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Human Pluripotent Stem Cells: Advances in Chondrogenic Differentiation and Articular Cartilage Regeneration

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Abstract Articular chondral lesions are major risk factors for the development of osteoarthritis (OA). Multiple adult cellbased approaches have been attempted to restore hyaline cartilage and prevent progressive degeneration; however, the formation of permanent cartilage has not yet been achieved. A scalable source of cartilage progenitors may have far-reaching potential to advance joint cartilage therapy as well as disease modeling and would be expected to facilitate the discovery of novel therapeutics to stimulate cartilage regeneration or prevent degeneration. Because of their unlimited proliferative capacity and pluripotency, human pluripotent stem cells have become an attractive therapeutic option as a source for consistently uniform cells with high chondrogenic capacity. This review focuses on the recent progress using development-based paradigms to control the differentiation of human pluripotent stem cells to an articular chondrocyte fate. We highlight recent findings that demonstrate the promise for using pluripotent stem cell-based replacement for hyaline cartilage repair.

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Abbreviations

ACAN	Aggrecan
ACI	Autologous chondrocyte implantation
bFGF	Basic fibroblast growth factor
BMP	Bone morphogenetic protein
BMPR1B	Bone morphogenetic protein receptor 1 beta
BMSC	Bone marrow-derived MSCs
ESC	Embryonic stem cells
GDF	Growth and differentiation factor
iPSC	Induced pluripotent stem cells
ISCT	International Society for Cellular Therapy
LIF	Leukemia inhibitory factor
MSCs	Mesenchymal stem cells
OA	Osteoarthritis
PDGF	Platelet-derived growth factor
PSC	Pluripotent stem cells
TGF	Transforming growth factor
VEGF	Vascular endothelial growth factor

Introduction

Osteoarthritis (OA) is a common and debilitating joint disease for which primary risk factors are traumatic joint injury or mechanical disruption of joint tissues. There is currently no cure for OA. Although the true prevalence of articular chondral lesions in the general public is unknown, approximately 60 % of patients undergoing knee arthroscopy have evidence of cartilage lesions [1, 2]. The natural history of articular cartilage lesions is poorly understood, as the means for evaluating these lesions (MRI, arthroscopy) are not commonly used for surveillance due to cost implications. A prevailing notion is that chondral lesions increase in size and predispose patients to developing OA. Supporting this idea is evidence that articular cartilage has limited regenerative

capacity, is poorly vascularized, and has a small cell-to-matrix volume and a very low mitotic rate [3]. Treatment options for OA patients such as total joint arthroplasty provide excellent outcomes by ameliorating pain and improving function. However, many patients sustain cartilage injuries when they are young and are not good candidates for these procedures as they would outlive their implant and would require multiple revision surgeries [2, 4–6]. Currently, the prevalence of patients living in the USA with a total hip or total knee arthroplasty is estimated to be 7.2 million, which is higher than the prevalence of stroke (6.8 million) and heart failure (5.1 million) and approaches that of myocardial infarction (7.6 million) [7]. Thus, OA is an important public health issue, and there remains an urgent and growing need to develop regenerative techniques for articular cartilage to treat symptomatic patients and potentially circumvent the onset of OA among individuals predisposed to developing OA.

Multiple strategies have been attempted for joint cartilage surface restoration, with the goal of improving joint function and delaying or preventing degeneration. However, regeneration of hyaline cartilage has not been achieved. Introduced by Brittberg et al. in 1994, implantation of in vitro expanded autologous human articular chondrocytes (hACs) is a widely used surgical procedure to treat focal chondral lesions in the knee joint [8]. Autologous chondrocyte implantation (ACI) involves extraction of a small cartilage biopsy from a non-load-bearing site within the affected knee joint and in vitro expansion of the isolated chondrocytes using "good manufacturing practice" (GMP) laboratory procedures, which is then followed by implantation of these cells to the defect site in a second surgical procedure [8]. The repair tissue is often fibrocartilaginous, with little hyaline cartilage restoration [9, 10]. Fibrocartilage generally deteriorates over time because of its inferior structural and mechanical properties, which increases the likelihood of OA and the need for further surgical intervention.

