Review Article

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The Potential of Human Embryonic Stem Cells for Articular Cartilage Repair and Osteoarthritis Treatment

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Abstract

Osteoarthritis is a debilitating joint disease present in epidemic proportions worldwide. Osteoarthritis results from degeneration of the articular cartilage of the joint surfaces due to acute trauma, or chronic wear and tear. Due to limited ability of cartilage to repair itself, and lack of available treatments, there is an urgent need for development of approaches to repair articular cartilage damage due to injury or osteoarthritic disease. Cell-based repair strategies are among the most promising of these approaches. Various adult cell sources for cartilage repair are proposed including autologous adult chondrocytes as well as adult Mesenchymal Stem Cells (MSC). Disadvantages such as destructive harvest protocols; poor proliferation, and particularly for MSC, considerable cellular heterogeneity, have limited success of these cell types for cartilage repair. Chondrogenic cells derived from human embryonic stem cells (hESC) offer a highly proliferative cell source, which when directed into the chondrogenic lineage, could provide an ideal source of cells for cartilage repair. Chondrogenic cells derived from human induced pluripotent stem cells (iPSC) offer additional advantages for patient-specific therapy. Recently protocols have been established for directed differentiation of hESC into the chondrogenic lineage. Harnessing the potential of hESC-derived chondrogenic cells will require comprehensive testing of their efficacy for *in vivo* cartilage repair, as well as considerations of safety and immunogenicity of the cells. Use of pro-chondrogenic factors and/or bioactive scaffolds may assist in optimizing cartilage repair by chondrogenic cells. Repair of cartilage damage in osteoarthritis is a special challenge because of the widespread damage and presence of signals and stressors which disrupt normal joint homeostasis. Particular promise in cell-based repair of osteoarthritis may be provided by chondrogenic progenitor cells which may mimic endogenous repair responses. This review discusses the current status of cell-based cartilage repair strategies and in particular the potential role of hESC-derived chondrocytes or chondroprogenitor cells for treatment of articular cartilage damage due to injury and osteoarthritis.

Keywords: Human embryonic stem cells; hESC; Induced pluripotent stem cells; iPSC; Cartilage repair; Osteoarthritis; Chondroprogenitors

Abbreviations: hESC: Human Embryonic Stem Cells; iPSC: Induced Pluripotent Stem Cells; MSC: Mesenchymal Stem Cells; OATS: Osteoarticular Transfer System; ACI: Autologous Chondrocyte Replacement; MACI: Matrix Assisted Chondrocyte Implantation; EB: Embryoid Body; FACS: Fluorescence-Activated Cell Sorting; GMP: Good Manufacturing Practice; SOP: Standard Operating Procedure; MEF: Mouse Embryonic Fibroblasts; FBS: Fetal Bovine Serum; KSR: Knockout Serum Replacement; MHC: Major Histocompatibility Complex; HLA: Human Leukocyte Antigen; MMP: Matrix Metalloproteases; BMP: Bone Morphogenetic Protein; TGF-B: Transforming Growth Factor- Beta; IGF: Insulin-Like Growth Factor; FGF: Fibroblast Growth Factor; ADAMS: A Disintegrin and Metalloproteinase; VEGF: Vascular Endothelial Growth Factor

Introduction

The problem of cartilage injury and osteoarthritis

Articular cartilage is the unique type of hyaline cartilage present at the joint surfaces of the long bones that is essential for normal joint mobility and resistance to compression forces [1]. Osteoarthritis is a debilitating joint disease caused by degeneration of the articular cartilage. Most osteoarthritis is associated with age-related wear-and-tear [2] but osteoarthritis can also occur as a consequence of prior acute joint injury [3]. Cartilage degeneration in osteoarthritis is mediated by disruption of the normal signals which regulate joint homeostasis, leading to decreased articular cartilage matrix synthesis and increased matrix proteolytic activity, and causing net articular cartilage loss. These structural changes lead to pain and disabling loss of mobility of the affected joints [4].

