes in alginate culture under low oxygen tension. Osteoarthritis Cartilage **10,** 13, 2002.
68. Häuselmann, H.J., Fernandes, R.J., Mok, S.S., Schmid, T.M., Block, J.A., Aydelotte, M.B., Kuettner, K.E., and Thonar, E.J. Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads. J Cell Sci **107,** 17, 1994.
69. Bonaventure, J., Kadhom, N., Cohen-Solal, L., Ng, K.H., Bourguignon, J., Lasselin, C., and Freisinger, P. Re-expression of cartilage-specific genes by dedifferentiated human articular chondrocytes cultured in alginate beads. Exp Cell Res **212,** 97, 1994.
70. Murphy, C.L., and Sambanis, A. Effect of oxygen tension and alginate encapsulation on restoration of the differentiated phenotype of passaged chondrocytes. Tissue Eng **7,** 791, 2001.
71. Schulze-Tanzil, G., Mobasheri, A., de Souza, P., John, T., and Shakibaei, M. Loss of chondrogenic potential in dedifferentiated chondrocytes correlates with deficient Shc-Erk interaction and apoptosis. Osteoarthritis Cartilage **12,** 448, 2004.
72. Na, K., Park, J.H., Kim, S.W., Sun, B.K., Woo, D.G., Chung, H.M., and Park, K.H. Delivery of dexamethasone, ascorbate, and growth factor (TGF beta-3) in thermoreversible hydrogel constructs embedded with rabbit chondrocytes. Biomaterials **27,** 5951, 2006.
73. Foldager, C.B., Nielsen, A.B., Munir, S., Ulrich-Vinther, M., Søballe, K., Bünger, C., and Lind, M. Combined 3D and hypoxic culture improves cartilage-specific gene expression in human chondrocytes. Acta Orthop **82,** 234, 2011.
74. Murphy, C.L., and Polak, J.M. Control of human articular chondrocyte differentiation by reduced oxygen tension. J Cell Physiol **199,** 451, 2004.
75. Lafont, J.E., Talma, S., and Murphy, C.L. Hypoxia-inducible factor 2alpha is essential for hypoxic induction of the human articular chondrocyte phenotype. Arthritis Rheum **56,** 3297, 2007.
76. Kellner, K., Liebsch, G., Klimant, I., Wolfbeis, O.S., Blunk, T., Schulz, M.B., and Göpferich, A. Determination of oxygen gradients in engineered tissue using a fluorescent sensor. Biotechnol Bioeng **80,** 73, 2002.
77. Dunkelman, N.S., Zimber, M.P., Lebaron, R.G., Pavelec, R., Kwan, M., and Purchio, A.F. Cartilage production by rabbit articular chondrocytes on polyglycolic acid scaffolds in a closed bioreactor system. Biotechnol Bioeng **46,** 299, 1995.
78. Marlovits, S., Tichy, B., Truppe, M., Gruber, D., and Schlegel, W. Collagen expression in tissue engineered cartilage of aged human articular chondrocytes in a rotating bioreactor. Int J Artif Organs **26,** 319, 2003.
79. Vunjak-Novakovic, G., Martin, I., Obradovic, B., Treppo, S., Grodzinsky, A.J., Langer, R., and Freed, L.E. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. J Orthop Res **17,** 130, 1999.
80. Démarteau, O., Wendt, D., Braccini, A., Jakob, M., Schäfer, D., Heberer, M., and Martin, I. Dynamic compression of cartilage constructs engineered from expanded human articular chondrocytes. Biochem Biophys Res Commun **310,** 580, 2003.
81. Davisson, T., Kunig, S., Chen, A., Sah, R., and Ratcliffe, A. Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. J Orthop Res **20,** 842, 2002.
82. Gan, L., and Kandel, R.A. In vitro cartilage tissue formation by co-culture of primary and passaged chondrocytes. Tissue Eng **13,** 831, 2007.
83. Hendriks, J.A.A., Miclea, R.L., Schotel, R., de Bruijn, E., Moroni, L., Karperien, M., Riesle, J., and van Blitterswijk, C.A. Primary chondrocytes enhance cartilage tissue formation upon co-culture with a range of cell types. Soft Matter **6,** 5080, 2010.
84. Tsuchiya, K., Chen, G., Ushida, T., Matsuno, T., and Tateishi, T. The effect of coculture of chondrocytes with mesenchymal stem cells on their cartilaginous phenotype *in vitro*. Mater Sci Eng C Mater Biol Appl **24,** 391, 2004.