Adult mesenchymal stem cells, commonly isolated from bone marrow, have been extensively examined as an alternative to either autologous or allogeneic chondrocytes for regeneration of articular cartilage. These cells offer advantages of ease of harvest using minimally invasive procedures, low immunogenicity, high proliferative indices, and an intrinsic chondrogenic capacity that can be exploited to yield vast quantities of chondroprogenitors to repair cartilage defects [11]. However, chondrogenic differentiation in bone marrow-derived MSCs (BMSCs) follows an endochondral pathway, yielding transient cartilage expressing markers of hypertrophy, mineralization, and catabolic enzymes detrimental to maintenance of permanent cartilage [12–16]. Donor-to-donor variability, as well as age-dependent decline in replicative and differentiation capacity, may also contribute to variable clinical outcomes when using adult mesenchymal stem cell (MSC) for cartilage repair [17, 18]. Over the past decade,

accumulating evidence also supports the presence of a resident stem/progenitor cell population within intact articular cartilage with the potential to respond to injury and to repair small lesions [19–26]. However, the means through which the expansion and differentiation of these cells can be effectively controlled to promote endogenous tissue repair remains unknown.

The Induced Pluripotent Stem Cell Revolution

Embryonic stem cells (ESCs) provide the unprecedented means to study mechanisms of human lineage commitment and cell specification; however, obvious ethical issues and a lack of patient specificity impede their clinical utility. Yamanaka's Nobel prize winning discovery that pluripotency can be induced in virtually any somatic cell through the transient ectopic expression of four reprogramming factors (Oct3/4, Klf4, Sox2, Myc; Nanog and Lin28 can replace c-Myc and Klf4) [27–29] has transformative potential for personalized regenerative medicine and disease modeling. These induced pluripotent stem cells (iPSCs) offer an ethically unencumbered source of patient-specific cells, which display hallmark features of ESCs, including expression of canonical pluripotency markers (OCT4, SSEA-4, NANOG, TRA-1-60, TRA-I-80). iPSCs also provide unlimited self-renewal and the ability to form derivatives of each of the three germ layers. Viral transduction has been the approach traditionally used to introduce the reprogramming factors, though this earlier approach may hinder their clinical use in patients. However, recent advances in reprogramming now offer safe and efficient transgene-free reprogramming techniques using synthetic messenger RNA, microRNA, proteins, and supplementation with small molecule compounds [30–33].

Unlike human primary cells, specialized cell types can be generated from highly renewable sources of iPSCs. Thus, cell reprogramming provides the unique ability to model diverse human diseases and to perform large-scale small molecule and drug screening using cells from panels of human patients representing the spectrum of a particular disease and drug response [34]. For instance, a drug screen using a library of human iPSC-derived cardiomyocytes indicated that healthy and diseased individuals exhibit different susceptibilities to cardiotoxic drugs [35]. Such studies have tremendous value as human disease-specific iPSC-derived cells may provide more accurate prediction of adverse drug responses as compared to standard clinical assays. Moreover, animal models often lack human disease relevance; thus, the use of human iPSCs may better capture the complexity of disease pathophysiology [34]. Applications of iPSCs and ESCs in high-throughput small molecule screening have also led to the identification of novel molecules that promote differentiation to specific lineages, including cardiomyocytes and insulin-

producing pancreatic b cells [36–38], as well as the identification of molecules that inhibit pluripotency [39]. These discoveries are critical for developing effective cell replacement strategies, as well as for the selective removal of undifferentiated cells prior to in vivo transplantation of iPSC-derived specialized cell types. Indeed, a major step toward the realizing the regenerative potential of iPSCs came in 2014 with a pioneering clinical study in Japan, where a patient suffering from age-related macular degeneration received the first iPSC-derived transplant. Progress toward the use of human iPSCs in (i) cartilage disease modeling, (ii) the discovery of new drugs that promote cartilage formation or prevent cartilage degeneration, and (iii) cartilage regenerative therapy is predicated upon the derivation of homogeneous articular-like chondrocytes which exhibit functional and genomic integrity. Knowledge gleaned from the developmental biology of articular cartilage has been applied to establish the molecular signaling and culture conditions to promote differentiation of human ESCs and iPSCs toward the chondrogenic lineage [40•, 41••, 42•, 43–50]. Indeed, human iPSCs may provide an unparalleled source of progenitors to model articular development, since the bona fide cartilage progenitors present only early in development are otherwise inaccessible [40•]. Below, we highlight recent findings that demonstrate the promise for using pluripotent stem cell (PSC)-based replacement for hyaline cartilage repair.

