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Concise Review

Challenges Facing the Translation of Embryonic Stem Cell Therapy for the Treatment of Cartilage Lesions

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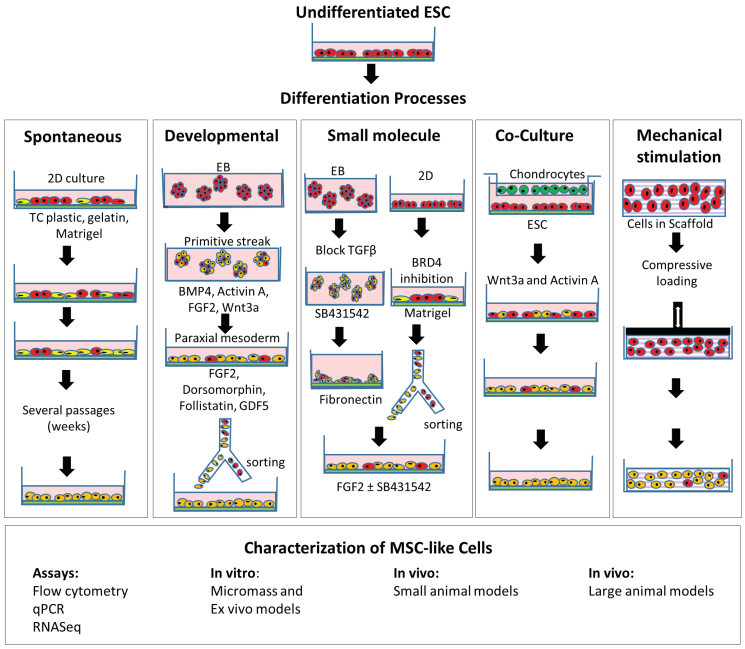
Abstract

Osteoarthritis is a common disease resulting in significant disability without approved disease-modifying treatment (other than total joint replacement). Stem cell-based therapy is being actively explored for the repair of cartilage lesions in the treatment and prevention of osteoarthritis. Embryonic stem cells are a very attractive source as they address many of the limitations inherent in autologous stem cells, such as variability in function and limited expansion. Over the past 20 years, there has been widespread interest in differentiating ESC into mesenchymal stem cells and chondroprogenitors with successful in vitro, ex vivo, and early animal studies. However, to date, none have progressed to clinical trials. In this review, we compare and contrast the various approaches to differentiating ESC; and discuss the benefits and drawbacks of each approach. Approaches relying on spontaneous differentiation are simpler but not as efficient as more targeted approaches. Methods replicating developmental biology are more efficient and reproducible but involve many steps in a complicated process. The small-molecule approach, arguably, combines the advantages of the above two methods because of the relative efficiency, reproducibility, and simplicity. To better understand the reasons for lack of progression to clinical applications, we explore technical, scientific, clinical, and regulatory challenges that remain to be overcome to achieve success in clinical applications.

Graphical Abstract



Overview of methods used to derive MSC-like cells. Spontaneous differentiation protocols involving long-term culture for emergence and selection of an MSC phenotype; **Developmental** differentiation emulates the progression of embryonic development of mesoderm and can be combined with cell selection via flow activated cell sorting (FACS); **Small molecules** target temporal blocking of ALK-5 signaling; **Co-culture** of ESC with chondrocytes leverages paracrine signaling; and **Mechanical stimulation** activates differentiation pathways. Evidence of differentiation is typically screened using flow cytometry, gene expression profiling, and histologic analysis of 3D cultures. Differentiated cell lines are initially screened in small animal models. For clinical application, proof of concept and safety studies need to be conducted in large animal models. EB = Embryoid bodies.

