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Isolation, Characterization, and Differentiation of Stem Cells for Cartilage Regeneration

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Abstract

The goal of tissue engineering is to create a functional replacement for tissues damaged by injury or disease. In many cases, impaired tissues cannot provide viable cells, leading to the investigation of stem cells as a possible alternative. Cartilage, in particular, may benefit from the use of stem cells since the tissue has low cellularity and cannot effectively repair itself. To address this need, researchers are investigating the chondrogenic capabilities of several multipotent stem cell sources, including adult and extra-embryonic mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs). Comparative studies indicate that each cell type has advantages and disadvantages, and while direct comparisons are difficult to make, published data suggest some sources may be more promising for cartilage regeneration than others. In this review, we identify current approaches for isolating and chondrogenically differentiating MSCs from bone marrow, fat, synovium, muscle, and peripheral blood, as well as cells from extra-embryonic tissues, ESCs, and iPSCs. Additionally, we assess chondrogenic induction with growth factors, identifying standard cocktails used for each stem cell type. Cell-only (pellet) and scaffold-based studies are also included, as is a discussion of *in vivo* results.

Keywords

regenerative medicine; cell-based therapies; tissue engineering; chondrogenesis

Introduction

Stem cells are promising cell sources for many tissue engineering applications, including cartilage regeneration. Embryonic stem cells (ESCs), adult and extra-embryonic mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs) have all been investigated as potential cell sources for cartilage applications (Fig. 1). The motivation for exploring the regenerative potential of these cells results from the lack of effective therapies currently available for patients suffering from joint diseases. One example is osteoarthritis, a pathology characterized by the degradation of hyaline cartilage.⁷⁹ Aside from total joint replacement, possible forms of treatment include microfracture, which allows for subchondral MSCs to populate the defect, and autologous chondrocyte implantation, which is a treatment using *ex-vivo* expanded chondrocytes and, potentially, stem cells. Unfortunately, both procedures can result in the formation of fibrocartilage, a mechanically inferior tissue to healthy hyaline cartilage. Tissue engineering approaches using primary chondrocytes are non-ideal since undamaged cartilage has to be destroyed to obtain the cells, and *in vitro* expansion is necessary to achieve sufficient cell numbers. This process

also takes precious weeks, results in dedifferentiation, and raises the risk of contamination. Stem cells have become an attractive therapeutic alternative due to their relative abundance and multipotent capabilities, specifically their ability to undergo chondrogenesis.¹⁴⁸ An ideal stem cell source has yet to be identified, as each has strengths and weaknesses. Studies have characterized these populations extensively, highlighting large variations in the different cell types, such as ease of isolation, differentiation potential, and surface marker expressions. Additional research has led to progress within all stem cell fields to optimize growth factor cocktails and delivery systems, although to varying degrees of success.

To induce stem cell chondrogenesis, many *in vitro* strategies have been explored, including mechanical stimulation, the use of scaffolds or growth factors, or a combination of these techniques.¹¹⁰ The most frequently used method of induction is treatment with chondrogenic medium in a pellet culture system.²⁷ Induction medium typically consists of insulin, transferrin, and selenous acid (ITS), dexamethasone, ascorbic acid, and sodium pyruvate, in addition to growth factors.^{90, 148} Many growth factors have been considered for chondrogenic differentiation, as reviewed by Danisovic et al.³⁰ The most well-characterized and implemented growth factors are part of the transforming growth factor-beta (TGF- β) superfamily, including TGF- β 1, 2 and 3, as well as bone morphogenic proteins (BMPs). This review includes the reported optimal growth factors for chondrogenesis, identifying specific cocktails for each stem cell type. Following differentiation, chondrogenesis is confirmed by the presence of extracellular matrix, specifically type II collagen, proteoglycans, and glycosaminoglycans (GAGs), as reviewed by Vater et al.¹⁵⁵ Many methods are used to assess these components, the most common of which are stains specific to proteoglycans, such as toluidine blue, and stains that bind to GAGs or sulfated GAGs, such as alcian blue and safranin-O. An additional assay commonly used to measure GAG synthesis is 1,9-dimethyl methylene blue (DMMB), which can be used to provide quantitative data via spectrophotometry. We will use these reports of matrix synthesis to evaluate the relative effectiveness of stem cell type and culture environment for inducing the chondrocytic phenotype.

This review also seeks to highlight the differences inherent among human stem cell populations currently being investigated for cartilage applications, though for areas with limited human studies we will report results from animal models. Isolation procedures and surface marker expressions will be summarized for each cell type, as they are stem cell-specific. In addition, due to the extensive use of *in vitro* differentiation by means of pellet culture and 3D scaffolds, evaluation of studies successfully inducing chondrogenesis by these methods will be included. It has also been shown that *in vitro* chondrogenic ability is not always indicative of function *in vivo*, due to loss of the chondrocytic phenotype upon implantation.¹¹⁴ As such, recent *in vivo* studies will be reviewed to stress the feasibility and variability of stem cell use. While previous articles have assessed stem cells for cartilage tissue engineering, these typically focus on only the most common sources. Consideration of many stem cell types is necessary to evaluate the progress and potential of these populations. A compilation of our findings has been included in Table 1.

Adult Stem Cell Sources

Adult stem cells are promising cell sources for cartilage repair due to their multipotency, lack of tumorigenicity, ease of isolation, and applicability to autologous transplantation procedures, which removes the risk of rejection associated with allogeneic sources.⁶² Unfortunately, these cell types do have disadvantages.¹¹¹ When compared with alternative sources, such as extra embryonic stem cells or embryonic and embryonic-like stem cells, adult stem cells have limited self-renewal capacities. Additionally, as a person ages, these cells exhibit decreased proliferation rates and lessened differentiation potential and, in some

cases, have similar regenerative characteristics as those associated with diseased patients.^{64, 111}

Bone Marrow-Derived Stem Cells

Though their existence was discovered much earlier by Friedenstein et al., bone marrow-derived mesenchymal stem cells (BMSCs) were first characterized by Pittenger et al. in the late 1990s and remain the most commonly used stem cell for cartilage tissue engineering.^{9, 17, 46, 118} The cells were identified by positive expression of STRO-1, CD105, CD44, CD71, CD90, CD106, CD120a, and CD124, and negative expression of hematopoietic markers, though later studies concluded negative expression of endothelial markers as well (Table 2).^{4, 118} Additionally, cells possessed the ability to adhere to tissue culture plastic, as well as expand *in vitro*, and differentiate along mesenchymal lineages.¹⁰ To obtain cells for assessment, Pittenger and colleagues employed a density centrifugation gradient, yielding approximately 0.001-0.01% of cells from the initial population. Low cell yield is still a persistent obstacle within the field, since it necessitates *in vitro* expansion to obtain sufficient cell numbers for therapies, a process that results in decreased differentiation potential.²⁴ Due to this setback, alternative methods have been pursued in an attempt to enhance cell yield efficiency and purification of cell populations.^{24, 106} Some approaches include frequent medium changes, plastic adherence, cell sorting by surface markers, as well as exposure of cells to cytotoxic reagents during expansion.^{24, 50, 56, 106} Many physical characteristics have also been used to identify BMSCs, including cell size and mechanical properties.^{31, 96, 146, 172} To date, however, BMSC isolation from marrow, regularly harvested from the iliac crest, is still typically conducted by density gradient centrifugation.¹⁰⁵ Subsequently, to further purify the population, cells are plated on tissue culture plastic where non-adhered cells are later washed away.

The use of chondrogenic induction factors has been heavily investigated *in vitro*, as reviewed by Puetzer et al., leading to continuous modification of cocktails and culture environments.¹¹⁹ One of the earliest studies attempting to optimize chondrogenic differentiation compared BMSC pellets exposed to TGF- β 1, β 2 or β 3.¹³ While chondrogenesis was induced under all conditions, after 21 days, it was discovered that TGF- β 2 and β 3 promoted the greatest GAG production, about twice that of pellets treated with TGF- β 1. To enhance differentiation with TGF- β 3, it was found that particular BMPs, most effectively BMP-2, could be incorporated.¹³³ Other uses for growth factors have also been considered, such as delaying impaired potency, a prominent issue after long-term expansion.¹³⁸ Sochaga et al. expanded BMSCs with fibroblast growth factor-2 (FGF-2) supplemented media and found that not only did this increase proliferation rates, but it also successfully delayed the loss of chondrogenesis. Furthermore, Cooke et al. investigated the ability of co-culturing BMSCs with juvenile chondrocytes to enhance differentiation in pellet culture, without additional growth factors.²⁸ A two-fold increase in GAG levels was observed in comparison to pellets containing only BMSCs. This was a greater degree of induction than BMSC pellets exposed to TGF- β 1.

Scaffolds of various materials have successfully induced chondrogenic differentiation of seeded BMSCs *in vitro*. Many biomaterials have been used to date, including collagen, a material known for its biocompatibility and ability to degrade.¹²¹ Ng et al. investigated differences between type I collagen and type II collagen scaffolds.¹¹¹ After treatment of porcine BMSC-seeded constructs with TGF- β 1, type I collagen scaffolds were superior to type II collagen in terms of GAGs per DNA production, 122.6 vs. 100.7 μ g GAGs/ μ g DNA. Other groups have combined scaffold materials to test for enhanced potency. Zhou et al. incorporated hydroxyapatite (HA) into their collagen scaffold to better mimic osteochondral tissue.¹⁷⁶ After 4 weeks of treatment with TGF- β 3, scaffolds with HA exhibited a two-fold increase of GAG production in comparison with those solely comprising of collagen.