Development-Based Strategies to Recapitulate Chondrogenesis in Human Pluripotent Stem Cells

Chondrogenesis during embryogenesis is a tightly regulated process involving the recruitment and migration of mesenchymal cells, condensation of progenitors to form compact nodules, commitment of the primordial mesenchymal cells to the chondrocyte lineage, and differentiation to proliferating prechondrocytes [51, 52]. Growth factors are critical for regulating the discrete stages of the chondrogenic differentiation program. For instance, fibroblast growth factor (FGF2) signaling plays a key role in the development of mesenchymal progenitors, while BMP4 has been shown to mediate mesoderm specification and instruct the uncommitted progenitors to the chondrogenic lineage [53]. Following commitment of chondrogenesis, the cells undergo differentiation via the endochondral ossification pathway that leads to the formation of bone or are differentiated toward the articular cartilage fate. Based on cell lineage tracking experiments in developmental model systems, articular chondrocytes, as well as other components of the synovial joint, are known to arise from a specialized population of condensed mesenchymal progenitors known as interzone cells [54]. Signaling through the transforming growth factor (TGF) β pathway is required for the initial formation of joint interzone cells and the maintenance of phenotypic

interzone markers such as *Noggin*, *Wnt9a*, and *growth and differentiation factor-5* (*Gdf5*) [55]. Downstream intracellular signaling from BMPs and TGFβ exerts their chondrogenic effects on progenitor cells through distinct gene regulatory pathways, such as regulation of SOX and RUNX family of transcription factors. Articular chondrocytes do not undergo hypertrophy and maturation, as seen in growth plate chondrocytes. It is well established that TGFβ inhibits chondrocyte maturation, whereas BMP signaling exerts positive effects on chondrocyte maturation. Thus, the sequential or concurrent activation of TGFβ and BMP pathways has been used as a strategy to promote chondrogenesis in various adult and pluripotent stem cells.

Several studies have identified the molecular signature of the primitive human mesodermal progenitor populations from human PSCs, and this knowledge has facilitated the development of more efficient strategies to promote chondrogenic differentiation. Earlier studies by Evseenko and colleagues identified the CD326-CD56+ population as the earliest multipotent mesoderm-committed progenitor population that arises from day 3.5 differentiated human ESCs during the process of epithelial-to-mesenchymal transition mediated by recombinant Activin A, BMP4, VEGF, and FGF2 [56]. In vitro functional assays demonstrated the full potential of this population, giving rise to all mesodermal lineages including chondrocytes. Additional studies have revealed that precise stage-specific modulation of multiple signaling pathways downstream of the early mesendoderm population can recapitulate the human developmental chondrogenic program in ESCs and iPSCs [40•, 41••, 42•, 46, 57••, 58]. Oldershaw et al. devised a three-step, chemically defined and serum-free protocol for directed differentiation of human ESCs through a transient primitive streak/mesendoderm stage, followed by controlled differentiation to a multipotent mesoderm, and subsequent differentiation of the mesoderm intermediates to chondrocytic cells within three-dimensional aggregates [42•]. Requiring temporal supplementation of seven different growth factors (Activin A, bFGF, WNT3a, BMP4, NEUROTROPHIN-4 (NT4), FOLLISTATIN, growth and differentiation factor-5 (GDF)), this approach has been used by multiple groups for efficient production of differentiated chondrocytes from independent lines of human embryonic stem cells (hESCs) and iPSCs [40•, 41••, 42•, 46, 57••, 58]. Lee et al. further optimized this approach to achieve improved survival and chondrogenic differentiation of human iPSCs, which showed enrichment of SOX9+CD140+CD44+ chondrocytes expressing phenotypic markers of articular chondrocytes (i.e., *WNT9a*, *SOTDC1*, *COL2A1*, *ACAN*), while lacking expression of markers of hypertrophic chondrocytes (*COL10A1*) and fibrocartilage (*COL1A1*) [58]. Transplantation of these iPSC-derived chondrocytes into immunodeficient mice was found to maintain a stable cartilage phenotype without evidence of tumorigenicity after 4 weeks [58]. A comprehensive transcriptional