85. Wu, L., Leijten, J.C., Georgi, N., Post, J.N., van Blitterswijk, C.A., and Karperien, M. Trophic effects of mesenchymal stem cells increase chondrocyte proliferation and matrix formation. Tissue Eng Part A **17,** 1425, 2011.
86. Wu, L., Prins, H.J., Helder, M.N., van Blitterswijk, C.A., and Karperien, M. Trophic effects of mesenchymal stem cells in chondrocyte co-cultures are independent of culture conditions and cell sources. Tissue Eng Part A **18,** 1542, 2012.
87. Sekiya, I., Vuoristo, J.T., Larson, B.L., and Prockop, D.J. *In vitro* cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. Proc Natl Acad Sci U S A **99,** 4397, 2002.
88. Dahlin, R.L., Ni, M., Meretoja, V.V., Kasper, F.K., and Mikos, A.G. TGF-beta3-induced chondrogenesis in co-cultures of chondrocytes and mesenchymal stem cells on biodegradable scaffolds. Biomaterials **35,** 123, 2014.
89. Xu, L., Wang, Q., Xu, F., Ye, Z., Zhou, Y., and Tan, W.S. Mesenchymal stem cells downregulate articular chondrocyte differentiation in noncontact coculture systems: implications in cartilage tissue regeneration. Stem Cells Dev **22,** 1657, 2013.
90. Liao, J., Guo, X., Grande-Allen, K.J., Kasper, F.K., and Mikos, A.G. Bioactive polymer/extracellular matrix scaffolds fabricated with a flow perfusion bioreactor for cartilage tissue engineering. Biomaterials **31,** 8911, 2010.
91. Thorpe, S.D., Buckley, C.T., Vinardell, T., O'Brien, F.J., Campbell, V.A., and Kelly, D.J. The response of bone marrow-derived mesenchymal stem cells to dynamic compression following TGF-beta3 induced chondrogenic differentiation. Ann Biomed Eng **38,** 2896, 2010.
92. Huang, A.H., Stein, A., Tuan, R.S., and Mauck, R.L. Transient exposure to transforming growth factor beta 3 improves the mechanical properties of mesenchymal stem cell-laden cartilage constructs in a density-dependent manner. Tissue Eng Part A **15,** 3461, 2009.
93. Chen, W.C., Yao, C.L., Wei, Y.H., and Chu, I.M. Evaluating osteochondral defect repair potential of autologous rabbit bone marrow cells on type II collagen scaffold. Cytotechnology **63,** 13, 2011.
94. Yang, W., Gomes, R.R., Brown, A.J., Burdett, A.R., Alicknavitch, M., Farach-Carson, M.C., and Carson, D.D. Chondrogenic differentiation on perlecan domain I, collagen II, and bone morphogenetic protein-2-based matrices. Tissue Eng **12,** 2009, 2006.
95. Schätti, O., Grad, S., Goldhahn, J., Salzmann, G., Li, Z., Alini, M., and Stoddart, M.J. A combination of shear and dynamic compression leads to mechanically induced chondrogenesis of human mesenchymal stem cells. Eur Cell Mater **22,** 214, 2011.
96. Bian, L., Zhai, D.Y., Tous, E., Rai, R., Mauck, R.L., and Burdick, J.A. Enhanced MSC chondrogenesis following delivery of TGF-beta3 from alginate microspheres within hyaluronic acid hydrogels *in vitro* and *in vivo*. Biomaterials **32,** 6425, 2011.
97. Kanichai, M., Ferguson, D., Prendergast, P.J., and Campbell, V.A. Hypoxia promotes chondrogenesis in rat mesenchymal stem cells: a role for AKT and hypoxia-inducible factor (HIF)-1alpha. J Cell Physiol **216,** 708, 2008.
98. Meyer, E.G., Buckley, C.T., Thorpe, S.D., and Kelly, D.J. Low oxygen tension is a more potent promoter of chondrogenic differentiation than dynamic compression. J Biomech **43,** 2516, 2010.
99. Lee, H.H., Chang, C.C., Shieh, M.J., Wang, J.P., Chen, Y.T., Young, T.H., and Hung, S.C. Hypoxia enhances chondrogenesis and prevents terminal differentiation through PI3K/Akt/FoxO dependent anti-apoptotic effect. Sci Rep **3,** 2683, 2013.
100. Kupcsik, L., Stoddart, M.J., Li, Z., Benneker, L.M., and Alini, M. Improving chondrogenesis: potential and limitations of SOX9 gene transfer and mechanical stimulation. Tissue Eng Part A **16,** 1845, 2010.
101. Huang, C.Y., Hagar, K.L., Frost, L.E., Sun, Y., and Cheung, H.S. Effects of cyclic compressive loading on chondrogenesis of rabbit bone-marrow derived mesenchymal stem cells. Stem Cells **22,** 313, 2004.
102. Huang, C.Y., Reuben, P.M., and Cheung, H.S. Temporal expression patterns and corresponding protein inductions of early responsive genes in rabbit bone marrow-derived mesenchymal stem cells under cyclic compressive loading. Stem Cells **23,** 1113, 2005.
103. Mauck, R.L., Nicoll, S.B., Seyhan, S.L., Ateshian, G.A., and Hung, C.T. Synergistic action of growth factors and dynamic loading for articular cartilage tissue engineering. Tissue Eng **9,** 597, 2003.
104. Bian, L., Zhai, D.Y., Mauck, R.L., and Burdick, J.A. Coculture of human mesenchymal stem cells and articular chondrocytes reduces hypertrophy and enhances functional properties of engineered cartilage. Tissue Eng Part A **17,** 1137, 2011.
105. Aung, A., Gupta, G., Majid, G., and Varghese, S. Osteoarthritic chondrocyte-secreted morphogens induce chondrogenic differentiation of human mesenchymal stem cells. Arthritis Rheum **63,** 148, 2011.
106. Sabatino, M.A., Santoro, R., Gueven, S., Jaquiery, C., Wendt, D.J., Martin, I., Moretti, M., and Barbero, A. Cartilage graft engineering by co-culturing primary human articular chondrocytes with human bone marrow stromal cells. J Tissue Eng Regen Med 2012 [Epub ahead of print]; DOI: 10.1002/term.1661.
107. Cooke, M.E., Allon, A.A., Cheng, T., Kuo, A.C., Kim, H.T., Vail, T.P., Marcucio, R.S., Schneider, R.A., Lotz, J.C., and Alliston, T. Structured three-dimensional co-culture of mesenchymal stem cells with chondrocytes promotes chondrogenic differentiation without hypertrophy. Osteoarthritis Cartilage **19,** 1210, 2011.
108. Giovannini, S., Diaz-Romero, J., Aigner, T., Heini, P., Mainil-Varlet, P., and Nesic, D. Micromass co-culture of human articular chondrocytes and human bone marrow mesenchymal stem cells to investigate stable neocartilage tissue formation *in vitro*. Eur Cell Mater **20,** 245, 2010.
109. Lettry, V., Hosoya, K., Takagi, S., and Okumura, M. Coculture of equine mesenchymal stem cells and mature equine articular chondrocytes results in improved chondrogenic differentiation of the stem cells. Jpn J Vet Res **58,** 5, 2010.
110. Yang, H.N., Park, J.S., Na, K., Woo, D.G., Kwon, Y.D., and Park, K.H. The use of green fluorescence gene (GFP)-modified rabbit mesenchymal stem cells (rMSCs) co-cultured with chondrocytes in hydrogel constructs to reveal the chondrogenesis of MSCs. Biomaterials **30,** 6374, 2009.
111. Mo, X.T., Guo, S.C., Xie, H.Q., Deng, L., Zhi, W., Xiang, Z., Li, X.Q., and Yang, Z.M. Variations in the ratios of co-cultured mesenchymal stem cells and chondrocytes regulate