Osteoarthritis is present in epidemic proportions in this country and around the world, and is a major cause of decreased quality of life in adults [2]. Currently, around 27 million adults in the U.S. suffer from osteoarthritis [5]. This number is expected to rise due to aging of the population [6,7] and increasing prevalence of obesity [8]. Effects of obesity are not solely mechanical, as high fat diets have been shown to cause changes in articular chondrocyte function independent of weight gain [9] and can induce production of inflammatory cytokines [10]. Other dietary factors such as low intake of Vitamin D have also been associated with increase osteoarthritis incidence [11]. Specific population groups at high risk for osteoarthritis include women, who have a 3 times higher osteoarthritis prevalence than men [12,13] and populations with bias for the disease due to genetic ethnicity (especially African Americans) [14], or due to socio-economic status [15].

The limited intrinsic ability of cartilage tissue to repair itself poses unique challenges to treatment of cartilage injury and osteoarthritis.

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The lack of effective treatments for osteoarthritis demonstrates a critical unmet need for an osteoarthritis therapy. A critical barrier to success in cell-based articular cartilage repair is identification of a readily available exogenous source of cells with the ability to seamlessly repair articular cartilage damage and restore long-term joint function.

Adult Cells for Treatment of Cartilage Injury or Osteoarthritis

Adult chondrocytes for cartilage repair

As most acute injuries to the articular cartilage ultimately progress to osteoarthritis [16], one approach to disease intervention is a preventative strategy to repair articular cartilage damage following acute injury, using cartilage grafts or implanted chondrocytes to replace focally damaged lesions. These treatments are clinically available, but are limited to repair of localized articular cartilage injury in otherwise generally healthy and relatively young patients and are not available for the typical osteoarthritis patient who is older and has more widespread damage. Moreover, the effectiveness of these treatments is limited by disadvantages inherent in the approaches and in the quality of the repair achieved. For example, surgical replacement of the damaged cartilage with cartilage plugs (allo- or auto-grafts, aka mosaicplasty or Osteoarticular Transfer System OATS) [17], tends to result in poor integration with the host tissue. Allografts have the added disadvantages of potential disease transmission, and karyotyping of post-mortem chondrocytes has revealed high rates of chromosome abnormalities [18] which may contribute to their poor performance. A major disadvantage to autografts is donor-site morbidity at the location of tissue harvest. Autologous Chondrocyte Implantation (ACI), in which articular chondrocytes are harvested from the patient, expanded *in vitro*, and re-implanted into the defect [19], has been FDA approved (as Carticel) for use in the clinic since 1997, and depending on the study [20,21] has been suggested to provide improvement in clinical outcome for patients with acute articular cartilage injury. However, ACI has not been widely accepted and recent meta-analysis of studies examining long term outcomes fail to demonstrate clinical improvement following use of ACI for repair of full thickness defects in the knee [22,23].

Modifications to ACI aimed to improve the approach include a second-generation strategy involving implantation of the cells under a synthetic collagen membrane (Matrix Assisted Chondrocyte Implantation, MACI) [24] and a third generation approach in which the cells are maintained and implanted in a supportive three dimensional hyaluronan scaffold (Hyalograft C) [25]. Another approach sorts the cells prior to implantation through their expression of certain chondrogenic markers (Characterized Chondrocyte Implantation) [26]. These modifications emphasize the importance of the nature of the cells used for repair, and the local signals which influence their repair capacity. A major drawback of ACI and related approaches is their continued reliance on adult autologous chondrocytes as the "gold-standard" cell source for articular cartilage repair. Disadvantages of adult chondrocytes as a cell source for repair include the requirement for initial harvest of the patient's cells, which causes donor site morbidity, and the need for the harvested cells, which do not proliferate well, to be expanded *ex vivo* for up to 6 weeks prior to surgical implantation, increasing expense and lengthening duration of patient disability. Adult chondrocytes are known for their propensity to de-differentiate during culture and expansion, loosing characteristics of the cartilage phenotype such as critical synthesis of appropriate cartilage matrix proteins. This tendency is exacerbated in articular chondrocytes obtained from older patients [27]. Indeed, it has been suggested that osteoarthritis is an ageing disorder of the chondrocyte itself [28,29], as mitotic activity and telomere length, an indicator of cellular age, were found to decline in chondrocytes obtained from progressively older patients [28]. Age-related changes in chondrocytes may result from cumulative oxidative stress causing DNA damage [30], or from disruption of cellular processes involving mitochondrial function which can lead to apoptosis [30,31]. The presence of age-related intrinsic changes in chondrocytes obtained from older donors is a further challenge to the use of adult autologous chondrocytes for repair of cartilage injury.