profiling of the intermediate populations during differentiation toward chondrocyte maturation provided new mechanistic insights into how the SOX9 regulatory network operates in the early mesodermal and mature chondrocyte fates [58]. Using iPSC lines generated by episomal reprogramming, Yamashita et al. devised a similar but simplified approach based on the initial mesendodermal differentiation in response to WNT3a and Activin A [42•] but followed this with differentiation to the chondrocytic lineage by temporal exposure of suspension cultures to ascorbic acid, BMP2, TGFβ1, and GDF5 [59••]. The use of a chondrocyte-specific COL11a2-eGFP reporter and gene expression analyses confirmed that this approach generated a highly purified population of lubricin/PRG4 expressing articular-like chondrocytes, which lacked the expression of type X collagen [59••].

In 2013, Evessenko and colleagues were the first to characterize the earliest stages of human chondrogenic development from human embryonic distal limbs, and the developing embryonic femoral bone epiphyses, by applying microarray screening and immunophenotypic profiling of laser-captured cells [40•]. Comprehensive transcriptional profiling and functional assays led to the identification of unique combinations of cell surface markers that can identify and differentiate between the earliest prechondrocytes ($CD146^{low/neg}CD166^{low/neg}CD73^+CD44^{low}$), immature periarticular chondrocytes ($BMPR1B^+LIFR^+$), and hypertrophic chondrocytes ($BMPR1B^{neg}LIFR^{neg}$). Based on these results, it was then possible to isolate primitive prechondrocytes and definitive resting chondrocytes from differentiating PSC cultures. Moreover, this study revealed that temporal modulation of LIF, TGFβ, and BMP signaling pathways in iPSC-derived cartilage progenitors could promote the development of immature chondrocytes, while concurrently inhibiting chondrocyte hypertrophy and maturation. Subsequent work by the Keller Laboratory has also provided critical insights into the molecular landmarks and requisite signaling for controlled specification of human ESCs to the articular chondrocyte versus hypertrophic chondrocyte fate [41••]. Sustained TGFβ3 signaling in human ESC-derived mesenchymal progenitors in micromass was needed to specify a population expressing hallmark markers of interzone cells (i.e., *GDF5*, *WNT9A*, *ERG*, *SOSTDC1*, *DCX*) and subsequently of articular chondrocytes (i.e., *PRG4/lubricin*, *cartilage intermediate layer protein 2/CILP2*, *COL2A1*) [41••]. In contrast, treatment with BMP4 promoted a hypertrophic chondrocyte fate, confirmed by high level of expression of hypertrophy genes including alkaline phosphatase (*ALP*), *COL10A1*, and *RUNX2*, as well as increased apoptosis indicative of an endochondral pathway. Moreover, distinct differences in the quality of cartilage formed by the TGFβ- versus BMP4-treated cells were observed following subcutaneous implantation of the two populations in immune-deficient mice. Consistent with their in vitro observations, 12-week grafts from BMP4-treated cells

were found to initiate endochondral ossification, whereas TGFβ3-treated grafts maintained a proteoglycan- and type II collagen-rich ECM with expression of lubricin/PRG4, with no evidence of chondrocyte hypertrophy or calcification. Thus, there is a continued promise that this approach may be translated for generation of transplantable cells to regenerate damaged hyaline cartilage.