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Significance Statement

Over the past 10 years, there has been widespread interest in differentiating embryonic stem cells into mesenchymal cells. We review the various approaches to differentiating embryonic stem cells and compare and contrast the benefits and drawbacks of each approach. We also list technical, scientific, clinical, and regulatory challenges that remain to be overcome to achieve success in clinical applications. Osteoarthritis is a common disease resulting in significant disability and no approved disease-modifying treatment. This review will benefit readers interested in cell therapy for osteoarthritis, the present status of embryonic stem cell therapy, and the reasons for lack of progression to clinical applications

Cell-Based Repair of Cartilage: The Need for a Suitable Cell Source

Current clinical approaches to address cartilage defects can be classified into repair, replacement, or regeneration. The present standard of care is to induce repair via microfracture of the subchondral bone which encourages marrow cell infiltration into the defect resulting in the formation of fibrocartilage.[1] Replacement involves autologous or allogeneic grafts of healthy cartilage or osteochondral tissue, usually in defects larger than those that can be treated with microfracture.[2-4] Regeneration is achieved by harvesting, expanding, and subsequent implantation of chondrocytes in suspension (autologous chondrocyte implantation (ACI))[5,6] or chondrocytes cultured on a scaffold system (matrix-induced ACI).[7] Other cell sources explored include bone marrow aspirate concentrate (BMAC) and allogeneic cells such as umbilical cord MSCs (CartiStem).[8,9] Several challenges remain for current cartilage treatment methods, most notably the lack of consistent hyaline cartilage formation and the relatively high re-operation rates for young patients (reviewed in ref. [10]).

The clinical efficacy of cell-based cartilage repair is reliant on an effective cell source that is yet to be universally accepted.[1,11] Several autologous cell sources with chondrogenic potential have their respective advantages and drawbacks. Terminally differentiated cells such as chondrocytes are attractive due to their inherent phenotype; however, limited cell expansion, loss of phenotype after multiple passages,[12,13] harvest site morbidity,[14,15] and requirements for enzymatic isolation[13] tend to limit their clinical potential (Table 1).

Adult-derived mesenchymal stem cells (MSC) are an alternate source of chondroprogenitors. MSC have cartilage-forming capacity and can be derived from bone marrow, adipose, synovial membrane, or other tissues with less invasive techniques.[16-18] Conversely, site harvest morbidity can be an issue, there are limitations on extensive cell expansion before senescence and reduction in cartilage forming capacity,[19,20] and adult MSC tend to undergo hypertrophic differentiation.[21] Adult MSC are the most extensively studied stem cells in regenerative medicine with relatively few ethical, immunogenic, and teratogenic risks but with limitations on their ability to form cartilage tissue following extended culture.[11,22] The clinical applications of adult MSC are therefore limited to using early passage cells.

Alternative cell sources are embryonic stem cells (ESC) and induced pluripotent stem cells (iPSCs), which have been used to generate MSC-like cells with cartilage tissue forming capacity (Tables 1-6). Embryonic stem cell-derived mesenchymal stem cells (ES-MSC) are a potentially universal source for cell-based cartilage regeneration, overcoming the inherent variability and limited availability of cells in autologous applications. Furthermore, these ES-MSC possess immunosuppressive and enhanced proliferative capacity in comparison to adult MSC.[23]

There are obvious ethical, regulatory, and clinical concerns associated with the use of ESC for cartilage tissue regeneration.[24] The major issues arise from the generation of these

Table 1. Therapeutic approaches for cartilage lesions.

	ESC	MACI	De Novo	MSC	CartiStem
Cell source	Embryonic	Arthroscopic biopsy of healthy cartilage	Juvenile allograft tissues	Bone marrow, adipose, synovial tissues	Umbilical cord blood
Harvest site morbidity	None	Arthroscopic biopsy	None	Needle aspiration; liposuction	None
Proliferation capacity	Very high	Limited	N/A	Limited to early passages	Very high
Chondrogenic differentiation potential	Good	Good	Not needed	Good	Good
Status in translation to clinical use	Preclinical	FDA approved	FDA approved	Permitted by FDA for certain applications	FDA approved
Potential for immune response	Yes	No	Yes	No	Yes
Commercialization/Potential for off-the-shelf therapy	Yes	No	Yes	No	Yes
Ethical considerations	Destruction of embryos	None	None	None	None
Regulatory challenges	High	FDA approved	FDA approved	Only permitted for minimally manipulated cells	FDA approved
Safety and potential risks in addition to surgical implantation	Risk of teratoma formation	Small surgical risk of biopsy	Minimal	Small surgical risk of needle aspiration	Minimal

cells under conditions involving exposure to animal cells and products, undesirable differentiation, local or systemic biodistribution, or teratoma formation. ESC lines that have been developed using xeno-free processes may be more promising for clinical use. In this review, we focus on methods used to derive ES-MSC cell lines with cartilage forming potential, assess the strengths and weaknesses of these methods, and list the existing challenges for translating ES-MSC to a clinical therapy for cartilage repair.