Additionally, encapsulation of cells by TGF- β 3-incorporated fibrin hydrogels can also be used, as shown by Park et al.¹¹⁴ GAG and relative type II collagen production revealed successful chondrogenic differentiation, 6 and 4.5 $\mu\text{g}/\mu\text{g}$ DNA, respectively. Other materials include, but are not limited to, chitosan, alginate and polyglycolic acid (PGA).^{135, 159, 165}

In the field of BMSC cartilage tissue engineering, there have been many encouraging studies using novel approaches that indicate these cells can treat cartilage pathologies *in vivo*. One study using a leporine (rabbit) model analyzed the use of BMSCs, among other stem cell types, to treat osteochondral defects.⁹² Scaffolds were comprised of demineralized bone matrix integrated with TGF- β 1. After 12 weeks, BMSC-seeded scaffolds were well-integrated into the surrounding tissue and exhibited robust expression of type II collagen. An alternative study by Igarashi et al., conducted without growth factors, treated osteochondral defects in a canine model.⁶¹ Their approach consisted of an *in situ* forming gel based on ultra-purified alginate containing autologous BMSCs. After 16 weeks of recovery, analysis of the treated area was reported as being smooth, firm, and glossy-white. Mechanical testing of the regenerated cartilage determined structural integrity was still inferior to that of native cartilage (9.2 vs. 12.2 MPa). Advances like these are what have enabled BMSC research in cartilage tissue engineering to reach clinical trials, where many are currently in progress.¹

Adipose-Derived Stem Cells

Adipose-derived stem cells (ASCs) were first pursued by Zuk et al. in 2001 as an alternative to BMSCs in an attempt to overcome their limitations.¹⁷⁸ In contrast to bone marrow, not only is adipose tissue readily available, but it can be harvested using minimally invasive surgical procedures such as liposuction, and yields significantly more cells overall. Initial studies found that while ASCs exhibit the functional characteristics of stem cells, like multipotency and extended proliferation, they express some surface marker dissimilarities when compared to BMSCs.^{13,37} Unlike BMSCs, ASCs show positive expression of CD49d and negative expression of CD106, although there is considerable overlap of other markers. To obtain these cells, protocols from the 1960's, originally designed for fat pads, are used and have since been modified for subcutaneous lipoaspirate, the standard source of ASCs.^{16, 71} However, more recently, ASCs from alternative locations have been isolated using similar procedures, concluding that variations in differentiation abilities exist among ASCs derived from epididymis, perinephrium, and subcutaneous adipose tissue.⁷¹ Following extraction of fat tissue, the process of ASC isolation involves enzymatic digestion with type I collagenase, followed by subsequent centrifugation and washing steps.¹⁶ The isolated cells generate a heterogeneous population including ASCs, fibroblasts, adipocytes, smooth muscle cells, and endothelial cells.¹⁶⁹ The negative effects of this cell contamination, including reduced proliferation and differentiation potential, continue to limit the use of ASCs for therapeutic applications.¹²² Seeking to reduce these effects, protocols that aid in purification have been proposed, such as additional washing steps, density centrifugation, and sorting by surface markers.^{5, 49, 122}

Many *in vitro* studies have been conducted to investigate the chondrogenic ability of ASCs. As such, cocktails have been refined to promote optimal differentiation. After reviewing current literature, there are few studies comparing cocktails directly, though many confirming that they have been used successfully, as reviewed by Puetzer et al.¹¹⁹ Commonly used growth factors are TGF- β 1, BMP-6, or a combined growth factor cocktail of TGF- β 3 and BMP-6.⁴¹ Additionally, TGF- β 2 in conjunction with BMP-7 has proven to be a potent inducer of chondrogenesis.⁷⁷ Kim et al. assessed chondrogenic induction after treatment with numerous members of the TGF- β superfamily, including BMP-2, 6, 7 and TGF- β 2. Individually, GAG amounts were at least twice those of control pellets for all factors. Combining BMP-2 and TGF- β 2 resulted in a three-fold increase in GAG production

when compared with controls, similar to BMSC pellets. Additionally, as seen with BMSCs, FGF-2 can be administered at low concentrations during expansion of ASCs to enhance proliferation rates and induce chondrogenesis.²² However, chondrogenic induction can be hindered by FGF-2 when combined with BMP-6 and TGF- β 1.⁵⁴ It has also been shown that chondrocytes can enhance chondrogenesis of ASCs by means of soluble factors, or direct interaction through co-culture.⁸⁸ Lee et al. assessed differentiation of ASCs in co-culture with chondrocytes, after exposure to chondrocytic conditioned medium, or exposure to a cocktail of TGF- β 2 and BMP-7. The co-culture condition resulted in a 37% increase in GAG production as compared with controls, while ASC pellets exposed to conditioned medium resulted in a 25% increase. However, the growth factor condition induced the greatest degree of chondrogenesis, increasing GAG levels by 50% over controls.

Many materials have been employed as scaffolds to obtain chondrogenic differentiation of ASCs. While early groups studied the ability of agarose, and gelatin to function as a scaffold material for ASC chondrogenesis, modification of these materials has since occurred.¹⁰ Nieto-Aguilar et al. combined fibrin and agarose to produce a novel hydrogel for such applications. Seeded scaffolds were cultured in chondrogenic induction medium supplemented with TGF- β 1 for extended periods. Though early assessment confirmed no differentiation had occurred, after 20 days, 98.7% and 86.7% of cells were positive for expression of GAGs and type II collagen, respectively. Recently, mechanically stimulated chitosan/gelatin scaffolds induced potent chondrogenesis of insulin-like growth factor-1 (IGF-1) induced ASCs.⁹¹ Collagen and proteoglycan levels were increased roughly three-fold from controls when assessing scaffolds not exposed to mechanical stimulation. An even greater increase, four-fold, was seen when comparing mechanically stimulated scaffolds with controls. Poly(lactic-co-glycolic acid) (PLGA) has also been used as a scaffold material.¹¹⁶ Park et al. recently observed chondrogenic differentiation of leporine ASCs in PLGA scaffolds incorporating dexamethasone and TGF- β 1. Though incorporation of TGF- β 1 yielded higher GAGs per DNA production than PLGA scaffolds without growth factors (0.15 vs. 0.25 μ g GAGs/ μ g DNA), seeded scaffolds incorporating only dexamethasone produced the most GAGs per DNA ($\sim$ 0.275 μ g GAGs/ μ g DNA).

In addition to cartilage assessment *in vitro*, past studies have demonstrated that ASCs are capable of producing cartilage *in vivo* using numerous approaches. Jung et al. examined the influence of fibrin glue on the *in vivo* chondrogenesis of ASCs when implanted subcutaneously in a nude murine model.⁷⁰ Prior to implantation, the stem cells were differentiated *in vitro* by media supplemented with TGF- β 1. Post-operation, constructs were qualitatively analyzed at 12 weeks, with the resulting tissue appearing to be structurally sound and glossy. Mehlhorn et al. assessed chondrogenesis of ASCs seeded on PLGA scaffolds.¹⁰¹ Differentiation was induced *in vitro* by means of TGF- β 1 supplemented medium after cells had migrated into the polymer matrix. Analysis of implants after 8 weeks revealed the presence of type II collagen and proteoglycans in scaffolds, suggesting successful differentiation and maintained chondrogenic phenotype. TGF- β 1 was also investigated during an *in vivo* study to assess treatment of leporine defects with various MSC sources.⁹² The growth factor was incorporated into demineralized bone matrix scaffolds, and subsequently seeded with MSCs. While ASC-treated defects were superior to controls, defects treated with BMSCs were observed to have greater surface architecture and integration.

Synovial Membrane-Derived Stem Cells

De Bari et al. first characterized synovial membrane-derived mesenchymal stem cells (SDSCs) in 2001 by examining their multipotency, proliferation rates, and surface marker expressions.³² Many commonalities with BMSCs were found, though later studies determined SDSCs to express CD49d, whereas BMSCs do not.^{32, 102} After collagenase

digestion and subsequent expansion, which is the isolation procedure predominantly used today, analysis by De Bari et al. revealed many advantageous characteristics of these cells for tissue engineering applications.³² SDSCs possessed considerable proliferative abilities, as seen by maintained growth rates over 30 population doublings, and multi-lineage differentiation capabilities. Since this early study, many groups have determined and confirmed superior chondrogenic and proliferative abilities compared to other MSC types, further supporting the importance of SDSC research.^{42, 128} Like with many adult stem cell types, isolating SDSCs yields a heterogeneous population, of which fibroblasts and macrophages are persistent contaminants.^{43, 69} To overcome this concern, many studies have investigated enrichment through surface marker expression. For this approach, cells are either sorted for negative expression of CD14 shortly after cell isolation or are expanded prior to sorting to exclude macrophages.⁴³

In terms of *in vitro* differentiation, beginning with De Bari et al., TGF- β 1 was shown to enhance chondrogenesis, as evidenced by GAG production.³² Later, *in vitro* differentiation was optimized after treatment with several cytokines, including TGF- β 3 and BMP-2.¹³⁷ Induction with TGF- β 3, alone, resulted in pellets averaging 0.8 mm in diameter, larger than controls averaging roughly 0.5 mm in diameter. With the addition of BMP-2, average pellet diameter strikingly increased to roughly 1.7 mm. Significant GAG and type II collagen production was also noted. Alternatively, BMP-2 has been combined with TGF- β 1 to induce differentiation of SDSCs. To discern the influence of passage on chondrogenesis, Han et al. differentiated cells from P0-P8, determining the maximum potential at P1, yielding roughly 37 μ g GAGs/ μ g DNA.⁵¹ Additionally, chondrogenic differentiation of porcine-derived cells by treatment with TGF- β 1 has been reported.¹⁴

Few recent studies have investigated the chondrogenic differentiation of SDSC-seeded scaffolds. Qi et al. recently determined chondrogenesis of CD105+ murine SDSCs integrated into chitosan-alginate scaffolds.¹²⁰ Following a two-week culture period in medium containing TGF- β 3 and BMP-2, expression of type II collagen was observed, as well as GAG production, averaging 5.5 μ g GAGs/ μ g DNA. SDSCs have also been seeded in polyethylene glycol diacrylate-based hydrogels and phosphoester-polyethylene glycol-based hydrogels.⁴² While both successfully induced chondrogenic differentiation, the latter proved to be superior, as shown by type II collagen and proteoglycan production.

In vivo studies have further confirmed the chondrogenic abilities of these cells. Koga et al. treated cartilage defects in a leporine model after suspending SDSCs in a collagen gel.⁸¹ Defects exhibited profuse matrix production after 4 weeks, increasing after 12 weeks. This repair was similar to BMSC-treated defects. Li et al. investigated SDSC treatment of defects in a leporine model, as well, with demineralized bone matrix-seeded scaffolds, incorporating TGF- β 1.⁹² The results from this group were not as successful as Koga et al., as BMSC-treated defects were superior in integration as well as cartilage production. Studies have also been conducted specifically to enhance integration of SDSCs in leporine knee osteochondral defects.¹³⁶ It was found that not only did cells adhere faster with the addition of magnesium, but cartilage production of SDSCs was expedited, resulting in greater matrix production.