Human Pluripotent Stem Cells Are a Source of Multipotent Mesenchymal-Like Progenitors

Methods for specification and prospective isolation of cartilage-committed progenitors from a heterogeneous population of differentiating human PSCs are technically challenging, as they require temporal fine-tuning of multiple signaling pathways. Thus, alternative approaches for generating scalable populations of multipotent cells with high chondrogenic capacity have also been explored. Applying diverse methodologies, multiple groups have now shown that the epithelial-to-mesenchymal transition step in differentiation of human iPSCs and hESCs could be achieved by (a) differentiating with or without an EB intermediate (EB) [43, 44, 60–65], (b) enriching an intermediate hemangioblast population [66, 67], (c) adding a small molecule inhibitor of TGF signaling [68], and (d) co-culturing with mesenchymal cell lines [69]. Collectively, these approaches have demonstrated that the loss of pluripotency and acquisition of the characteristic MSC-like morphology is associated with expression of MSC antigens (positive for CD90, CD73, CD105, CD29, CD44, CD49, CD166 and negative for CD34 and CD45), as well as the capacity for multi-lineage differentiation in vitro and in vivo. Chondrogenic differentiation within 3D cultures of PSC-derived MSCs has been induced via singular and combined treatment with growth factors, known to play important roles in initiating embryonic mesenchymal condensations and cartilage nodule formation (i.e., TGFβ1, TGFβ3, BMP2, BMP4, BMP6, and GDF5) [44, 46, 70–72]. However, few studies have demonstrated controlled differentiation of human PSC-derived MSCs to articular-like chondrocytes for cartilage defect repair [61].

A key question in the field is whether there exists molecular and functional equivalency between human iPSC-derived MSCs and adult MSCs. To gain insight, Diederich et al. performed side-by-side genomic and functional comparisons of adult BMSCs and MSCs generated from human BMSC-iPSCs. The study design used human donor-specific iPSCs generated from BMSCs, as well as multiple methods to derive MSCs from iPSCs to circumvent confounding effects attributed to donor variability or method of MSC derivation. Comparative analyses revealed distinct transcriptomic as well as functional differences between the two sources [73]. iPSC-derived MSCs were generally less responsive to traditional

BMSC chondrogenic differentiation protocols [73]. Although functional outcomes in vivo were not available, the authors speculated that compliance with criteria put forth by the International Society for Cellular Therapy (ISCT) [74] may not be sufficient to confirm derivation of bonafide MSCs from iPSCs. In another comparative in vitro study using combinations of TGFβ3, BMP, and PDGF to drive chondrogenic differentiation, superior formation of hyaline-like cartilage tissue was observed in human ESC-derived paraxial mesoderm-like cells as compared to either adult MSCs or hESC-derived MSC-like cells (the latter lacking mesendoderm identity) [45]. These functional disparities in vitro may point to a need for more sophisticated factor-based regimens to direct lineage-specific hPSC differentiation or the need for alternative methods to enrich the chondrogenic progenitors from hPSCs [45, 73].

Others have postulated that human iPSCs bear an epigenetic advantage over adult stem cells for large-scale generation of chondrocytes that do not exhibit a propensity for hypertrophy. While examining differences in chondrogenesis between BMSC and human induced pluripotent cells (hiPSCs), Ko and colleagues demonstrated greater expression of signature chondrogenic markers (i.e., *SOX9*, *COL2A1*, *ACAN*) and lower levels of hypertrophic markers (i.e., *COL10A1*, *RUNX2*) in chondro-induced iPSCs versus BMSC pellets [64]. Differences in the maturation status were attributed to epigenetic disparities between the two cell sources at the level of DNA methylation. Enhanced methylation of the *COL10A1* gene promoter was also present in human iPSC-derived chondrocytes compared with BMSCs, which corresponded to reduced *COL10A1* gene expression seen in human iPSC-derived chondrocytes [64]. Whether the epigenetic disparities among the cell sources may be used to predict a superior regenerative potential or better quality cartilage in vivo remains to be determined.