Methods to Derive MSC-Like Cells from ESC

Several approaches, with varying degrees of complexity, have been described to differentiate embryonic stem cells (ESC) to an MSC-like or a chondroprogenitor phenotype for cartilage repair. The simplest approaches leverage the spontaneous induction of differentiation in ESC. While these methods may be easier to implement, the mechanisms are not well understood or have not been fully characterized. More elaborate methods simulate the key developmental signaling events, involve co-culturing with chondrocytes, applying mechanical stimulation, or enriching selective populations via cell sorting. An overview of the various approaches is provided in Fig. 1. Details of cell sources and differentiation factors are listed in Tables 2-6.

Spontaneous Differentiation

ESC in 2D culture spontaneously differentiate. We derived MSC-like cells by culturing mechanically dissected ESC colonies on standard tissue culture plastic.[25] MSC-markers (CD73, CD90, CD44, and CD105) began to appear by passage 3; and by passage 9, the percentage of MSC-like cells had increased to 95%. These ES-MSC cells underwent chondrogenic differentiation in high density cultures and repaired articular defects in ex vivo human cartilage explants. Others have reported success with enhancing spontaneous differentiation by selective pressure favoring fast growing cells with the ability to attach and proliferate on gelatin, vitronectin, Matrigel, or Geltrex coatings.[26-29] Culture on cell feeder layers such as murine bone marrow stromal cells (OP9) or irradiated mouse embryonic fibroblasts can also induce differentiation into ES-MSC.[30,31]

Developmental Approaches

The developmental approach involves a more directed recapitulation of the steps in embryonic development, namely the formation of primitive streak and paraxial mesoderm. Primitive streak differentiation is usually achieved by exposing ESC to several molecules such as Activin A, BMP2, BMP4, FGF2, and Wnt3a.[32-38] Differentiation toward a paraxial mesoderm fate is induced by adding bFGF and follistatin, inhibiting BMP receptors with noggin or small-molecule dorsomorphin (DM), or inhibiting activin receptor-like kinase (ALK).[32,33,35-39] The resulting cells are expanded into fibroblastic cells with an MSC-like phenotype in monolayer on standard tissue culture plastic or on substrates such as gelatin and fibronectin. Although more complicated, these developmental approaches appear to be robust, reproducible, well characterized, and feasible in inducing a mesenchymal phenotype and providing a source of chondroprogenitor cells. Chondrogenesis has been documented via expression of chondrogenic genes, and histologic analysis of 3D cultures in vitro or in vivo after subcutaneous implantation into immune deficient mice, or osteochondral defects in mice or rats.

Fluorescence-activated (FACS) and magnetic-activated cell sorting (MACS) have also been implemented to select mesoderm and pre-chondrogenic populations after mesoderm and mesenchymal induction.[34,40,41] Petrigliano et al.[41] used MACS to purify hESC-derived mesodermal cells and exclude epithelial (CD326+) and cardiovascular mesodermal (CD309+) cells. Cartilage regenerative capacity was shown by implanting ESC-derived chondrospheres or cell-seeded porcine collagen I/III membranes into full thickness chondral lesions in minipigs. After 6 months, the resultant repair tissue contained proteoglycans and collagen type II, evidence of stratification with a superficial region and good integration with the surrounding host tissue. In general, sorting with FACS and MACS results in a greater percentage of MSC-like cells (>95%), although the net cell population can be small (15-25% of the starting ESC).

Developmental approaches have been widely studied, are especially informative for understanding ESC differentiation, and have led to more targeted methods to generate chondroprogenitors.

Small Molecules

Small molecules can accelerate the derivation of chondroprogenitors, bypassing some of the steps in developmental approaches. Studies have used the ALK-inhibitor SB431542 (SB) to specifically drive ESCs to a mesoderm fate and derive MSC-like cells with cartilage forming capacity.[42-44] FGF and TGFβ pathways are essential to maintain ESCs in a pluripotent state.[45-47] To induce ESC differentiation, FGF is replaced with SB to inhibit SMAD 2/3 signaling by binding to the ATP-binding domains of activin receptor-like kinase (ALK) receptors 4, 5, and 7, while increasing the SMAD 1/5/8 signaling pathway.[48] BMP4 expression increases after SB treatment, which is required for mesodermal fate.[49]

Inactivating ALK receptors also reduces NANOG promoter activity and leads to a loss of OCT4, SOX2, and NANOG expression (typically needed to maintain ESC in an undifferentiated state).[44,45,50] The collective influence of SB treatment on differentiation pathways therefore induces a mesoderm fate.