the expression of cartilaginous and osseous phenotype in alginate constructs. Bone **45,** 42, 2009.
112. Fischer, J., Dickhut, A., Rickert, M., and Richter, W. Human articular chondrocytes secrete parathyroid hormone-related protein and inhibit hypertrophy of mesenchymal stem cells in coculture during chondrogenesis. Arthritis Rheum **62,** 2696, 2010.
113. Chen, W.H., Lai, M.T., Wu, A.T., Wu, C.C., Gelovani, J.G., Lin, C.T., Hung, S.C., Chiu, W.T., and Deng, W.P. *In vitro* stage-specific chondrogenesis of mesenchymal stem cells committed to chondrocytes. Arthritis Rheum **60,** 450, 2009.
114. Qing, C., Wei-ding, C., and Wei-min, F. Co-culture of chondrocytes and bone marrow mesenchymal stem cells *in vitro* enhances the expression of cartilaginous extracellular matrix components. Braz J Med Biol Res **44,** 303, 2011.
115. Rothenberg, A.R., Ouyang, L., and Elisseeff, J.H. Mesenchymal stem cell stimulation of tissue growth depends on differentiation state. Stem Cells Dev **20,** 405, 2011.
116. Levorson, E.J., Santoro, M., Kasper, F.K., and Mikos, A.G. Direct and indirect co-culture of chondrocytes and mesenchymal stem cells for the generation of polymer/extracellular matrix hybrid constructs. Acta Biomater **10,** 1824, 2014.
117. Levorson, E.J., Hu, O., Mountziaris, P.M., Kasper, F.K., and Mikos, A.G. Cell-derived polymer/extracellular matrix composite scaffolds for cartilage regeneration, Part 2: construct devitalization and determination of chondroinductive capacity. Tissue Eng Part C Methods **20,** 358, 2014.
118. Bekkers, J.E., Tsuchida, A.I., van Rijen, M.H., Vonk, L.A., Dhert, W.J., Creemers, L.B., and Saris, D.B. Single-stage cell-based cartilage regeneration using a combination of chondrons and mesenchymal stromal cells: comparison with microfracture. Am J Sports Med **41,** 2158, 2013.
119. Dehne, T., Schenk, R., Perka, C., Morawietz, L., Pruss, A., Sittinger, M., Kaps, C., and Ringe, J. Gene expression profiling of primary human articular chondrocytes in high-density micromasses reveals patterns of recovery, maintenance, re- and dedifferentiation. Gene **462,** 8, 2010.

Address correspondence to:
Antonios G. Mikos, PhD
Department of Bioengineering–MS 142
Rice University
6100 Main Street
P.O. Box 1892
Houston, TX 77005

E-mail: mikos@rice.edu

Received: January 14, 2014
Accepted: May 13, 2014
Online Publication Date: June 23, 2014