Muscle-Derived Stem Cells

The discovery of muscle-derived stem cells (MDSCs) in mammals, isolated using a freeze/thaw procedure, occurred in the mid-1990s.¹¹⁷ Though this study confirmed potency of these cells, it was not until years later that their surface markers were extensively characterized.⁶⁷ Seeking to expedite the isolation procedure, Young et al. determined that MDSCs express CD10, CD13, CD56, and Sca-1, while lacking CD45, similar to BMSCs.¹⁷¹ As reviewed by Wu et al., additional surface marker expressions include CD34, desmin, MyoD, CD144, and c-Kit, though papers have also contradicted this positive expression.¹⁶³

Additional markers, distinct from BMSCs, have yet to be determined. Because of their unique surface marker expression within muscle-derived populations, fluorescence-activated cell sorting (FACS) is occasionally used to select for MDSCs, though can be unreliable due to overlapping expression with non-MDSCs.²⁰ However, the most common isolation method is the modified preplate technique. Preplating attempts to isolate MDSC populations based on their adherence rates to tissue culture plastic or collagen-coated flasks, whereby stem cells are the slowly adhering fraction, obtained 4-5 days post-harvest.⁴⁷ Cells are initially released from biopsies by means of enzymatic digestion.¹⁰³ A recent study aiming to purify MDSC populations further discovered that Percoll density gradient centrifugation should also be considered as a viable option.²⁰

In vitro chondrogenesis of MDSCs has been accomplished in a few studies, generally incorporating BMP-4. Kuroda et al. isolated cells from murine muscle biopsies and transduced them with BMP-4.⁸⁵ Colonies were then formed and subsequently differentiated with and without the addition of TGF- β 1. In control media, cultures of BMP-4-transduced cells had more type II collagen-producing colonies than those of non-transduced cells. Additionally, exposure to TGF- β 1 did not enhance this effect. Similar to the Kuroda et al., another group transduced murine MDSCs with BMP-4, and then co-pelleted these cells with either vascular endothelial growth factor (VEGF)-blocked or VEGF-producing MDSCs and subsequently treated with TGF- β 3.⁸⁴ It was found that pelleting with VEGF-blocked MDSCs enhanced differentiation two-fold when compared to pelleting with VEGF-producing cells or pellets consisting solely of unmodified MDSCs. Recently, Ye et al. isolated murine MDSCs for chondrogenic assessment.¹⁶⁶ Following induction of chondrogenesis with TGF- β 1 for 2 weeks, a 25-fold increase in type II collagen gene expression was observed over controls. Alternatively, Lu et al. confirmed that TGF- β 1 induced chondrogenesis in human MDSCs.⁹³

Few groups have recently explored scaffolds for chondrogenic differentiation of MDSCs. Alginate beads have been used successfully, while treating MDSCs with TGF- β 1.⁷ Following 21 days, staining with alcian blue and assaying for aggrecan revealed presence of chondrogenic markers. A study by Nawata et al. incorporated BMP-2 into type I collagen scaffolds.¹⁰⁹ Toluidine blue staining and type II collagen expression confirmed chondrogenesis.

Many of the previously described MDSC studies investigated the chondrogenic potential of MDSCs *in vivo*, in addition to *in vitro*. Kuroda et al. determined the ability of differentiated MDSCs to treat osteochondral defects in a murine model when mixed with fibrin glue.⁸⁵ After 24 weeks, the cells were well-integrated into the surrounding tissue, and regenerated cartilage looked white, glossy, and smooth. Similar repair was seen by Kubo et al. after treating osteochondral defects with VEGF-blocked cells, in addition to fibrin-embedded BMP-4-transduced cells.⁸⁴ In a gender study by Matsumoto et al., cells derived from both males and females were transduced with BMP-4 and mixed with fibrin glue before treating osteochondral defects in nude rats. Unlike defects treated with male MDSCs, those treated with female-derived MDSCs did not heal effectively.⁹⁸ A more recent study by Li et al. compared *in vivo* chondrogenesis of cells derived from various source tissues.⁹² Leporine knee defects were treated with demineralized bone matrix scaffolds seeded with undifferentiated MSCs. BMSCs proved to be superior to MDSCs on many levels including integration and surface architecture.

Peripheral Blood Stem Cells

The field of peripheral blood mesenchymal stem cells (PBMSCs), an idea initially proposed in 2000 by Zvaifler et al., is relatively new, as their existence is still being investigated due to contradicting reports in early studies.^{58, 127, 179} These non-hematopoietic cells isolated

from peripheral blood have been determined to lack STRO-1, unique from BMSCs, and can be isolated from blood samples using Ficoll or Percoll gradients and subsequent plating to further isolate by means of plastic adherence.^{52, 53, 58, 151} While this method successfully isolates PBMSCs, it results in extremely low yields (0.0002% of initial population), thus, groups have made many modifications to address this issue.⁹⁴ FACS has been explored to isolate cells based on expression of various surface markers, specifically positive expression of CD133 or negative expression of CD14.^{58, 151} Fibrin microbeads have also been used successfully, due to their ability to bind to matrix-dependent cells.⁷³ In this study, treating patients with granulocyte colony-stimulating factor increased cell yield dramatically, although yield was still only 0.5% of the initial population.

Chondrogenesis of these cells has been achieved *in vitro* using a variety of methods, many similar to those used for BMSCs, though without the incorporation of scaffolds. It was determined that PBMSCs in 2-D culture induced along the chondrogenic lineage by TGF- β 3 express type II collagen precursors, though contradicting results of this finding exist.^{25, 123} Chong et al. also analyzed the effect of TGF- β 3 on the differentiation of pelleted human PBMSCs.²⁶ Average GAG concentrations of PBMSCs pellets were found to be similar to those of BMSCs, roughly 3 and 3.25 μ g GAGs/ μ g DNA, respectively. Unfortunately, both of these levels were still lower than those of chondrocyte pellets, 4 μ g GAGs/ μ g DNA. Tondreau et al. induced chondrogenesis of PBMSCs by treatment with TGF- β 1. Following 2-3 weeks of culture, the presence of GAGs indicated successful differentiation. Though few groups have assessed the function of these cells *in vivo*, an encouraging study was recently conducted by Saw et al.¹³⁰ Patients with chondral defects first underwent subchondral drilling. PBMSCs were harvested one week after the procedure, and then re-injected intraarticularly with hyaluronic acid once a week for 5 weeks. Long-term, post-operative assessment concluded regeneration of hyaline cartilage at the treatment site. Patients reported minimal adverse side effects apart from discomfort.

Extra-Embryonic Stem Cell Sources

Cells from extra-embryonic sources have recently been proposed for the purposes of regenerative medicine. These cells are isolated from tissue discarded after birth and are characterized as being in a more developmentally primitive state⁶². This translates into higher proliferation rates and, potentially, increased multi-lineage differentiation capabilities. Unfortunately, extra embryonic stem cells must be cryogenically stored prior to autologous use, which can be associated with high costs and potentially result in deleterious effects.

Wharton's Jelly Stem Cells

Though isolation of cells was first achieved in 1991, characterization of MSCs derived from Wharton's jelly, the mucoid connective tissue surrounding the umbilical vein, was first conducted by Wang et al. in 2004.^{99, 157} These cells isolated from Wharton's jelly, also termed umbilical cord matrix, were determined to express surface markers similar to BMSCs, though a more recent study reported lack of CD18 expression (Table 3).^{44, 86} In their initial study, Wang and colleagues investigated not only surface marker expression, but also the differentiation potential of Wharton's jelly stem cells (WJSCs) along the chondrogenic lineage.¹⁵⁷ Pelleted cells were exposed to medium supplemented with TGF- β 1 for 3 weeks and then assessed by immunohistochemical analysis. Staining illustrated rich deposits of type II collagen in both control and experimental pellets. Alternatively, Baksh et al. analyzed the differentiation of TGF- β 3 treated pellets.¹² Compared with controls, GAG levels of WJSC pellets were 4 times as high. Additional studies have been conducted incorporating the use of scaffolds. Fong et al. fabricated polycaprolactone (PCL)/type I

collagen nanofibrous scaffolds prior to seeding WJSCs and observed significant production of cartilage matrix molecules.⁴⁵

Though these studies suggest the therapeutic potential of WJSCs for hyaline cartilage applications, more promising results were observed for successful fibrocartilage fabrication, indicated by type I collagen production.^{11, 158} An early study by Bailey et al. explored the potential of PGA scaffolds seeded with WJSCs to generate temporomandibular joint condylar cartilage.¹¹ Four weeks after seeding scaffolds with TGF- β 1-differentiated cells, quantification of GAG content revealed higher levels in WJSC scaffolds than those seeded with chondrocytes, approximately 3 μ g and 1 μ g, respectively. Additionally, WJSC scaffolds exhibited a significant amount of type I collagen, more than was seen in chondrocyte pellets, whereas type II collagen production was nominal in both. These results inspired further investigation by the same group to determine optimal seeding density.¹⁵⁸ Concentrations higher than 25 million cells/mL successfully sustained GAG production while simultaneously enhancing the mechanical integrity of the construct.

Umbilical Cord Blood Stem Cells

Confirmed homing capacity of MSCs and circulatory properties of hematopoietic stem cells inspired Erices et al. to investigate whether umbilical cord blood could also serve as a stem cell source, though the presence of mesenchymal-like cells had previously been reported.^{40, 167} Expression of common mesenchymal stem cell surface markers was seen, and again confirmed in a study directly comparing those of BMSCs and umbilical cord blood mesenchymal stem cells, but expression of CD49d has been observed in umbilical cord blood stem cells.^{89, 100, 156} Further assessment of surface markers by Chang et al. determined the existence of two MSC subpopulations within umbilical cord blood, one being positive for expression of CD90 and the other being negative.¹⁹ Similar chondrogenic and osteogenic abilities were observed between populations. Conversely, adipogenic differentiation potential was greater in the population expressing CD90. While these studies have successfully obtained MSCs from umbilical cord blood for analysis, low or even undetectable levels of MSCs in cord blood continue to hinder their clinical use.^{40, 64, 174} Research within the field has continued, however, due to the availability of the tissue and their superior proliferative capacity.⁷⁴ *In vitro* chondrogenesis has been investigated and achieved using various growth cocktails. In the presence of TGF- β 1, pelleted umbilical cord blood stem cells are capable of producing twice as much type II collagen as control pellets, similar to ASCs, though BMSCs under the same conditions produce three times as much as their controls.¹²⁴ Differentiation with TGF- β 3 and BMP-2 was also analyzed by Zhang et al.¹⁷⁴ The pellet mass obtained from three different donors was quantified following differentiation with TGF- β 3 and BMP-2, TGF- β 3 alone, and BMP-2 alone. It was noted consistently for all donors that BMP-2 promoted the largest pellets. However, this also led to production of hypertrophic chondrocytes. Collagen scaffolds were also seeded with differentiated umbilical cord blood stem cells and then implanted subcutaneously in a murine model. Post-operative assessment three weeks later confirmed matrix section, as well as type II collagen production. The constructs were visually white, stiff, and glistening, features resembling cartilage. Most recently, De Mara et al. compared the chondrogenic induction efficiencies of BMP-2 and BMP-6 on umbilical cord blood stem cells.³⁴ Type II collagen expression was superior in cell monolayers exposed to BMP-2, as shown by western blotting. No comments on manifestation of hypertrophic chondrocytes were made.