The main differences among studies reporting the use of SB are duration of exposure and differentiation of ESC as embryoid bodies (EBs) or single cells in monolayer culture. Although all these methods led to emergence of MSC-like cell phenotypes, the efficiency of generating MSC and the requirement for EBs versus monolayer expansion must be weighed against the level of evidence for chondrogenesis. Mahmood et al. exposed EBs to SB before seeding on fibronectin[42]; Chen et al. did not require any specific coating of culture plates[44]; and Sanchez et al. avoided using EBs but required cell sorting.[43] Differences in the reporting of mesenchymal cell markers, however, make it challenging to compare the purity of MSC populations among these methods.

Other small molecules that have been used to produce chondroprogenitors are a glycogen synthase kinase 3 inhibitor (CHIR99021), a retinoid acid receptor agonist (TTNPB), and a combination of bromodomain-containing protein 4 (BRD4) inhibitors (LLY-507 and AZD5153)[51,52] (Table 4).

Co-culture

Co-culturing with chondrocytes can also direct differentiation in pluripotent cells.[53-56] Co-culture with primary human or bovine chondrocytes in culture well inserts induced chondrogenic differentiation in pluripotent cells.[55-57] ESC or iPSC co-cultured with irradiated human chondrocytes in

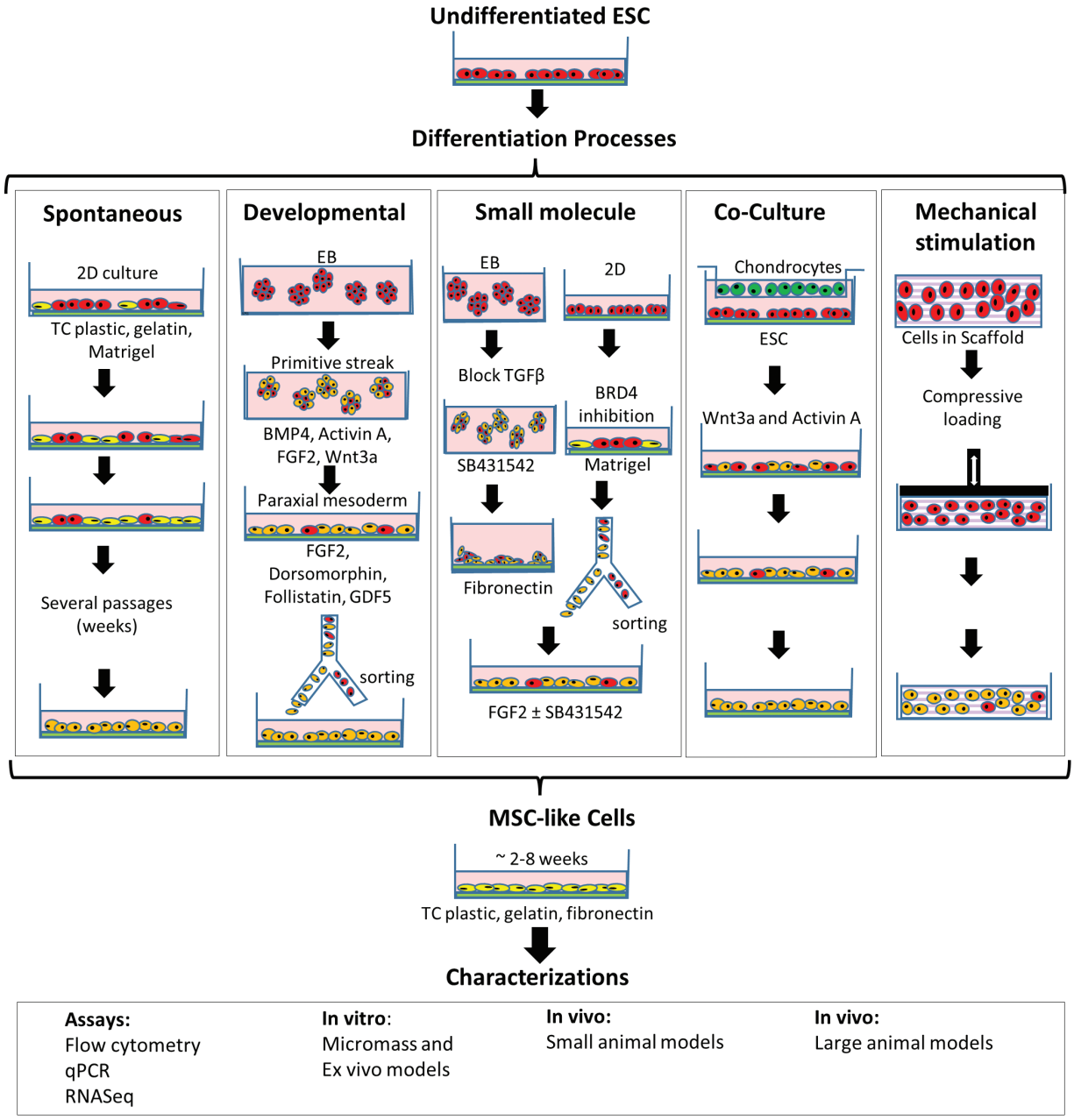

Figure 1. Overview of methods used to derive MSC-like cells. Spontaneous differentiation protocols involve long-term culture for emergence and selection of an MSC phenotype; developmental differentiation emulates the progression of embryonic development of mesoderm and can be combined with cell selection via flow activated cell sorting (FACS); Small-molecules target temporal blocking of ALK-5 signaling; coculture of ESC with chondrocytes leverages paracrine signaling; and mechanical stimulation activates differentiation pathways. Evidence of differentiation is typically screened using flow cytometry, gene expression profiling, and histologic analysis of 3D cultures. Differentiated cell lines are initially screened in small animal models. For clinical application, proof of concept and safety studies need to be conducted in large animal models. EB = embryoid bodies.