Epigenetic Regulation of Chondrogenic Differentiation

Epigenetic mechanisms including DNA methylation, chromatin remodeling, and histone modifications serve a fundamental role in the control of stem cell maintenance and differentiation. The epigenomic landscape in human PSCs, their differentiated progeny, and a broad range of adult cell types including BMSC-derived chondrocytes have been systematically determined, revealing developmental and tissue-specific regulation of lineage-control genes. It is anticipated that targeting the epigenetic machinery may present new avenues to fine-tune chondrocyte cell fate decisions in differentiating PSCS. Histone posttranslational modifications (PTM) play a fundamental role in the control of gene expression by altering chromatin conformation and accessibility of the transcriptional

machinery [75, 76]. Combinations of PTMs such as acetylation (ac) and methylation (me) on lysine (K) residues in histone H3 constitute an "epigenetic code" for transcriptional activation or repression. Genome-wide mapping of the quantified epigenetic changes during chondrogenic differentiation in adult bone marrow-derived MSCs revealed that histone PTMs constitute a primary mechanism for induction of cartilage lineage genes in differentiating mesenchymal progenitors [77•, 78]. Several recent studies have also indicated that targeting of chemically modifiable chromatin modifiers, such as histone lysine demethylases (i.e., KDM4B, JMJD3, PHF2), may provide new strategies to improve chondrogenesis and control maintenance of the chondrocyte phenotype [79–83]. However, the epigenetic regulation of chondrogenesis in human PSCs remains largely unexplored. Thus, with emerging knowledge of the epigenetic landmarks and critical modifiers distinguishing the distinct stages of chondrogenic lineage progression, we may be better poised to limit the risk of tumorigenicity, enhance chondrogenic capacity, and exert more precise control over the specification of articular-like chondrocytes in human pluripotent stem cells.

Pluripotent Stem Cell-Mediated Repair of Articular Cartilage Damage

Scaffold-cell complexes and scaffoldless approaches have been used to assess the potential of human pluripotent stem cells in engineered cartilage formation and cartilage defect repair [41••, 57••, 58, 59••, 61, 64, 84–86, 87•, 88]. Several recent studies demonstrated the efficacy of human ESCs and iPSCs to mediate the repair of cartilage defects in various animal models. Fibrin gel-encapsulated hESC-derived chondroprogenitors generated via a chemically defined and feeder-free culture system promoted the repair of focal defects in rats with hyaline-like cartilage, without evidence of tumor formation [85]. However, hESC-mediated cartilage repair was not equivalent to uninjured cartilage. TGFβ3-mediated chondro-induced iPSCs implanted as pellets or within alginate hydrogel promoted the restoration of the articular cartilage surface 12 weeks following implantation into osteochondral defects in immunosuppressed rats [64]. In other studies, histological analyses also indicated reduced proteoglycan content in the iPSC-derived tissue when compared with adjacent host tissue [64]. Yamashita et al. recently used scaffold-free, stage-dependent chondrocyte particles derived from COL11A2-EGFP iPSCs to evaluate their ability to repair osteochondral defects in nude rats and minipigs [59••]. Cartilaginous particles differentiated from iPSC-derived mesendodermal cells treated with TGFβ, GDF5, and BMP2 were transplanted into defects created in the articular cartilage of SCID rats, without tumor formation or ectopic tissue formation. Immunohistochemical showed strong expression of type II collagen at 12 weeks post-implantation, as well as preservation of hyaline-like cartilage structure, and good

integration between tissue formed by transplanted cells and rat articular cartilage. The hiPSC-derived chondrocytes also showed promise in filling defects in minipigs 4 weeks after implantation, yet demonstration of long-lasting mechanically stable hyaline-like cartilage using this approach will require further studies.

Although hyaline-like cartilage regeneration in vivo has been demonstrated following orthotopic transplantation of pluripotent stem cell-derived chondroprogenitor and chondrocytes, without signs of tumor growth [57••, 59••, 64], the potential carry-over of undifferentiated and undefined cell types remains a valid concern. Saito and colleagues recently reported development of a large immature teratoma 16 weeks following transplantation of iPSC-derived chondrocytes into a full-thickness cartilage defect in the murine knee joint [87•]. This corresponded to an incidence of 6.7 %. These findings emphasize the critical need for refined and standardized methods to purify homogenous populations of human pluripotent stem cell-derived chondrocytes, as well as more thorough characterizations of the transplants over an extended follow-up period, and removal of undifferentiated cells prior to clinical use.