Adult mesenchymal stem cells for cartilage repair

Mesenchymal Stem Cells (MSC) obtained from bone marrow, synovium, fat, or umbilical cord, have also been proposed as a cell source for cartilage repair [32-34]. MSC from different sources may have distinct potential for differentiation into the chondrogenic lineage, and adipocyte-derived MSC have been suggested to be most chondrogenic [35]. MSC may be differentiated into chondrocytes using growth factors such as Bone Morphogenetic protein (BMP), Transforming Growth Factor-Beta (TGF-β), Insulin-like Growth Factor (IGF) or fibroblast growth Factor (FGF) [36-39], or using scaffolds [40] which may release pro-chondrogenic signals such as TGF-β [41]. MSC have immunosuppressive potential [42] and may be useful in allogenic as well as autologous therapies. These are important considerations in favor of use of MSC for cartilage injury. However MSC have significant disadvantages as a cell source for cartilage repair. MSC tend to proliferate poorly *in vitro*, especially when obtained from older patients [43,44], making expansion to a sufficient number for repair difficult. Obtaining MSC from bone marrow, the most commonly used source, is an invasive procedure, and MSC present in bone marrow aspirates are rare and estimated at less than 0.01% of the cell population [45]. Purification of MSC may be necessary to obtain sufficient cells with robust chondrogenic potential [46], and methods of MSC harvest, isolation and purification need to be developed which optimize cell viability and performance [47-49]. The most significant disadvantage of MSCs may be that by definition, they are a heterogenous mix of adipogenic, osteogenic and chondrogenic progenitors. The presence of non-chondrogenic cells in MSC dilutes the pool of available cells with cartilage repair potential, which may explain formation of non-uniform or atypic cartilage by MSC [50]. Consistent with this possibility, the clinical procedure known as microfracture or Pridie drilling, which involve repeated puncture of the injured articular cartilage to promote influx of marrow cells from the subchondral bone, results in formation of repair tissue comprised of fibrocartilage, rather than hyaline cartilage [51]. Although micro fracture can provide short term clinical improvement for cartilage damage [52], outcomes analysis after 1-2 years indicate poor clinical function and visually worsened articular cartilage damage [53], consistent with the inability of this procedure to provide long-term, durable repair. A small clinical trial has reported that MSC implanted directly into damaged knee articular cartilage does not lead to tumor formation [54], however, the effectiveness of MSC in repairing articular cartilage damage requires further study.

Human Embryonic Stem Cells (Hesc) as a Source of Cells for Cartilage Repair

Potential of hESC for treatment of cartilage injury

Because of their unlimited capacity for self-renewal while maintaining potential for chondrogenic differentiation, Human Embryonic Stem Cells (hESC) could provide essentially limitless numbers of cells with potential for cartilage repair, and may offer an

ideal alternative cell source for cartilage repair compared to MSC, or to the adult chondrocytes used in ACI. In order to achieve this potential, procedures for efficient directed differentiation of pluripotent cells into the chondrogenic lineage are needed. Protocols have been reported for chondrogenic differentiation of hESC under various conditions, typically involving supplementation with growth factors such as TGF-β and BMPs. Most approaches utilize an intermediate step involving Embryoid Body (EB) formation [55-60]. Embryoid bodies contain cells from all three embryonic germ layers including ectoderm and endoderm, which may introduce non-chondrogenic cell types, potentially requiring cell sorting or FACS to purify the mesenchymal progenitors [58,59]. Procedures have also been reported in which either EB steps, co-culture with other cell types, cell sorting or prolonged culture periods in monolayer are used to derive an MSC-like intermediate from the hESC, which may then be directed into the chondrogenic lineage [61-70]. Gene expression profiling suggests these MSC-like hESC-derived cells represent a more primitive lineage than adult MSC, and hence may be even more heterogeneous [71]. Pellet culture has also been used to direct the chondrogenic differentiation of hESC [72]. Pellet culture may be less optimal for directing chondrogenic differentiation of progenitor cells than other approaches, such as high density micromass culture, which is a standard method for differentiation of embryonic limb mesenchymal cells into chondrogenic cells [73]. Comparison of chondrogenic differentiation by MSC in micromass culture vs. pellet culture revealed superior uniformity of chondrogenic differentiation using the micromass approach [74], suggesting high density micromass culture may be an optimal condition for directed hESC differentiation into chondrocytes.