high-density pellet culture or encapsulated in cellulose and alginate have also exhibited successful chondrogenic commitment resulting in matrix deposition and morphology typical of cartilage.[54] Xenogenic cell sources have also been explored for chondrogenic coculture, although unlikely to be suitable for clinical translation (Table 5).

Mechanical Stress

Mechanical stimulation has major effects on cell proliferation and differentiation and has been used to produce chondroprogenitors. Twenty-four hours of static compression applied to murine ESC in a PDMS scaffold upregulated chondrogenic genes COL2A1, SOX9, and ACAN and downregulated hypertrophic chondrogenic markers RUNX2, COL10A1, and MMP13, and the pluripotent marker OCT4.[58] Mechanical stimulation and selective control of matrix elasticity are therefore emerging areas of interest in the production of chondroprogenitors and may provide additional selectivity for the desired phenotype.

Assessing Chondrogenesis

In vitro, ex vivo, and in vivo models are commonly used to assess chondrogenesis and cartilage regeneration. In vitro

Table 2. Spontaneous approach.

Cells used	Substrate	References
H9	Standard TC Culture	Olee et al.[25]
SA167, SA002.5, SA461	Gelatin	de Peppo et al.[80]
SA001, SA002, SA002.5, AS034, AS034.1.1, SA121, SA167, SA348, SA461, SA611.	Gelatin	Karlsson et al.[81]
H9	Gelatin	Nakagawa et al.[26]
HUES9	Gelatin	Hwang et al.[82]
Umbilical cord-derived iPSC	Gelatin	Rim et al.[83]
Adipose & Fibroblast-derived iPSC	Matrigel	Nejadnik et al.[27]
Fibroblast-derived iPSC	MEFs	Guzzo and Drissi[84]
H1, WA1	Matrigel	Lee et al.[30]
H1, H7, H9	Matrigel	Trivedi and Hematti[85]
H9	MEFs	Gibson et al.[31]
Nasal mucosa MSC-derived iPSC	Geltrex	Jakob et al.[28]
QCTS-hESC-2	Vitronectin	Xing et al.[29]

Table 3. Developmental approach.