Amniotic Fluid Stem Cells

The earliest report of amniotic fluid-derived stem cells (AFSCs) is by In 't Anker in 2003, though amniotic fluid cells expressing OCT-4 were publicized just prior and were further characterized by De Coppi et al. a few years later.^{33, 65} Cells were isolated from second

trimester fluid after centrifugation and subsequent culturing. Flow cytometry revealed positive expression of MSC markers, however, positive expression of CD117 and negative expression of CD10 were also determined, unique from BMSCs.^{8, 33, 112, 153} The ability of AFSCs to undergo chondrogenesis was studied in depth by Kolambkar et al. in 2007 by comparing the effects of TGF- β 1, TGF- β 3, and BMP-2.⁸² The combination of TGF- β 3 and BMP-2, and TGF- β 3 alone, produced the lowest increase in GAG production, 1.9- and 2.2-fold greater than controls, whereas TGF- β 1 alone produced a 2.7-fold increase. Staining for type II collagen was substantial in pellets exposed to TGF- β 1 or β 3. However, minimal staining was found after treatment with the combination of TGF- β 3 and BMP-2. Tsai et al. also differentiated AFSCs using TGF- β 3 and confirmed proteoglycan production after 3 weeks.¹⁵² Because AFSC research is still preliminary, aside from the few studies investigating *in vitro* chondrogenesis, cartilage repair applications have not been investigated thoroughly.¹⁶² An *in vivo* study has been conducted by Park et al., encapsulating cells with TGF- β 3-containing fibrin gels.¹¹³ While *in vitro* analysis revealed substantial GAG levels, on par with BMSCs, post-implantation assessment determined GAG production of BMSCs to be superior to AFSCs *in vivo*, roughly 7 and 4 μ g GAGs/ μ g DNA.

Placenta-Derived Mesenchymal Stem Cells

In 2003, Zhang et al. first identified MSCs in term placenta.¹⁷⁵ Soon after, further characterization was performed by In 't Anker et al., when they investigated the specific membranes of the placenta, as the amnion and chorion are of fetal origin, and the decidua portions are of maternal origin.⁶⁴ Cells were isolated simply by mincing the membranes and then filtering the resulting suspension. Their surface markers have been characterized to include many BMSC markers, with the exception of negative expression of STRO-1 and SSEA-4.¹³⁴ Since that time, though many encouraging studies have been conducted in the field of placenta-derived mesenchymal stem cells (PMSCs), very few targeted cartilage applications.⁵⁷ Groups have studied the amnion and the chorion, determining characteristic differences between the two sources, as well as their ability to differentiate. Portman-Lanz et al. determined that chondrogenesis of chorion-derived cells with TGF- β 1 was superior to amnion-derived cells. Conversely, Cavallo et al. discovered that with the addition of 10% serum, chorion-derived cells do not undergo chondrogenesis. However, type II collagen gene expression of amnion-derived cells was determined to increase by roughly 15-fold.¹⁸ Alternatively, amnion-derived cells have been differentiated successfully after exposure to TGF- β 3, as seen by type II collagen production.⁶

Embryonic and Embryonic-Like Stem Cell Sources

Embryonic and embryonic-like stem cells, such as induced pluripotent stem cells (iPSCs), have been considered for regenerative medicine applications due to their pluripotency and capacity to continuously self-renew.¹¹¹ Use of ESCs requires immunosuppressants, but iPSCs can be transformed from patient-specific cells into embryonic-like cells such that autologous transplantation is possible.³⁹ However, tumorigenicity is an unwavering concern for both cell types, in addition to transmission of zoonotic factors introduced during the expansion process, since murine feeder layers are essential for maintaining proliferation rates and pluripotency.^{39, 125, 148} The therapeutic versatility of ESCs and iPSCs may outweigh these concerns in the long run for tissues that cannot be repaired using other cell types.

Embryonic Stem Cells

The field of human ESC research stems from their discovery by Thomson et al., who successfully isolated lines of human ESCs and determined their pluripotent characteristics.¹⁴⁴ Characteristically, ESCs express SSEA3, SSEA4, TRA-1-60, TRA-1-8,

OCT4, SOX2 and NANOG as reviewed by Draper et al. and De Miguel et al. (Table 4).^{35, 38} Steps to isolate these cells from an *in vitro* fertilization-produced embryo include culturing until it reaches the blastocyst stage, whereby cell masses can then be isolated.¹⁴⁴ Currently, mechanical isolation, by which the blastocyst is plated and trophectoderm cells are subsequently removed, is used to obtain these cells without the use of zoonotic components, a feature of other techniques such as immunosurgery that involve use of animal enzymes.¹⁴⁰ Expansion of ESCs on tissue culture plastic, unlike adult stem cells, adversely leads to rapid differentiation. Early preventative measures, including the addition of a mouse embryonic fibroblast feeder layer or conditioned media, were proven to be effective, though risk of zoonotic pathogens were a concern for clinical applications.¹²⁵ Alternatives, such as use of human-derived cells, were proven to be a safer method.¹³⁹

Due to their tumorigenic tendencies upon implantation, well-controlled ESC differentiation along the chondrogenic lineage *in vitro* is essential.¹⁴⁸ While recent findings using ESCs for cartilage engineering are inferior to BMSC, ASC, and SDSC results, elucidation of optimal chondrogenic conditions may make ESCs a viable option in the future (Fig. 2). Many approaches incorporating growth factors have been assessed, most commonly employing the use of embryoid body (EB) systems, a method highly dependent on the stage of development and frequently leading to heterogeneous differentiation.^{48, 148} A few groups have attempted to promote chondrogenesis without EB formation. An initial study conducted by Yang et al. compared stem cells directly differentiated, without EB formation, by TGF- β 1.¹⁶⁴ Under both conditions, chondrogenesis did not occur. In contrast, Gong et al. successfully induced chondrogenesis with TGF- β 1 using a high-density adherent micromass culture system.⁴⁸ However, this induction was accomplished by supplementing media with BMP-2, as well. Moreover, in the same study, it was shown that BMP-2 alone was capable of stimulating chondrogenic differentiation. Type II collagen production of BMP-2-treated cells was seven times that of control pellets. Chondrogenesis has also been induced by BMP-2, using EB formation, after first treating with TGF- β 3.⁸⁰ However, it was found that treatment with TGF- β 1 and IGF-1 after TGF- β 3 was more effective, as seen by a two-fold increase in type II collagen production. Alternatively, using a gelatin-coated flask, Nakagawa et al. induced chondrogenesis of ESCs cultured with BMP-7, and even more so when combined with TGF- β 1.¹⁰⁷ Analysis determined generation of high levels of GAGs per DNA, reaching roughly 21 μ g GAGs/ μ g DNA with BMP-7 alone, and 41 μ g GAGs/ μ g DNA when treated with BMP-7 combined with TGF- β 1. As normalized to GAPDH levels, RT-PCR also revealed a seven-fold increase in mRNA expression of type II collagen.

Incorporating scaffolds into the differentiation process has been conducted with much success. Tigli et al. assessed ESC differentiation after seeding onto either chitosan or silk-fibroin scaffolds incorporated with BMP-6.¹³¹ Following 4 weeks of differentiation, histological and RT-PCR analysis identified chitosan to be inferior in terms of chondrogenesis. Upon investigating the effect of modulus on chondrogenic differentiation, Nam et al. cited the ability of low modulus PCL scaffolds to induce chondrogenesis, as seen by upregulated type II collagen and aggrecan expression.¹⁰⁸ Conversely, neither type I collagen nor type II collagen scaffolds successfully induced differentiation of ESCs.¹¹¹ While constructs were also treated with TGF- β 1, type II collagen and GAG production were not observed.

Successful demonstration of ESC chondrogenesis *in vivo* has been conducted numerous times. Hwang et al. obtained chondrogenic-committed cells using an ESC-chondrocyte co-culture system and subsequently seeded PEG-RGD scaffolds prior to subcutaneous implantation in a murine model.⁶⁰ Analysis of implanted hydrogels after 24 weeks confirmed an increase in pellet weight and long-term chondrogenic commitment. A more recent study reported encouraging results when treating osteochondral defects in a murine

model using ESCs embedded in a hyaluronic acid-based hydrogel.¹⁴⁹ Prior to implantation, scaffolds were treated *in vitro* with various cocktails, whereby BMP-7 and TGF- β 1 were deemed superior. Assessment after 12 weeks revealed exceptional integration of the scaffold, and successful treatment of the defect, as seen by regenerated, hyaline-like cartilage.

Induced Pluripotent Stem Cells

In the late 1990s, Wilmut et al. transferred cell nuclei from an embryonic cell line to adult lamb somatic cells, thereby successfully producing a viable offspring.¹⁶¹ This study confirmed the potential of an adult cell to be reprogrammed into an embryonic cell. These results inspired Takahashi, a decade later, to investigate the factors that cause this phenomenon.¹⁴² After assessing genes known to maintain pluripotency of embryonic stem cells, such as OCT3/4, c-Myc, SOX2, and KLF4, they successfully generated pluripotent cells from murine embryonic and adult fibroblasts, termed induced pluripotent stem cells (iPSCs). Soon after, they, along with Yu et al., reproduced these results in adult human fibroblasts.^{141, 173} However, Yu et al. were the first to report pluripotency in cells reprogrammed to express OCT4, SOX2, NANOG and LIN28.¹⁷³ These results supported the potential of these cells to be used for regenerative medicine applications, and confirmed similar surface marker expression to ESCs. Unfortunately, this method was burdened by a low success rate, prompting others to explore alternative routes, such as using microRNAs and creating new vectors.³⁹ One of the largest obstacles that still remains with iPSCs is the tumorigenic tendencies linked to the factors employed, further stressing the need for alternative techniques. Few groups have induced chondrogenesis of iPSCs, though Wei et al. cited chondrogenic differentiation of induced osteoarthritic chondrocytes following *in vitro* and *in vivo* assessment.¹⁶⁰ *In vitro*, cells were transfected with TGF- β 1 and embedded in alginate with chondrocytes. RT-PCR determined type II collagen and GAG levels to be comparable to chondrocytes and two-fold greater than iPSCs cultured without chondrocytes. Following two weeks of *in vitro* differentiation, constructs were implanted subcutaneously in a murine model. Post-operative assessment 6 weeks later confirmed chondrogenesis had been achieved, as seen by type II collagen production. Teramura et al. differentiated murine iPSCs along the chondrogenic lineage by treating cells in a micromass culture with STEMPRO® chondrogenic medium.¹⁴³ Following 2 weeks of treatment, type II collagen expression was increased 20-fold from non-induced iPSC cultures. While these groups have cited successful chondrogenic differentiation, the relative novelty of the field restricts assessment of their potential, though future exploration remains promising.