A protocol we have developed utilizes micromass culture, in the presence of TGF-β and BMP, to direct the rapid and substantially uniform differentiation of hESC into the chondrogenic lineage without prior EB formation [75]. The hESC-derived chondrogenic cells obtained through this approach produce abundant cartilage matrix as determined by Alcian blue staining and type II collagen (Col2a1) immunohistochemistry, and express high message levels of Col2a1 as well as the definitive cartilage marker, aggrecan. Direct comparison revealed that the extent of chondrogenic differentiation achieved by the non-EB derived hESC was markedly superior to that obtained by EB-derived hESC [75], consistent with the idea that generation of non-chondrogenic cells via the EB and/or MSC-like intermediates used in most differentiation protocols may contribute to heterogeneity of the resultant cell population.

In addition to avoidance of cellular heterogeneity, another important aspect of a differentiation protocol aimed to produce chondrogenic cells for cartilage repair is avoidance of chondrocyte hypertrophy. Chondrocyte hypertrophy is the terminal differentiation process through which endochondral ossification occurs during normal long bone development [76,77]. In this process, the chondrocytes of the cartilage models of the limb skeletal elements undergo progressive enlargement (hypertrophy), accompanied by changes in their gene expression profile including induction of expression of type X collagen, a definitive marker of hypertrophy. Hypertrophic chondrocytes also express signals including Vascular Endothelial Growth Factor (VEGF) and Matrix Metallo Proteinases (MMPs) which lead to vascular invasion of the cartilage and degradation of the matrix, prompting remodeling of the tissue and its replacement by bone [76,77]. Notably, hypertrophic chondrocytes are inappropriately found in osteoarthritic cartilage and their presence there is considered a hallmark of the disease [78,79]. Several protocols for chondrogenic differentiation of hESC including our own have confirmed appropriate low or absent expression of hypertrophic markers including type X collagen [58,60,75].

Significantly, promising results have been obtained in a recent study evaluating *in vivo* articular cartilage repair by chondrocytes derived from hESC [80]. The protocol used to generate the cells utilized EB formation and high density micromass culture in the presence of TGF-β [80]. The hESC-derived chondrocytes were embedded in a hyaluronan hydrogel and surgically implanted into articular cartilage defects generated in rats. After 12 weeks, the defect was filled by hyaline cartilage-like repair tissue with good integration with the adjacent cartilage [80]. Some potential problems noted in the study included low-level induction of type X collagen expression by the repair tissue, and surprising gradual loss of the human cells, despite chemical immune suppression to avoid graft rejection. Nonetheless, this encouraging study demonstrates the potential *in vivo* efficacy of a hESC-based cartilage repair strategy. Improvements will likely be realized through use of chondrogenic hESC generated in protocols which minimize potential heterogeneity [75], as well as through appreciation of the signals which control chondrocyte differentiation and hypertrophy, and a better understanding of hESC in graft-host responses.

Safety of hESC in cartilage repair

In order to gain clinical acceptance, hESC must be demonstrated to be a safe cell source for cartilage repair. Because stem cells, when undifferentiated, are pluripotent, they can form tumors consisting of cells from all three germ layers (teratomas). As tumor formation is not a characteristic of differentiated cells, hESC-derived chondrogenic cells may have low intrinsic tumor-forming potential. In addition, it has been suggested that signals present within the joint and articular cartilage function to promote the in situ chondrogenic differentiation of potential repair cells introduced into it [81], which may assist in retention or promotion of chondrogenic phenotype and suppression of tumorigenic potential. Indeed, pluripotent ES cells placed directly within articular cartilage defects in rats formed repair tissue, and not teratomas [81]. Moreover, implantation of MSC, which are multipotent cells, into human knee articular cartilage defects has failed to reveal tumor formation in a small clinical cohort after at least 10 years [54], and implantation of differentiated hESC into articular cartilage defects in a rat model also failed to lead to tumor formation [81]. Karyotyping can confirm chromosomal stability and absence of major genetic deletions or rearrangements which can occur following long term passaging of the pluripotent hESC used to derive the repair cells [82].