Cells used	Factors used	References
HUES1, HUES7, and HUES8	*Primitive stream/ Mesendoderm:* WNT3A, Act-A, BMP4, FGF2, *Mesoderm:* FGF2, BMP4, NT4	Oldershaw et al.[36]
H1, H9, and HES3	*Mesoderm:* BMP4, VEGF, and bFGF, ActA. *FACS:* CD326- CD56+ and CD73+ CD105+ CD34– cells	Evseenko et al.[34]
H1, UCLA3, HIPS23	*Mesoderm:* bFGF, ActA, Noggin and Wnt3a. *MACS:* CD166+CD146+PDGFR-a+KDRneg	Wu et al[40]
ESI-017	*Mesoderm:* bFGF, Wnt3a, ActA, Noggin *MACS:* CD326+ and CD309+ depletion	Petrigliano et al[41]
HUES1, MAN7, human fibroblast-derived iPSC	See: Oldershaw et al.[36]	Cheng et al.[32] (modification of Oldershaw et al.[36])
HUES1, MAN7	Substitution of BMP2	Wang et al.[37] (modification of Oldershaw et al.[36])
409B2, 604B1, HDF-11, KF4009-1 iPSCs	Adjusted Oldershaw & Umeda protocols	Yamashita et al.[76]
H9	*Primitive Streak:* BMP4	Faial et al.[49]
HES2, H7	*Primitive Streak:* ActA, BMP4, FGF *Mesoderm:* DM, FGF,	Craft et al.[38]
hiPSC (ATCC, BJFF, and STAN)	*Primitive Streak:* Act, CHIR99021, FGF2 *Mesoderm:* SB505124, CHIR99021 FGF2, DM *Chondroprogenitor:* SB505124, WntC59, PD173074, DM, Purmorphamine, BMP4	Wu et al.[39]
H1	*Mesoderm (Days 1-3):* FGF2, ActA, Wnt3a *(Day 3-7):* Add Noggin, remove ActA *Chondrogenic (Day 11-14):*FGF2, SHH, BMP4 *(Day 14+):* IGF2, FGF2, LIF, TGFb1, BMP4	Ferguson et al.[35]

models typically consist of high-density pellet cultures, cell-seeded scaffolds, or cell encapsulation in hydrogels under chondrogenic conditions. Ex vivo explants can additionally assess integration of neotissue with host tissue which is one of the major factors limiting clinical success.[25] Establishing chondrogenic potential in human explants also overcomes a potential weakness of implanting human cells in animal models. Chondrogenesis has been assessed in vivo by implantation of pellet cultures,[31,55] or cell-seeded hydrogels,[32,37] into osteochondral defects[32,37] or subcutaneous tissue.[38,42]

Validation in large animal models represents a major gap in the translation of ES-MSC for cartilage regeneration. One notable exception, Petrigliano et al.,[41] implanted ESC derived chondrocytes into full thickness cartilage defects in Yucatan minipigs with encouraging results. Such validation of cartilage regeneration and healing in large mammals such as porcine, ovine, and caprine will be necessary before translation to clinical trials.

While the assessment of chondrogenesis is widely described, the differences in models, measurement techniques, and analysis make it difficult to compare efficacy among different studies. There is therefore a critical need for standardization of chondrogenic methods for clinical translation. Finally, we lack validation of these models and outcome measures. Why do cells that generate apparently compelling histology in vitro fail to do so in vivo? In all likelihood, the majority of negative animal results are not reported.

Translation of ESC-Derived Chondroprogenitors to the Clinic

Clinical translation of any cell therapy involves definitive demonstration of safety and efficacy in preclinical models, followed by clinical trials in a phased approach. This process involves strict compliance with the rules and regulations as required by the corresponding government agencies. In the US, for example, the Food and Drug Administration (FDA) specifies adherence to current Good Manufacturing Practices (cGMP) for all cell-lines intended for use in clinical trials. Meeting the criteria for the final release of cell-based products requires extensive testing to ensure sterility, identity, purity, potency, and genetic stability (Reviewed in[59]; ISSCR Guidelines 2016). Even stricter criteria are necessary for ESC-derived cell therapy due to the additional safety concerns of teratoma formation, undesired differentiation, proliferation out of the implantation site, local and systemic biodistribution, and off-target effects.[60]

Preclinical Requirements for ESC-Derived Cell Therapy

The FDA has published general guidelines for clinical use of cartilage repair products that are valuable to design

Table 4. Small-molecule approach.