Transdifferentiation of adult cells has been explored as an alternative approach to generate large quantities of chondrogenic progenitors that do not carry risk of teratoma formation [89•]. It is currently thought that transdifferentiation, or lineage conversion, can occur without transition through a pluripotent state. This possibility would provide an additional level of safety; however, this process has been called into question by several recent studies [90, 91]. Outani et al. determined that retroviral delivery of two reprogramming factors (c-MYC, KLF4) and the master cartilage regulatory SOX9 was sufficient for lineage reprogramming of human dermal fibroblasts into proliferative induced chondrogenic cells [89•]. Following transplantation of induced chondrogenic cells into the subcutaneous spaces of nude mice, stable homogenous hyaline-like cartilage tissue formation was observed at 3 months, without evidence of tumors [89•]. Moreover, the induced chondrogenic cells formed cartilaginous tissue when implanted into articular cartilage defects in SCID rats and did not respond to osteogenic conditions. To date, only viral methods have been used to introduce the necessary reprogramming factors for conversion of fibroblasts to chondrocytes. Further studies are necessary to evaluate whether induced chondrogenic cells can be generated through the use of non-viral, integration-free methods and provide long-term in vivo hyaline cartilage repair.

There has been significant growth in the field of articular cartilage regeneration through the use of animal models of cartilage defects. However, some critical limitations remain. A recent review article by Sakata et al. provides an excellent summary of animal models of articular cartilage defects and some key perspectives regarding the challenges in interpreting these models [92]. Currently, there is no standardized outcome assessment protocol for true articular cartilage regeneration. A brief survey of the literature demonstrates that there are a

plurality of morphometric stains, immunohistochemical approaches, gene expression analysis targets, and histologic grading scales utilized in the assessment of cartilage regeneration [93–96]. Unfortunately, there is significant variation in the specific outcome measures that are assessed for any particular study. With such heterogeneity, direct comparisons of the quality of repair tissue amongst multiple studies are highly challenging. Clearly, in some scenarios, a unique assessment may be needed to test a certain hypothesis. However, there is a critical need in the field for a basic consensus protocol, indicating which specific tests are absolutely required, for the accurate assessment of cartilage regeneration and the direct comparison with previous work.

Conclusions and Future Perspectives

Continued research on the instructive signaling cues and epigenetic mechanisms that control chondrogenic lineage commitment and articular chondrocyte fate specification will prove essential for advancing our fundamental knowledge of cartilage development as well as regenerative applications of the future. Many different strategies employing varying combinations of growth factors, progenitor-like populations, and culture conditions are currently being investigated for the formation of hyaline cartilage from human embryonic and induced pluripotent stem cells. The "optimal" strategy for producing articular-like chondrocytes that maintain functional and genomic integrity in vivo and contribute to articular cartilage defect repair remains elusive and will require more comprehensive studies in vitro and in vivo. Despite the significant progress made toward to the use of safer viral-free and non-integrating reprogramming methods, concerns about the genome integrity of human iPSC-differentiated progeny and their safety still persist. Thus, there remains a critical need to apply more rigorous characterization of the differentiated cells as well as the removal of contaminating undifferentiated and undefined cells prior to in vivo transplantations. The idea that iPSCs may be used as an "off-the-shelf" product for cartilage repair will also require consideration of the time, effort, and costly resources to fully reprogram patient-derived cells, perform critical quality control analyses, and generate the desired cell type in high purity and sufficient quantity. Lineage reprogramming into cartilage-specific progenitor cells may provide a more efficient and reproducible approach and warrants further investigations.

Currently, there are no preclinical or clinical studies examining the potential for human iPSCs to repair cartilage. Indeed, several laboratories have begun to establish human iPSCs as platforms to model OA and to identify new targets for treating OA. Perhaps, the most imminent translational application of human iPSCs comes as a scalable source of patient- and disease-specific cells for small molecule and drug screening

platforms to identify novel disease-modifying drugs [97]. Cartilage progenitors and articular chondrocytes derived from panels of patient-specific iPSCs may provide new opportunities for the discovery of molecules that stimulate chondrogenesis or drugs that prevent cartilage degeneration.

Compliance with Ethical Standards

Conflict of Interest Rosa M. Guzzo and Michael B. O'Sullivan declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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