The demand to realize the therapeutic potential of hESC will require development of new, clinically-compliant hESC lines for research and potential therapeutic use [83]. hESC are defined by their common pluripotent characteristics, including considerable similarity in pluripotent gene expression profiles [84] and shared functional ability to differentiate into cell lineages from each germ layer [85]. Despite these commonalities, genomic sequencing and microarray profiling have revealed differences in gene expression and in lineage-specific differentiation potential among hESC lines [86-89]. These subtle differences in differentiation potential may relate to inherent differences in the source of the cells used for derivation, or to changes acquired during long-term culture [90]. These observations emphasize the importance of comparing differentiation potential and cellular function of hESC derivatives generated from multiple lines. Remarkably, studies show that the majority of hESC research has been done with very few lines [91]. More than 50% of studies published between 1998 and 2008 used only three lines (H1, H7 and H9), derived by Thomson and colleagues and provided by WiCell Research Institute, and another 40% of studies during this period utilized only eight additional lines [91]. Comparing and potentially identifying a particularly efficient

hESC line for chondrogenic differentiation or repair would be a benefit for generation of chondrogenic cells for clinical use.

Clinical application of hESC will depend on utilization of GMP (Good Manufacturing Practices) and SOP (Standard Operating Procedures), and establishment of facilities to derive, expand, bank and supply pluripotent lines. There is a need for optimization of techniques and culture systems used to derive and maintain pluripotent hES cells. These include the development of culture methodologies which avoid use of undefined and/or xenogenic components such as feeder layers or animal products, which introduce contaminants precluding use of the cells or their derivatives for human therapeutic applications [92,93]. Feeder layers, typically Mouse Embryonic Fibroblasts (MEFs), were initially shown to be required for maintenance of human stem cell viability and pluripotency [85] but have been replaced in some protocols by semi-defined culture substrates including Matrigel [94,95] or other xeno-free or defined substrates such as human laminin or Cell Start [96]. Additional optimization of human stem cell culture methodologies may employ various approaches for enzymatic digestion of the pluripotent colonies to single cells for passaging, and using animal-free enzyme replacements [97,98]. Utilization of such modifications will assist in developing clinically-compliant hESC for human therapeutic use [99].

Potential for immune rejection of hESC-derived cartilage repair cells

An important consideration in a hESC-based therapy is the potential for the cells, which are allogenic, to elicit an immune response. Graft immune rejection is due to expression of Major Histocompatibility (MHC) antigens which are recognized by the host as foreign, leading to stimulation of host immune cell proliferation and activity. An important consideration in generation of an immune response is the physical accessibility of the host immune cells to the grafted cells. In this regard, the synovial joints has been suggested to be an immune-privileged site, as it poorly vascularized and separated from the rest of the body by a physical capsule. Moreover, the presence of pericellular matrix which surrounds cartilage cells, and the location of their engraftment within the local environment of the cartilage defect, may provide additional protection from host immune surveillance [100,101]. A recent study has reported that injections of human MSC into the knee joints of osteoarthritic guinea pigs, which resulted in integration of the cells into the damaged cartilage, were well tolerated and did not induce local inflammation [102]. A rejection response also depends on the extent and ability of engrafted cells to present their MHC antigens to the host. hESC (and MSC) have intrinsic systemic immunosuppressive activity [103], and as undifferentiated hESC express very low levels of MHC antigens [104], hESC and their derivatives are themselves considered inherently immune-privileged [105,106]. Though levels of MHC antigens increase upon differentiation of hESC, they are still below the level expressed by other somatic cells [104]. Human chondrocytes in particular show weak or incomplete expression of MHC antigens, as they express class I MHC but do not normally express co-stimulatory class II MHC [107], and accordingly lack a component typically required for generation of a robust rejection response. Thus unlike applications of hESC-based therapy in tissues like heart or other organs which are readily accessible by the host immune system, and which might require use of more powerful immune-suppressive drugs with potential undesirable side effects [108,109], it is conceivable that use of hESC in cartilage repair may be achievable with minimal immune suppression through the use of allogenic cells that are closely but not identically matched. National and international incentives are being planned for establishment of banks of hESC lines obtained from donors with diverse races and ethnicities, which can be used to provide hESCs with MHC haplotype matching for most individuals [91]. Studies estimate that such a bank would require between 150-170 hESC lines to provide HLA (Human Leukocyte Antigen) matching for populations in the United Kingdom and Japan with minimal immune suppression required [110,111], and an established 188-line hESC bank created in China is planned to provide HLA matching for 25-50% of the population without immune suppression [112].