Conclusion

Since their discovery, stem cells have been considered propitious candidates for many tissue engineering applications, especially cartilage regeneration. Currently, there is a great deal of research supporting this favoritism. However, further investigation is necessary to improve upon current methods of differentiation and, ultimately, regeneration. This literature review confirms great variability in results from stem cell studies (Fig. 2). Individual findings are influenced by how the cells were handled, including isolation procedures, surface marker analyses, and differentiation assessments, as well as the use of multiple growth factors to stimulate chondrogenesis (Table 5). Some differences can be attributed to the influence of distinct, stem cell niches prior to isolation. While MSCs from various tissues have been shown to play similar roles, such as repair and immunosuppression, the inherent differences among microenvironments could influence the properties of resident stem cells.⁸³ MSCs (e.g., BMSCs and ASCs) are not all the same cell type, as demonstrated by the widely differing responses observed when treated with the same growth factors. MSCs are not stem cells by the classical definition, due to their finite proliferative capacities. Instead, they can

be more accurately described as multipotent, progenitor cells. This description is supported by a study showing that cells from adipose tissue differentiate through different molecular mechanisms than those from skin, while still reaching the same desired cell type.⁶⁶ Results depict contrasting stem cell properties, potentially caused by microenvironments or idiosyncrasies of these cells aligning to their roles in the tissue. The goal of this review was to provide a comprehensive summary of stem cell sources for chondrogenesis while addressing these characteristic differences. However, new tissues are continuously being investigated as promising cell depots. Likewise, previously established stem cell sources, such as skin, are showing promise in the area of cartilage regeneration.¹²⁹

Many modifications should be considered in order to enable standardization and reliable comparisons within the field. This is a difficult proposition, however, since investigators have distinct goals for their projects, and allowing direct comparisons to other stem cell sources may not be one of them. With respect to 3D environments for chondrogenesis, employing basic pellet cultures, rather than scaffolds, could provide standardization that allows greater insight into chondrogenic abilities of each cell type. This would eliminate interactions between cells and scaffold materials, permitting assessment of baseline differentiation. Additionally, consistency among reported parameters should be improved upon. Though mechanical properties are considered supremely important for determining tissue functionality, type II collagen and sGAG quantification should be reported as well, potentially using a quantitative, enzyme-linked immunosorbent assay (ELISA) or DMMB assay, respectively. These can be further normalized by quantifying data on a per cell basis, rather than total amounts, which can vary based on number of cells and size of the pellet. True control samples, where stem cells are not induced, would also enhance standardization, enabling examination of fold-increases that can be used for cross-study comparisons. Furthermore, wet weight and dry weight should be considered, since the hydration of cartilage tissue is an informative parameter. Though current studies comparing stem cells have provided great insight, treatment with universal cocktails hinders the ability to identify optimal stem cell sources for cartilage repair, as direct comparisons are difficult to make. Additionally, many *in vivo* studies fail to assess regeneration using previously determined optimal conditions, specifically growth factors. This suggests that greater effort to adapt *in vitro* results to *in vivo* studies could improve upon cartilage regeneration, potentially revealing an optimal stem cell source for future therapies.

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References

1. ClinicalTrials.gov. 2012. <http://clinicaltrials.gov>
2. Afizah H, Yang Z, Hui JH, Ouyang HW, Lee EH. A comparison between the chondrogenic potential of human bone marrow stem cells (BMSCs) and adipose-derived stem cells (ADSCs) taken from the same donors. *Tissue Eng.* 2007; 13:659–666. [PubMed: 17371203]
3. Ahmed TA, Giulivi A, Griffith M, Hincke M. Fibrin glues in combination with mesenchymal stem cells to develop a tissue-engineered cartilage substitute. *Tissue engineering. Part A.* 2011; 17:323–335. [PubMed: 20799906]
4. Aicher WK, Buhning HJ, Hart M, Rolauffs B, Badke A, Klein G. Regeneration of cartilage and bone by defined subsets of mesenchymal stromal cells--potential and pitfalls. *Adv Drug Deliv Rev.* 2011; 63:342–351. [PubMed: 21184789]
5. Al Battah F, De Kock J, Ramboer E, Heymans A, Vanhaecke T, Rogiers V, Snykers S. Evaluation of the multipotent character of human adipose tissue-derived stem cells isolated by Ficoll gradient centrifugation and red blood cell lysis treatment. *Toxicology in vitro : an international journal published in association with BIBRA.* 2011; 25:1224–1230. [PubMed: 21645610]

6. Alviano F, Fossati V, Marchionni C, Arpinati M, Bonsi L, Franchina M, Lanzoni G, Cantoni S, Cavallini C, Bianchi F, Tazzari PL, Pasquinelli G, Foroni L, Ventura C, Grossi A, Bagnara GP. Term amniotic membrane is a high throughput source for multipotent mesenchymal stem cells with the ability to differentiate into endothelial cells in vitro. *Bmc Dev Biol.* 2007; 7
7. Andriamanalijaona R, Duval E, Raoudi M, Lecourt S, Vilquin JT, Marolleau JP, Pujol JP, Galera P, Boumediene K. Differentiation potential of human muscle-derived cells towards chondrogenic phenotype in alginate beads culture. *Osteoarthritis Cartilage.* 2008; 16:1509–1518.
8. Arnhold S, Gluer S, Hartmann K, Raabe O, Addicks K, Wenisch S, Hoopmann M. Amniotic-Fluid Stem Cells: Growth Dynamics and Differentiation Potential after a CD-117-Based Selection Procedure. *Stem cells international.* 2011; 2011:715341. [PubMed: 21437196]
9. Aroen A. Stem cell therapy for articular cartilage defects. *Brit Med Bull.* 2011; 99:227–240. [PubMed: 21821628]
10. Awad HA, Wickham MQ, Leddy HA, Gimble JM, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials.* 2004; 25:3211–3222. [PubMed: 14980416]
11. Bailey MM, Wang L, Bode CJ, Mitchell KE, Detamore MS. A comparison of human umbilical cord matrix stem cells and temporomandibular joint condylar Chondrocytes for tissue engineering temporomandibular joint condylar cartilage. *Tissue Eng.* 2007; 13:2003–2010. [PubMed: 17518722]
12. Baksh D, Yao R, Tuan RS. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. *Stem Cells.* 2007; 25:1384–1392. [PubMed: 17332507]
13. Barry F, Boynton RE, Liu BS, Murphy JM. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: Differentiation-dependent gene expression of matrix components. *Exp Cell Res.* 2001; 268:189–200. [PubMed: 11478845]
14. Bilgen B, Ren Y, Pei M, Aaron RK, Ciombor DM. CD14-negative isolation enhances chondrogenesis in synovial fibroblasts. *Tissue engineering. Part A.* 2009; 15:3261–3270. [PubMed: 19382853]
15. Brooke G, Tong H, Levesque JP, Atkinson K. Molecular trafficking mechanisms of multipotent mesenchymal stem cells derived from human bone marrow and placenta. *Stem cells and development.* 2008; 17:929–940. [PubMed: 18564033]
16. Bunnell BA, Flaat M, Gagliardi C, Patel B, Ripoll C. Adipose-derived stem cells: isolation, expansion and differentiation. *Methods.* 2008; 45:115–120. [PubMed: 18593609]
17. Caplan AI. Mesenchymal Stem-Cells. *J Orthopaed Res.* 1991; 9:641–650.
18. Cavallo C, Cuomo C, Fantini S, Ricci F, Tazzari PL, Lucarelli E, Donati D, Facchini A, Lisignoli G, Fornasari PM, Grigolo B, Moroni L. Comparison of Alternative Mesenchymal Stem Cell Sources for Cell Banking and Musculoskeletal Advanced Therapies. *Journal of cellular biochemistry.* 2011; 112:1418–1430. [PubMed: 21321995]
19. Chang YJ, Tseng CP, Hsu LF, Hsieh TB, Hwang SM. Characterization of two populations of mesenchymal progenitor cells in umbilical cord blood. *Cell Biol Int.* 2006; 30:495–499. [PubMed: 16731010]
20. Che X, Guo J, Wang B, Bai Y. Rapid isolation of muscle-derived stem cells by discontinuous Percoll density gradient centrifugation. *In vitro cellular & developmental biology. Animal.* 2011; 47:454–458. [PubMed: 21691920]
21. Cheng NC, Estes BT, Awad HA, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells by a porous scaffold derived from native articular cartilage extracellular matrix. *Tissue engineering. Part A.* 2009; 15:231–241. [PubMed: 18950290]
22. Chiou M, Xu Y, Longaker MT. Mitogenic and chondrogenic effects of fibroblast growth factor-2 in adipose-derived mesenchymal cells. *Biochem Biophys Res Commun.* 2006; 343:644–652. [PubMed: 16554022]
23. Choi JS, Kim BS, Kim JD, Choi YC, Lee HY, Cho YW. In vitro cartilage tissue engineering using adipose-derived extracellular matrix scaffolds seeded with adipose-derived stem cells. *Tissue engineering. Part A.* 2012; 18:80–92. [PubMed: 21905881]