Cells used	Small molecules	References
HUES9	SB431542	Mahmood et al[42]
H9, AND1, AND2, SHEF1; iPSC (iPS-CB-CD34#2, iAND-4, iMSUH001)	SB431542	Sánchez et al[43]
Mel1, HES3; iPSC (MR90CL2 and ES4CL1)	SB431542	Chen et al[44]
H9, Mel1	SB431542, BIO, Noggin	Umeda et al.[86]
hiPSC (7F3955, PB001, PB004)	CHIR99021, TTNPB	Kawata et al.[51]
H1, hiPSC	LLY-507 and AZD5153	Zhang et al.[52]

Table 5. Coculture approach.

Cells used	Coculture cell type	References
H1	Chondrocytes	Vats et al[56]
BG02	Chondrocytes	Hwang et al[55]
hESC HS306, hiPSC (UEFhfiPSC1.4)	Chondrocytes	Qu et al[87]
SA167, AS034, AS034.1	Irradiated chondrocytes	Bigdeli et al.[54]
OA chondrocyte-derived hiPSC	Chondrocytes	Wei et al[57]
H1, H9	OP9	Barberi et al[53]
H1, H9, H13	OP9	Vodyanik et al[88]
R1 (murine), FVB/N (murine)	Limb bud progenitor cells (murine)	Sui et al[89]
ES-D3 GL (murine)	Hepatic cells HEPA-1C1c7 (murine)	Lee et al[90]
A2B iPSC	Irradiated chondrocytes	Nguyen et al[91]

Table 6. Mechanical stressor approach.

Cells used	Applied stress	Reference
7AC5	24-h static compression (0.05 MPa)	McKee et al[58]

ESC-based cell therapy for clinical trials (https://www.fda.gov/media/82562/download). Appropriate and convincing preclinical data supporting safety and efficacy of the proposed therapy are the primary criteria. Additional considerations must be made during experimental design to improve the translation of results into a new drug application (NDA). Cartilage repair in animal models should show proof of biological response, durability, toxicology, dose response in a cartilage lesion of clinically relevant size and location, and with appropriate endpoints that will inform subsequent clinical trials. These criteria are most applicable in a large animal model in which the size, depth, and location of the cartilage lesion can be analogous to human patients and long-term durability (at least 1 year) can be convincingly documented. The animal model should reflect these considerations and strive to replicate the clinical features of human patients in clinical trials.

While the FDA guidelines are broadly applicable to all cartilage repair products, ESC-based cell therapy bears an additional level of burden, particularly in terms of risk for teratoma formation, off-target effects, and allogeneic response.[60] These safety studies must be carefully designed and executed as the clinical risks have yet to be fully established.

Derivation and Culture of ESC

A major concern related to derivation of earlier ESC lines was exposure to animal-derived materials, such as mouse embryonal fibroblasts (MEF) or fetal bovine serum (FBS) during culture,[61-63] or during cryopreservation.[64] To avoid this issue, human feeder cells have been used, including fetal muscle cells, fetal fibroblasts, adult fallopian tubal epithelial cells, and dermal fibroblasts.[65,66] To reduce additional contamination from animal-based products, serum- and xeno-free reagents are available for cell expansion and cryopreservation.[67,68] Matrigel, although widely used as a substrate for ESC culture and differentiation, is not suitable for clinical use because it is derived from mouse sarcoma cells.

As alternatives to Matrigel, several cell-free and xeno-free substrates have become available. These include extracellular glycoproteins such as vitronectin, laminin, fibronectin, and a number of synthetic substrates such as APMAAm, PMVE-alt-MA, PMEDSAH, and PAM_6*co*-PSS_2.[69,70]