Induced pluripotent stem cell (iPSC)-derived chondrogenic cells

Induced Pluripotent Stem Cells (iPSC), which are pluripotent stem cells derived from somatic cells, offer a compelling alternative to allogenic cells for regenerative medicine. iPSC have the advantage of being readily available from sources such as adult skin, and since they are obtained from a patient's own cells, they can provide patient-specific cell-mediated therapy [113,114]. Safe, therapeutic use of iPSC for clinical regenerative medicine will require development of procedures to induce pluripotency of the somatic cells, without modification of the cellular genome [115]. The standard methodology for induction of pluripotency in iPSC involves viral transduction using transcription factors including oncogenes [116,117], which can lead to insertional mutagenesis and increased risk of tumor formation [118]. Methodology for generation of iPSC is rapidly evolving, and reprogramming of adult somatic cells is now being demonstrated which avoids permanent transgene integration, using excisable plasmids, Cre-Lox mediated reprogramming, or mRNA or protein transfection [119,120]. These approaches show much promise for future production of clinical-grade iPSCs for regenerative therapy including cartilage repair.

Recently, *in vitro* differentiation of mouse [121,122] and human [123-126] iPSC into the chondrogenic lineage, in protocols using EB and/or MSC like intermediates, has been reported. The sources of cells used to derive the iPSC in these studies were mouse and human fibroblasts [121-123], human fetal brain-derived neural stem cells [124], and significantly, adult human synovial cells or chondrocytes from patients with osteoarthritis [125,126]. Interestingly, chondrogenic cultures of one of the two synovial-derived iPSC lines obtained from the osteoarthritic patients expressed high levels of type X collagen, indicating inappropriate hypertrophic differentiation [125]. This may reflect the disease state of the original cell source, consistent with the idea that an epigenetic memory is imprinted on the somatic cells from which the iPSC are derived [127]. Similarly, iPSC derived from osteoarthritic cartilage expressed high levels of the degenerative cartilage marker Vascular Endothelial Growth Factor (VEGF), even when maintained in a chondrocyte-supportive matrix and transfected with the pro-chondrogenic factor TGFβ1, leading the authors to suggest that the cells had a degenerative tendency reflective of their origin in diseased tissue [126]. This study also reported that co-culture of the iPSC-derived chondrocytes from osteoarthritic patients in a transwell system with normal adult chondrocytes, resulted in more robust expression of cartilage-specific genes and a loss of VEGF expression [126]. This result emphasizes the importance of pro-chondrogenic signals which may be useful in promoting or maintaining the chondrogenic phenotype by putative repair cells.

Optimization of Cell-Based Cartilage Repair Strategies

Viscosupplementation

Strategies for optimizing cartilage repair by exogenous cells include providing the cells with signals and conditions which promote their chondrogenic differentiation and/or maintenance of

the chondrocyte phenotype. A widely used approach for palliative therapy of osteoarthritis is viscosupplementation with hyaluronan, a very large glycosaminoglycan polymer [128] which is a major component of synovial fluid [129]. Hyaluronan levels in synovial fluid from osteoarthritis patients are reduced by 30-50% [130] providing the basis for intra-articular hyaluronan viscosupplementation as a clinical osteoarthritis treatment. The efficacy of hyaluronan viscosupplementation has been analyzed in numerous randomized controlled trials, and overall there is consistency of positive clinical benefit in terms of pain relief and improved function [131-134] with few deleterious side effects. However, statistically significant clinical benefit has not always been shown and duration of improvement appears to be one year or less [135,136]. While hyaluronan viscosupplementation may not be sufficient by itself to treat articular cartilage degeneration, it may offer promise in conjunction with exogenous cell based therapies, by enhancing the pro-chondrogenic environment within the joint. For example, a recent study comparing cartilage repair by exogenous MSC introduced into the osteoarthritic joints of guinea pigs with or without hyaluronan viscosupplementation found that only the animals treated with the combination therapy showed integration of the exogenous cells into damaged cartilage and subsequent articular cartilage repair [102]. Viscosupplementation with lubricin, another joint-lubricating compound, may also have potential for osteoarthritis treatment [137] as intra articular injection of lubricin slowed cartilage damage in a rat osteoarthritis model [138].