24. Choi WH, Choi BH, Min BH, Park SR. Low-intensity ultrasound increased colony forming unit-fibroblasts of mesenchymal stem cells during primary culture. *Tissue engineering. Part C, Methods*. 2011; 17:517–526. [PubMed: 21171932]
25. Choi YH, Burdick MD, Strieter RM. Human circulating fibrocytes have the capacity to differentiate osteoblasts and chondrocytes. *Int J Biochem Cell B*. 2010; 42:662–671.
26. Chong PP, Selvaratnam L, Abbas AA, Kamarul T. Human peripheral blood derived mesenchymal stem cells demonstrate similar characteristics and chondrogenic differentiation potential to bone marrow derived mesenchymal stem cells. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*. 2011
27. Coleman RM, Case ND, Guldborg RE. Hydrogel effects on bone marrow stromal cell response to chondrogenic growth factors. *Biomaterials*. 2007; 28:2077–2086. [PubMed: 17257670]
28. Cooke ME, Allon AA, Cheng T, Kuo AC, Kim HT, Vail TP, Marcucio RS, Schneider RA, Lotz JC, Alliston T. Structured three-dimensional co-culture of mesenchymal stem cells with chondrocytes promotes chondrogenic differentiation without hypertrophy. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2011; 19:1210–1218.
29. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A. Human mesenchymal stem cells modulate B-cell functions. *Blood*. 2006; 107:367–372. [PubMed: 16141348]
30. Danisovic L, Varga I, Polak S. Growth factors and chondrogenic differentiation of mesenchymal stem cells. *Tissue & cell*. 2012; 44:69–73. [PubMed: 22185680]
31. Darling EM, Topel M, Zauscher S, Vail TP, Guilak F. Viscoelastic properties of human mesenchymally-derived stem cells and primary osteoblasts, chondrocytes, and adipocytes. *J Biomech*. 2008; 41:454–464. [PubMed: 17825308]
32. De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum*. 2001; 44:1928–1942. [PubMed: 11508446]
33. De Coppi P, Bartsch G, Siddiqui MM, Xu T, Santos CC, Perin L, Mostoslavsky G, Serre AC, Snyder EY, Yoo JJ, Furth ME, Soker S, Atala A. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol*. 2007; 25:100–106. [PubMed: 17206138]
34. de Mara CS, Duarte AS, Sartori-Cintra AR, Luzo AC, Saad ST, Coimbra IB. Chondrogenesis from umbilical cord blood cells stimulated with BMP-2 and BMP-6. *Rheumatology international*. 2012
35. De Miguel MP, Fuentes-Julian S, Alcaina Y. Pluripotent stem cells: origin, maintenance and induction. *Stem cell reviews*. 2010; 6:633–649. [PubMed: 20669057]
36. Deasy BM, Yong LI, Huard J. Tissue engineering with muscle-derived stem cells. *Curr Opin Biotech*. 2004; 15:419–423. [PubMed: 15464371]
37. Diekman BO, Rowland CR, Lennon DP, Caplan AI, Guilak F. Chondrogenesis of Adult Stem Cells from Adipose Tissue and Bone Marrow: Induction by Growth Factors and Cartilage-Derived Matrix. *Tissue Eng Pt A*. 2010; 16:523–533.
38. Draper JS, Pigott C, Thomson JA, Andrews PW. Surface antigens of human embryonic stem cells: changes upon differentiation in culture. *J Anat*. 2002; 200:249–258. [PubMed: 12033729]
39. Ebben JD, Zorniak M, Clark PA, Kuo JS. Introduction to induced pluripotent stem cells: advancing the potential for personalized medicine. *World neurosurgery*. 2011; 76:270–275. [PubMed: 21986423]
40. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *British journal of haematology*. 2000; 109:235–242. [PubMed: 10848804]
41. Estes BT, Diekman BO, Gimble JM, Guilak F. Isolation of adipose-derived stem cells and their induction to a chondrogenic phenotype. *Nat Protoc*. 2010; 5:1294–1311. [PubMed: 20595958]
42. Fan JB, Ren L, Liang RS, Gong YH, Cai DZ, Wang DA. Chondrogenesis of Synovium-Derived Mesenchymal Stem Cells in Photopolymerizing Hydrogel Scaffolds. *J Biomat Sci-Polym E*. 2010; 21:1653–1667.
43. Fan JB, Varshney RR, Ren L, Cai DZ, Wang DA. Synovium-Derived Mesenchymal Stem Cells: A New Cell Source for Musculoskeletal Regeneration. *Tissue Eng Part B-Re*. 2009; 15:75–86.
44. Fong CY, Chak LL, Biswas A, Tan JH, Gauthaman K, Chan WK, Bongso A. Human Wharton's jelly stem cells have unique transcriptome profiles compared to human embryonic stem cells and other mesenchymal stem cells. *Stem Cell Rev*. 2011; 7:1–16. [PubMed: 20602182]

45. Fong CY, Subramanian A, Gauthaman K, Venugopal J, Biswas A, Ramakrishna S, Bongso A. Human umbilical cord Wharton's jelly stem cells undergo enhanced chondrogenic differentiation when grown on nanofibrous scaffolds and in a sequential two-stage culture medium environment. *Stem cell reviews*. 2012; 8:195–209. [PubMed: 21671058]
46. Friedenstein AJ, Gorskaja UF, Kulagina NN. Fibroblast Precursors in Normal and Irradiated Mouse Hematopoietic Organs. *Exp Hematol*. 1976; 4:267–274. [PubMed: 976387]
47. Gharaibeh B, Lu A, Tebbets J, Zheng B, Feduska J, Crisan M, Peault B, Cummins J, Huard J. Isolation of a slowly adhering cell fraction containing stem cells from murine skeletal muscle by the preplate technique. *Nat Protoc*. 2008; 3:1501–1509. [PubMed: 18772878]
48. Gong GC, Ferrari D, Dealy CN, Kosher RA. Direct and Progressive Differentiation of Human Embryonic Stem Cells Into the Chondrogenic Lineage. *Journal of cellular physiology*. 2010; 224:664–671. [PubMed: 20432462]
49. Griesche N, Luttmann W, Luttmann A, Stammermann T, Geiger H, Baer PC. A simple modification of the separation method reduces heterogeneity of adipose-derived stem cells. *Cells, tissues, organs*. 2010; 192:106–115. [PubMed: 20185896]
50. Gronthos S, Zannettino AC. A method to isolate and purify human bone marrow stromal stem cells. *Methods in molecular biology*. 2008; 449:45–57. [PubMed: 18370082]
51. Han HS, Lee S, Kim JH, Seong SC, Lee MC. Changes in Chondrogenic Phenotype and Gene Expression Profiles Associated with the In Vitro Expansion of Human Synovium-Derived Cells. *J Orthopaed Res*. 2010; 28:1283–1291.
52. Harichandan A, Buhning HJ. Prospective isolation of human MSC. *Best practice & research. Clinical haematology*. 2011; 24:25–36. [PubMed: 21396590]
53. He Q, Wan C, Li G. Concise review: multipotent mesenchymal stromal cells in blood. *Stem Cells*. 2007; 25:69–77. [PubMed: 16973831]
54. Hildner F, Peterbauer A, Wolbank S, Nurnberger S, Marlovits S, Redl H, van Griensven M, Gabriel C. FGF-2 abolishes the chondrogenic effect of combined BMP-6 and TGF-beta in human adipose derived stem cells. *J Biomed Mater Res A*. 2010; 94:978–987. [PubMed: 20730935]
55. Ho ST, Cool SM, Hui JH, Hutmacher DW. The influence of fibrin based hydrogels on the chondrogenic differentiation of human bone marrow stromal cells. *Biomaterials*. 2010; 31:38–47. [PubMed: 19800683]
56. Hsiao FSH, Cheng CC, Peng SY, Huang HY, Lian WS, Jan ML, Fang YT, Cheng ECH, Lee KH, Cheng WTK, Lin SP, Wu SC. Isolation of therapeutically functional mouse bone marrow mesenchymal stem cells within 3 h by an effective single-step plastic-adherent method. *Cell Proliferat*. 2010; 43:235–248.
57. Hsu SH, Huang TB, Cheng SJ, Weng SY, Tsai CL, Tseng CS, Chen DC, Liu TY, Fu KY, Yen BL. Chondrogenesis from Human Placenta-Derived Mesenchymal Stem Cells in Three-Dimensional Scaffolds for Cartilage Tissue Engineering. *Tissue Eng Pt A*. 2011; 17:1549–1560.
58. Hu G, Liu P, Feng J, Jin Y. A Novel Population of Mesenchymal Progenitors with Hematopoietic Potential Originated from CD14(-) Peripheral Blood Mononuclear Cells. *Int J Med Sci*. 2011; 8:16–29. [PubMed: 21209798]
59. Huang P, Lin LM, Wu XY, Tang QL, Feng XY, Lin GY, Lin X, Wang HW, Huang TH, Ma L. Differentiation of human umbilical cord Wharton's jelly-derived mesenchymal stem cells into germ-like cells in vitro. *Journal of cellular biochemistry*. 2010; 109:747–754. [PubMed: 20052672]
60. Hwang NS, Varghese S, Elisseeff J. Derivation of chondrogenically-committed cells from human embryonic cells for cartilage tissue regeneration. *Plos One*. 2008; 3:e2498. [PubMed: 18575581]
61. Igarashi T, Iwasaki N, Kawamura D, Kasahara Y, Tsukuda Y, Ohzawa N, Ito M, Izumisawa Y, Minami A. Repair of articular cartilage defects with a novel injectable in situ forming material in a canine model. *J Biomed Mater Res A*. 2012; 100:180–187. [PubMed: 22021195]
62. Ilancheran S, Moodley Y, Manuelpillai U. Human fetal membranes: a source of stem cells for tissue regeneration and repair? *Placenta*. 2009; 30:2–10. [PubMed: 18995896]
63. Im GI, Kim HJ, Lee JH. Chondrogenesis of adipose stem cells in a porous PLGA scaffold impregnated with plasmid DNA containing SOX trio (SOX-5,-6 and -9) genes. *Biomaterials*. 2011; 32:4385–4392. [PubMed: 21421267]

64. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells*. 2004; 22:1338–1345. [PubMed: 15579651]
65. in 'tAnker PS, Scherjon SA, Kleijburg-van der Keur C, Noort WA, Claas FHJ, Willemze R, Fibbe WE, Kanhai HHH. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. *Blood*. 2003; 102:1548–1549. [PubMed: 12900350]
66. Jaager K, Islam S, Zajac P, Linnarsson S, Neuman T. RNA-Seq Analysis Reveals Different Dynamics of Differentiation of Human Dermis- and Adipose-Derived Stromal Stem Cells. *Plos One*. 2012; 7:e38833. [PubMed: 22723894]
67. Jankowski RJ, Deasy BM, Huard J. Muscle-derived stem cells. *Gene Ther*. 2002; 9:642–647. [PubMed: 12032710]
68. Jin XB, Sun YS, Zhang K, Wang J, Shi TP, Ju XD, Lou SQ. Tissue engineered cartilage from hTGF beta2 transduced human adipose derived stem cells seeded in PLGA/alginate compound in vitro and in vivo. *J Biomed Mater Res A*. 2008; 86:1077–1087. [PubMed: 18080296]
69. Jones E, McGonagle D. Synovial mesenchymal stem cells in vivo: Potential key players for joint regeneration. *World Journal of Rheumatology*. 2011; 1:4–11.
70. Jung SN, Rhie JW, Kwon H, Jun YJ, Seo JW, Yoo G, Oh DY, Ahn ST, Woo J, Oh J. In Vivo Cartilage Formation Using Chondrogenic-Differentiated Human Adipose-Derived Mesenchymal Stem Cells Mixed With Fibrin Glue. *J Craniofac Surg*. 2010; 21:468–472. [PubMed: 20489452]
71. Kaewkhaw R, Scutt AM, Haycock JW. Anatomical site influences the differentiation of adipose-derived stem cells for Schwann-cell phenotype and function. *Glia*. 2011; 59:734–749. [PubMed: 21351157]
72. Karlsson C, Brantsing C, Svensson T, Brisby H, Asp J, Tallheden T, Lindahl A. Differentiation of human mesenchymal stem cells and articular chondrocytes: analysis of chondrogenic potential and expression pattern of differentiation-related transcription factors. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*. 2007; 25:152–163. [PubMed: 17072841]
73. Kassis I, Zangi L, Rivkin R, Levdansky L, Samuel S, Marx G, Gorodetsky R. Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. *Bone Marrow Transpl*. 2006; 37:967–976.
74. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*. 2006; 24:1294–1301. [PubMed: 16410387]
75. Khan WS, Adesida AB, Tew SR, Lowe ET, Hardingham TE. Bone Marrow-Derived Mesenchymal Stem Cells Express the Pericyte Marker 3G5 in Culture and Show Enhanced Chondrogenesis in Hypoxic Conditions. *J Orthopaed Res*. 2010; 28:834–840.
76. Kim HJ, Im GI. Chondrogenic differentiation of adipose tissue-derived mesenchymal stem cells: greater doses of growth factor are necessary. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*. 2009; 27:612–619. [PubMed: 18985688]
77. Kim HJ, Im GI. Combination of transforming growth factor-beta2 and bone morphogenetic protein 7 enhances chondrogenesis from adipose tissue-derived mesenchymal stem cells. *Tissue engineering. Part A*. 2009; 15:1543–1551. [PubMed: 19072523]
78. Kim HJ, Lee JH, Im GI. Chondrogenesis using mesenchymal stem cells and PCL scaffolds. *J Biomed Mater Res A*. 2010; 92:659–666. [PubMed: 19235210]
79. Knutsen G, Engebretsen L, Ludvigsen TC, Drogset JO, Grontvedt T, Solheim E, Strand T, Roberts S, Isaksen V, Johansen O. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. *J Bone Joint Surg Am*. 2004; 86-A:455–464. [PubMed: 14996869]
80. Koay EJ, Hoben GM, Athanasiou KA. Tissue engineering with chondrogenically differentiated human embryonic stem cells. *Stem Cells*. 2007; 25:2183–2190. [PubMed: 17540854]
81. Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, Sekiya I. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. *Cell and tissue research*. 2008; 333:207–215. [PubMed: 18560897]
82. Kolambkar YM, Peister A, Soker S, Atala A, Guldberg RE. Chondrogenic differentiation of amniotic fluid-derived stem cells. *J Mol Histol*. 2007; 38:405–413. [PubMed: 17668282]