To mitigate concerns of contamination with animal products, ESC lines have been successfully derived, cultured, and preserved in xeno-free conditions.[66,71-74] The generation of more clinically relevant cell lines has largely focused on key areas of ESC derivation, expansion, and storage that were traditionally dependent on animal products: 1) the separation of the inner cell mass (ICM) from the trophectoderm and zona pellicuda, 2) establishment of human feeder sources, 3) ESC expansion protocols, and 4) cryopreservation methods and reagents. Bypassing the use of animal products during ICM harvest has been accomplished by simple mechanical dissection,[74] chemical dissection of the zona pellicuda using acid solutions,[72] or laser drilling.[66,71] Traditional MEF layers have effectively been replaced with human feeder cells such as human foreskin and placental stromal fibroblasts, and fetal and umbilical cord tissue.[66,72,74] Various xeno-free media and reagents are now commercially available for hESC culture on human feeder cells, most being supplemented with human serum albumin and basic fibroblast growth factor (bFGF). GMP-grade human serum albumin[66] and many FDA-approved xeno-free reagents are available for slow cooling and vitrification, such as Cryostor10 used for HAD-C 100, 102, and 106 cell lines.[66]

Clinical Trials

The majority of hESC clinical trials to date have been conducted with earlier non-xeno free cell lines (H1, MA09, and I6).[75] More recently, completely xeno-free hESC have been used for Phase I/II clinical trials such as the Hadassah

hESC cell lines (HAD-C 100, 102, 106) for retinal degeneration and amyotrophic lateral sclerosis ([73]; clinicaltrials.gov). Clinical trials specific to ES-MSC are limited. A trial of hES-MSC for meniscus injury has been initiated as well as trials for interstitial cystitis and intrauterine adhesions (clinicaltrials.gov). To our knowledge, no clinical trials have been initiated using hESC-derived cells for cartilage tissue regeneration. Xeno-free clinical grade hESC may eliminate previous concerns associated with the use of animal products and expedite the use of hESC in clinical trials for cartilage regeneration.

Compared to the speed of development from discovery to animal studies, the last stage of clinical translation remains relatively sluggish. The major obstacles are technical challenges in cell manufacturing, regulatory hurdles in establishing safety, lack of translation to preclinical models, and lack of validation of preclinical models. Despite several reports of apparent success in animals, these potential therapies have not progressed to clinical trials. This lack of progress emphasizes the need to revisit the true value of these models. There is an unmet need for a validated translational pipeline that progresses predictably from in vitro, ex vivo, and small animal proof of concept to definitive preclinical studies in large animals.

Conclusions and Future Perspectives

Despite the potential advantages and numerous reports of ESC for chondrogenesis, no treatment has yet been approved for clinical studies. Ethical concerns regarding the source and derivation of embryonic cells have not been fully resolved. Technical issues with maintaining stemness during culture and storage, and safety risks such as teratogenesis and immune rejection require validation. A variety of approaches can differentiate ESC into MSC with potential for cartilage tissue formation and repair in vitro, ex vivo, and in animal models. To satisfy the requirements for scalable manufacturing, a clinical cell therapy candidate needs to be relatively simple, efficient, robust, and reproducible. Methods relying on spontaneous differentiation are simpler but not as efficient as more targeted approaches. Methods replicating developmental biology are more efficient and reproducible, but involve many steps in a complicated process. The small-molecule approach, arguably, combines the advantages of the above two methods because of the relative efficiency, reproducibility, and simplicity. Another important development in this field is the derivation of xeno-free cell lines and application of xeno-free conditions that are critical for clinical translation.

Cell sources need to be tested in appropriate animal models for safety, efficacy, and clinical relevance. Several small animal models have demonstrated in vivo evidence of cartilage regeneration using hESC.[31,32,37,55,56,76,77] However, animal studies have not progressed to clinical trials, suggesting a need to revisit the true value of these models. It is essential to establish a validated translational pipeline that progresses reliably from in vitro, ex vivo, and small animal proof of concept to definitive preclinical studies in large animals. One disadvantage is that the response of human cells in xenogenic models is not always predictable. Another disadvantage of traditional animal models is the surgical creation of artificial cartilage lesions in young healthy animals. One needs to carefully assess the translational value of these models in appropriately predicting safety and efficacy in clinical trials. The veterinary field contains reports of clinical applications of cell therapy in animals.[78,79] Animal models with clinical disease therefore may become increasingly valuable in assessing preclinical safety and efficacy to inform human clinical trials.

Author Contributions

S.G.: conceptualization, formal analysis, investigation, methodology, project administration, supervision, validation, writing. D.D.: conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing. J.K.: data curation, investigation, writing.

Conflict of Interest

The authors declared no potential conflicts of interest.

Data Availability

No new data were generated or analyzed in support of this research.

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