Matrices and scaffolds

Enhancement of pro-chondrogenic activity for cells used to repair cartilage damage may also be provided by scaffolds or bioactive matrices. Hyaluronan-containing matrices have been used to promote *in vitro* maintenance of the differentiated phenotype by adult chondrocytes, and to promote chondrogenic differentiation of MSC [139-142]. In addition, implantation of adult chondrocytes or MSC in hyaluronan-containing matrices is a standard approach to improve their ability to repair focal cartilage lesions in animals [143-145] and this approach has also been used to demonstrate successful repair of cartilage defects by hESC-derived chondrocytes [80]. A clinical modification to autologous chondrocyte implantation utilizes adult chondrocytes within a hyaluronan sponge (Hyalograft C) [146]. Matrix hyaluronan is thought to have direct pro-chondrogenic activities on the cells within it. Hyaluronan is a major component of embryonic and adult cartilage, and we have found, using a conditional genetic approach in transgenic mice, that absence of hyaluronan in developing cartilage perturbs chondrogenic differentiation [147]. Cellular functions of hyaluronan are mediated by binding to its cell surface receptor, CD44, which is expressed by chondrocytes [148]. Hyaluronan-CD44 interactions in articular chondrocytes stimulate synthesis of cartilage matrix [149], reduce inappropriate expression of matrix degradative enzymes such as MMPs [150,151], and activate pro-chondrogenic BMP activity [152]. In addition to hyaluronan, other matrices are also being tested for their abilities to provide pro-chondrogenic stimulation during cell-based cartilage repair, and which may also offer superior structural support which would be useful in withstanding mechanical loading of the joint during the repair process [153,154]. These include a myriad of scaffolds, sponges and nano fibers comprised of collagen, chitin, silk and/or synthetic polymers [155-160], which have been demonstrated to promote chondrogenesis of adult chondrocytes, MSC or hESC-derived cells *in vitro* [155-160].

Growth factors

Pro-chondrogenic growth factors may have utility in enhancing the

ability of exogenous cells to repair damaged articular cartilage [161,162]. Promising growth factors include FGF or BMP, as direct injection of these factors into the joint space improves the histological appearance of articular cartilage in animal models of osteoarthritis [163,164], and in a clinical trial, intra-articular injection of BMP7 into the knees of patients with symptomatic osteoarthritis led to improvement in osteoarthritis symptoms over placebo [165]. As a limiting factor in a direct injection approach may be the half-life of the injected compound or factor, other delivery strategies are being developed. One approach is to incorporate pro-chondrogenic factors into biomaterials. Biomaterial scaffolds impregnated with BMP, TGF-β, or IGF maintain the differentiated phenotype of adult chondrocytes and promote chondrogenesis by MSC *in vitro* [166-169], and successful *in vivo* repair of articular cartilage defects in animal models has been reported in some studies using MSC seeded into TGF-β-containing biomaterial scaffolds [169,170]. However, other studies using a similar approach did not result in improved *in vivo* cartilage repair [171,172], and in one case led to a foreign body reaction [171]. These studies suggest a better understanding is needed of the complexities inherent in biomaterial/cell/growth factor combinations which may influence cartilage repair outcomes. An exciting strategy for non-invasive biomaterial-mediated delivery of growth factors (or conceivably, factors together with cells) may be to encapsulate them in nanoparticles which are directly injectable. This approach has been used to achieve sustained delivery of Parathyroid hormone (PTH) into the joints of osteoarthritic rats [173]. Genetic approaches are also being developed to achieve expression of pro-chondrogenic growth factors or signals by exogenous repair cells themselves. These approaches have used adenovirus [174,175] or direct gene transfer [176-179], to express growth factors such as BMP, IGF or FGF, or pro-chondrogenic signals such as Sox9, in chondrocytes or MSC. Outcomes in studies in which repair of articular cartilage defects by the genetically modified cells have been examined are promising, particularly for gene transfer, suggesting that this approach may have potential for enhancing cell-based cartilage repair potential, provided its safety is rigorously assessed [180,181].

Challenges and Opportunities in Cell-Based Treatment of Osteoarthritis

The osteoarthritic joint is particularly challenging for cell-based articular cartilage restoration [182,183]. Cartilage damage in osteoarthritis is widespread and lesions are large, making surgical implantation approaches unfeasible. Mechanical abnormalities due to joint misalignment, bony remodeling, and ligament stretching may also be present which can cause localized regions of dynamic loading stress on the articular cartilage surface, interfering with repair. Moreover, the local environment of the osteoarthritic joint is considered hostile due to presence of signals which promote inflammation, suppress cartilage matrix synthesis and enhance matrix degradation. Despite these adverse factors, some success has been reported using adult human chondrocytes to repair articular cartilage defects in the joints of patients with early stage osteoarthritis [184], indicating the potential feasibility of cell-based approaches for cartilage injury even in the hostile environment of the chronically-osteoarthritic joint.

Paradoxically, osteoarthritic cartilage may offer particular opportunity for repair, as endogenous stem-cell like progenitor cells with chondrogenic potential have been found in osteoarthritic human cartilage which is not present in normal cartilage [185]. These cells may represent an endogenous repair response in osteoarthritis. Other studies have also identified endogenous chondroprogenitor cells in normal articular cartilage [186-188]. Accordingly, approaches using

Chondrogenic Progenitor Cell
Responses to Cartilage Injury

exogenous cells to augment or stimulate the host's own putative repair cells may be a useful strategy for repair of damaged articular cartilage. Exogenous cells which are themselves chondroprogenitors may offer particular promise, as progenitor cells are more proliferative than overtly differentiated chondrocytes [189,190], and may also be more responsive to signals which direct them towards their final differentiated fate, and/or maintain their function as articular chondrocytes seamlessly integrated into the repaired tissue [191,192]. For example, various types of MSC, introduced directly into the joint through direct injection, have been shown to integrate into damaged cartilage and participate in a repair response in the joints of animals with surgically-induced [193-195] or spontaneous [102] osteoarthritis, confirming that exogenous progenitor cells are capable of *in vivo* chondrogenic differentiation and participation in repair. Interestingly, undifferentiated MSC performed better at healing articular cartilage defects in an animal model than MSC which were pre-differentiated into chondrocytes *in vitro* prior to implantation [196], consistent with the idea that progenitor cells may offer superior utility in cartilage injury compared to fully differentiated chondrocytes. Although MSC are being extensively investigated as a cell source for cartilage repair, a major disadvantage of MSC is cellular heterogeneity. To tap the potential of progenitor cells for treatment of cartilage damage, it will be necessary to develop systems which maximize generation of large numbers of homogeneous chondroprogenitors. We have found that hESC maintained in high density micromass culture in the presence of BMP and TGF-β undergoes progressive differentiation into the chondrogenic lineage [98]. Analysis of molecular markers expressed by the hESC at different times in the *in vitro* progression confirmed that overt chondrogenic differentiation is preceded by a chondro progenitor stage [98]. Such a system may be useful in providing homogenous populations of chondroprogenitor cells at various stages of differentiation into the chondrogenic lineage which can be tested for potentially distinct capabilities for articular cartilage repair.

Conclusions

Exciting potential exists for use of hESC (and iPSC) in providing a source of cells for repair of damaged human articular cartilage in injury or osteoarthritic disease. Protocols are being developed for efficient directed differentiation of hESC into the chondrogenic lineage. The next steps include comprehensive testing of repair efficacy of hESC-derived chondrogenic cells in animal preclinical models, and optimization of repair through use of pro-chondrogenic factors or supportive bioactive scaffolds. Future consideration of hESC- or iPSC-based cartilage repair therapies must also address concerns of safety and potential immunogenicity. A fundamental question to be resolved is the optimal chondrogenic cell stage for repair: will this be the overtly differentiated chondrocyte or a chondroprogenitor cell? The potential for exogenous cells to stimulate endogenous cartilage repair responses must also be considered. Ongoing research to investigate these points will be essential for development of a reliable and effective hESC-based approach for clinical cartilage repair and treatment of osteoarthritis.

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