83. Krampera M, Pizzolo G, Aprili G, Franchini M. Mesenchymal stem cells for bone, cartilage, tendon and skeletal muscle repair. *Bone*. 2006; 39:678–683. [PubMed: 16765663]
84. Kubo S, Cooper GM, Matsumoto T, Phillippi JA, Corsi KA, Usas A, Li G, Fu FH, Huard J. Blocking vascular endothelial growth factor with soluble Flt-1 improves the chondrogenic potential of mouse skeletal muscle-derived stem cells. *Arthritis Rheum*. 2009; 60:155–165. [PubMed: 19116905]
85. Kuroda R, Usas A, Kubo S, Corsi K, Peng HR, Rose T, Cummins J, Fu FH, Huard J. Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. *Arthritis Rheum*. 2006; 54:433–442. [PubMed: 16447218]
86. La Rocca G, Anzalone R, Corrao S, Magno F, Loria T, Lo Iacono M, Di Stefano A, Giannuzzi P, Marasa L, Cappello F, Zummo G, Farina F. Isolation and characterization of Oct-4+/HLA-G+ mesenchymal stem cells from human umbilical cord matrix: differentiation potential and detection of new markers. *Histochem Cell Biol*. 2009; 131:267–282. [PubMed: 18836737]
87. Lee HJ, Yu C, Chansakul T, Varghese S, Hwang NS, Elisseff JH. Enhanced chondrogenic differentiation of embryonic stem cells by coculture with hepatic cells. *Stem cells and development*. 2008; 17:555–563. [PubMed: 18513166]
88. Lee JS, Im GI. Influence of chondrocytes on the chondrogenic differentiation of adipose stem cells. *Tissue engineering. Part A*. 2010; 16:3569–3577. [PubMed: 20597811]
89. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood*. 2004; 103:1669–1675. [PubMed: 14576065]
90. Lee S, Kim JH, Jo CH, Seong SC, Lee JC, Lee MC. Effect of Serum and Growth Factors on Chondrogenic Differentiation of Synovium-Derived Stromal Cells. *Tissue Eng Pt A*. 2009; 15:3401–3415.
91. Li JJ, Zhao Q, Wang EB, Zhang CH, Wang GB, Yuan Q. Dynamic compression of rabbit adipose-derived stem cells transfected with insulin-like growth factor 1 in chitosan/gelatin scaffolds induces chondrogenesis and matrix biosynthesis. *Journal of cellular physiology*. 2012; 227:2003–2012. [PubMed: 21751209]
92. Li Q, Tang J, Wang R, Bei C, Xin L, Zeng Y, Tang X. Comparing the chondrogenic potential in vivo of autogeneic mesenchymal stem cells derived from different tissues. *Artificial cells, blood substitutes, and immobilization biotechnology*. 2011; 39:31–38.
93. Lu SH, Yang AH, Wei CF, Chiang HS, Chancellor MB. Multi-potent differentiation of human purified muscle-derived cells: potential for tissue regeneration. *BJU international*. 2010; 105:1174–1180. [PubMed: 19712114]
94. Lund TC, Tolar J, Orchard PJ. Granulocyte colony-stimulating factor mobilized CFU-F can be found in the peripheral blood but have limited expansion potential. *Haematologica*. 2008; 93:908–912. [PubMed: 18403392]
95. Mahmoudifar N, Doran PM. Chondrogenic differentiation of human adipose-derived stem cells in polyglycolic acid mesh scaffolds under dynamic culture conditions. *Biomaterials*. 2010; 31:3858–3867. [PubMed: 20153043]
96. Maloney JM, Nikova D, Lautenschlager F, Clarke E, Langer R, Guck J, Van Vliet KJ. Mesenchymal Stem Cell Mechanics from the Attached to the Suspended State. *Biophys J*. 2010; 99:2479–2487. [PubMed: 20959088]
97. Marsano A, Millward-Sadler SJ, Salter DM, Adesida A, Hardingham T, Tognana E, Kon E, Chiari-Grisar C, Nehrer S, Jakob M, Martin I. Differential cartilaginous tissue formation by human synovial membrane, fat pad, meniscus cells and articular chondrocytes. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2007; 15:48–58.
98. Matsumoto T, Kubo S, Meszaros LB, Corsi KA, Cooper GM, Li G, Usas A, Osawa A, Fu FH, Huard J. The influence of sex on the chondrogenic potential of muscle-derived stem cells: implications for cartilage regeneration and repair. *Arthritis Rheum*. 2008; 58:3809–3819. [PubMed: 19035511]
99. McElreavey KD, Irvine AI, Ennis KT, McLean WH. Isolation, culture and characterisation of fibroblast-like cells derived from the Wharton's jelly portion of human umbilical cord. *Biochem Soc Trans*. 1991; 19:29S. [PubMed: 1709890]

100. McGuckin CP, Forraz N, Baradez MO, Navran S, Zhao J, Urban R, Tilton R, Denner L. Production of stem cells with embryonic characteristics from human umbilical cord blood. *Cell Proliferat.* 2005; 38:245–255.
101. Mehlhorn AT, Zwingmann J, Finkenzeller G, Niemeyer P, Dauner M, Stark B, Sudkamp NP, Schmal H. Chondrogenesis of Adipose-Derived Adult Stem Cells in a Poly-Lactide-Co-Glycolide Scaffold. *Tissue Eng Pt A.* 2009; 15:1159–1167.
102. Meng J, Adkin CF, Arechavala-Gomeza V, Boldrin L, Muntoni F, Morgan JE. The contribution of human synovial stem cells to skeletal muscle regeneration. *Neuromuscular disorders : NMD.* 2010; 20:6–15. [PubMed: 20034794]
103. Meng J, Adkin CF, Xu SW, Muntoni F, Morgan JE. Contribution of human muscle-derived cells to skeletal muscle regeneration in dystrophic host mice. *Plos One.* 2011; 6:e17454. [PubMed: 21408080]
104. Miyamoto C, Matsumoto T, Sakimura K, Shindo H. Osteogenic protein-1 with transforming growth factor-beta1: potent inducer of chondrogenesis of synovial mesenchymal stem cells in vitro. *Journal of orthopaedic science : official journal of the Japanese Orthopaedic Association.* 2007; 12:555–561. [PubMed: 18040638]
105. Mosna F, Sensebe L, Krampera M. Human bone marrow and adipose tissue mesenchymal stem cells: a user's guide. *Stem cells and development.* 2010; 19:1449–1470. [PubMed: 20486777]
106. Nadri S, Soleimani M, Hosseini RH, Massumi M, Atashi A, Izadpanah R. An efficient method for isolation of murine bone marrow mesenchymal stem cells. *The International journal of developmental biology.* 2007; 51:723–729. [PubMed: 17939119]
107. Nakagawa T, Lee SY, Reddi AH. Induction of Chondrogenesis From Human Embryonic Stem Cells Without Embryoid Body Formation by Bone Morphogenetic Protein 7 and Transforming Growth Factor beta 1. *Arthritis Rheum.* 2009; 60:3686–3692. [PubMed: 19950276]
108. Nam J, Johnson J, Lannutti JJ, Agarwal S. Modulation of embryonic mesenchymal progenitor cell differentiation via control over pure mechanical modulus in electrospun nanofibers. *Acta Biomater.* 2011; 7:1516–1524. [PubMed: 21109030]
109. Nawata M, Wakitani S, Nakaya H, Tanigami A, Seki T, Nakamura Y, Saito N, Sano K, Hidaka E, Takaoka K. Use of bone morphogenetic protein 2 and diffusion chambers to engineer cartilage tissue for the repair of defects in articular cartilage. *Arthritis Rheum.* 2005; 52:155–163. [PubMed: 15641068]
110. Nesic D, Whiteside R, Brittberg M, Wendt D, Martin I, Mainil-Varlet P. Cartilage tissue engineering for degenerative joint disease. *Adv Drug Deliver Rev.* 2006; 58:300–322.
111. Ng KK, Thatte HS, Spector M. Chondrogenic differentiation of adult mesenchymal stem cells and embryonic cells in collagen scaffolds. *J Biomed Mater Res A.* 2011; 99A:275–282. [PubMed: 21858916]
112. Pappa KI, Anagnou N. Novel sources of fetal stem cells: where do they fit on the developmental continuum? *Regen Med.* 2009; 4:423–433. [PubMed: 19438317]
113. Park JS, Shim MS, Shim SH, Yang HN, Jeon SY, Woo DG, Lee DR, Yoon TK, Park KH. Chondrogenic potential of stem cells derived from amniotic fluid, adipose tissue, or bone marrow encapsulated in fibrin gels containing TGF-beta 3. *Biomaterials.* 2011; 32:8139–8149. [PubMed: 21840589]
114. Park JS, Yang HN, Woo DG, Chung HM, Park KH. In vitro and in vivo chondrogenesis of rabbit bone marrow-derived stromal cells in fibrin matrix mixed with growth factor loaded in nanoparticles. *Tissue engineering. Part A.* 2009; 15:2163–2175. [PubMed: 19413492]
115. Park JS, Yang HN, Woo DG, Jeon SY, Park KH. The promotion of chondrogenesis, osteogenesis, and adipogenesis of human mesenchymal stem cells by multiple growth factors incorporated into nanosphere-coated microspheres. *Biomaterials.* 2011; 32:28–38. [PubMed: 20875915]
116. Park K, Cho KJ, Kim JJ, Kim IH, Han DK. Functional PLGA scaffolds for chondrogenesis of bone-marrow-derived mesenchymal stem cells. *Macromolecular bioscience.* 2009; 9:221–229. [PubMed: 19089870]
117. Pate DW, Southerland SS, Grande DA, Young HE, Lucas PA. Isolation and Differentiation of Mesenchymal Stem-Cells from Rabbit Muscle. *Clin Res.* 1993; 41:A347–A347.

118. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; 284:143–147. [PubMed: 10102814]
119. Puetzer JL, Petite JN, Lobo EG. Comparative Review of Growth Factors for Induction of Three-Dimensional In Vitro Chondrogenesis in Human Mesenchymal Stem Cells Isolated from Bone Marrow and Adipose Tissue. *Tissue Eng Part B-Re*. 2010; 16:435–444.
120. Qi J, Chen A, You H, Li K, Zhang D, Guo F. Proliferation and chondrogenic differentiation of CD105-positive enriched rat synovium-derived mesenchymal stem cells in three-dimensional porous scaffolds. *Biomed Mater*. 2011; 6:015006. [PubMed: 21205995]
121. Qi YY, Zhao TF, Xu K, Dai TY, Yan WQ. The restoration of full-thickness cartilage defects with mesenchymal stem cells (MSCs) loaded and cross-linked bilayer collagen scaffolds on rabbit model. *Mol Biol Rep*. 2012; 39:1231–1237. [PubMed: 21594730]
122. Rada T, Reis RL, Gomes AE. Novel method for the isolation of adipose stem cells (ASCs). *J Tissue Eng Regen M*. 2009; 3:158–159. [PubMed: 19111010]
123. Raghunath J, Sutherland J, Salih V, Mordan N, Butler PE, Seifalian AM. Chondrogenic potential of blood-acquired mesenchymal progenitor cells. *Journal of plastic, reconstructive & aesthetic surgery : JPRAS*. 2010; 63:841–847.
124. Rebelatto CK, Aguiar AM, Moretao MP, Senegaglia AC, Hansen P, Barchiki F, Oliveira J, Martins J, Kuligovski C, Mansur F, Christofis A, Amaral VF, Brofman PS, Goldenberg S, Nakao LS, Correa A. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. *Exp Biol Med (Maywood)*. 2008; 233:901–913. [PubMed: 18445775]
125. Richards M, Fong CY, Chan WK, Wong PC, Bongso A. Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells. *Nat Biotechnol*. 2002; 20:933–936. [PubMed: 12161760]
126. Riekstina U, Cakstina I, Parfejevs V, Hoogduijn M, Jankovskis G, Muiznieks I, Muceniece R, Ancans J. Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis. *Stem cell reviews*. 2009; 5:378–386. [PubMed: 20058201]
127. Roufosse CA, Direkze NC, Otto WR, Wright NA. Circulating mesenchymal stem cells. *The international journal of biochemistry & cell biology*. 2004; 36:585–597.
128. Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues - Superiority of synovium as a cell source. *Arthritis Rheum*. 2005; 52:2521–2529. [PubMed: 16052568]
129. Sanchez-Adams J, Athanasiou KA. Dermis isolated adult stem cells for cartilage tissue engineering. *Biomaterials*. 2012; 33:109–119. [PubMed: 21959004]
130. Saw KY, Anz A, Merican S, Tay YG, Ragavanaidu K, Jee CSY, McGuire DA. Articular Cartilage Regeneration With Autologous Peripheral Blood Progenitor Cells and Hyaluronic Acid After Arthroscopic Subchondral Drilling: A Report of 5 Cases With Histology. *Arthroscopy*. 2011; 27:493–506. [PubMed: 21334844]
131. Seda Tigli R, Ghosh S, Laha MM, Shevde NK, Daheron L, Gimble J, Gumusderelioglu M, Kaplan DL. Comparative chondrogenesis of human cell sources in 3D scaffolds. *J Tissue Eng Regen M*. 2009; 3:348–360. [PubMed: 19382119]
132. Sekiya I, Colter DC, Prockop DJ. BMP-6 enhances chondrogenesis in a subpopulation of human marrow stromal cells. *Biochem Biophys Res Commun*. 2001; 284:411–418. [PubMed: 11394894]
133. Sekiya I, Larson BL, Vuoristo JT, Reger RL, Prockop DJ. Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma. *Cell and tissue research*. 2005; 320:269–276. [PubMed: 15778851]
134. Semenov OV, Koestenbauer S, Riegel M, Zech N, Zimmermann R, Zisch AH, Malek A. Multipotent mesenchymal stem cells from human placenta: critical parameters for isolation and maintenance of stemness after isolation. *Am J Obstet Gynecol*. 2010; 202:193, e191–193, e113. [PubMed: 20035913]

135. Shen B, Wei A, Tao H, Diwan AD, Ma DD. BMP-2 enhances TGF-beta3-mediated chondrogenic differentiation of human bone marrow multipotent mesenchymal stromal cells in alginate bead culture. *Tissue engineering. Part A*. 2009; 15:1311–1320. [PubMed: 18950289]
136. Shimaya M, Muneta T, Ichinose S, Tsuji K, Sekiya I. Magnesium enhances adherence and cartilage formation of synovial mesenchymal stem cells through integrins. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2010; 18:1300–1309.
137. Shirasawa S, Sekiya I, Sakaguchi Y, Yagishita K, Ichinose S, Muneta T. In vitro chondrogenesis of human synovium-derived mesenchymal stem cells: optimal condition and comparison with bone marrow-derived cells. *Journal of cellular biochemistry*. 2006; 97:84–97. [PubMed: 16088956]
138. Solchaga LA, Penick K, Goldberg VM, Caplan AI, Welter JF. Fibroblast Growth Factor-2 Enhances Proliferation and Delays Loss of Chondrogenic Potential in Human Adult Bone-Marrow-Derived Mesenchymal Stem Cells. *Tissue Eng Pt A*. 2010; 16:1009–1019.
139. Strom S, Holm F, Bergstrom R, Stromberg AM, Hovatta O. Derivation of 30 human embryonic stem cell lines--improving the quality. *In vitro cellular & developmental biology. Animal*. 2010; 46:337–344. [PubMed: 20198446]
140. Strom S, Inzunza J, Grinnemo KH, Hohmberg K, Matilainen E, Stromberg AM, Blennow E, Hovatta O. Mechanical isolation of the inner cell mass is effective in derivation of new human embryonic stem cell lines. *Hum Reprod*. 2007; 22:3051–3058. [PubMed: 17959612]
141. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131:861–872. [PubMed: 18035408]
142. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
143. Teramura T, Onodera Y, Mihara T, Hosoi Y, Hamanishi C, Fukuda K. Induction of mesenchymal progenitor cells with chondrogenic property from mouse-induced pluripotent stem cells. *Cellular reprogramming*. 2010; 12:249–261. [PubMed: 20698767]
144. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282:1145–1147. [PubMed: 9804556]
145. Tigli RS, Cannizaro C, Gumusderelioglu M, Kaplan DL. Chondrogenesis in perfusion bioreactors using porous silk scaffolds and hESC-derived MSCs. *J Biomed Mater Res A*. 2011; 96:21–28. [PubMed: 20949478]
146. Titushkin I, Cho M. Modulation of cellular mechanics during osteogenic differentiation of human mesenchymal stem cells. *Biophys J*. 2007; 93:3693–3702. [PubMed: 17675345]
147. Tobita M, Orbay H, Mizuno H. Adipose-derived stem cells: current findings and future perspectives. *Discovery medicine*. 2011; 11:160–170. [PubMed: 21356171]
148. Toh WS, Lee EH, Cao T. Potential of human embryonic stem cells in cartilage tissue engineering and regenerative medicine. *Stem Cell Rev*. 2011; 7:544–559. [PubMed: 21188652]
149. Toh WS, Lee EH, Guo XM, Chan JKY, Yeow CH, Choo AB, Cao T. Cartilage repair using hyaluronan hydrogel-encapsulated human embryonic stem cell-derived chondrogenic cells. *Biomaterials*. 2010; 31:6968–6980. [PubMed: 20619789]
150. Toh WS, Yang Z, Liu H, Heng BC, Lee EH, Cao T. Effects of culture conditions and bone morphogenetic protein 2 on extent of chondrogenesis from human embryonic stem cells. *Stem Cells*. 2007; 25:950–960. [PubMed: 17218402]
151. Tondreau T, Meuleman N, Delforge A, Dejeneffe M, Leroy R, Massy M, Mortier C, Bron D, Lagneaux L. Mesenchymal stem cells derived from CD133-positive cells in mobilized peripheral blood and cord blood: Proliferation, Oct4 expression, and plasticity. *Stem Cells*. 2005; 23:1105–1112. [PubMed: 15955825]
152. Tsai MS, Hwang SM, Chen KD, Lee YS, Hsu LW, Chang YJ, Wang CN, Peng HH, Chang YL, Chao AS, Chang SD, Lee KD, Wang TH, Wang HS, Soong YK. Functional network analysis of the transcriptomes of mesenchymal stem cells derived from amniotic fluid, amniotic membrane, cord blood, and bone marrow. *Stem Cells*. 2007; 25:2511–2523. [PubMed: 17556597]

153. Tsai MS, Lee JL, Chang YJ, Hwang SM. Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. *Hum Reprod*. 2004; 19:1450–1456. [PubMed: 15105397]
154. Valonen PK, Moutos FT, Kusanagi A, Moretti MG, Diekmann BO, Welter JF, Caplan AI, Guilak F, Freed LE. In vitro generation of mechanically functional cartilage grafts based on adult human stem cells and 3D-woven poly(epsilon-caprolactone) scaffolds. *Biomaterials*. 2010; 31:2193–2200. [PubMed: 20034665]
155. Vater C, Kasten P, Stiehler M. Culture media for the differentiation of mesenchymal stromal cells. *Acta Biomater*. 2011; 7:463–477. [PubMed: 20688199]
156. Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W, Ho AD. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. *Exp Hematol*. 2005; 33:1402–1416. [PubMed: 16263424]
157. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, Fu YS, Lai MC, Chen CC. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells*. 2004; 22:1330–1337. [PubMed: 15579650]
158. Wang L, Seshareddy K, Weiss ML, Detamore MS. Effect of initial seeding density on human umbilical cord mesenchymal stromal cells for fibrocartilage tissue engineering. *Tissue engineering. Part A*. 2009; 15:1009–1017. [PubMed: 18759671]
159. Wang L, Tran I, Seshareddy K, Weiss ML, Detamore MS. A comparison of human bone marrow-derived mesenchymal stem cells and human umbilical cord-derived mesenchymal stromal cells for cartilage tissue engineering. *Tissue engineering. Part A*. 2009; 15:2259–2266. [PubMed: 19260778]
160. Wei Y, Zeng W, Wan R, Wang J, Zhou Q, Qiu S, Singh SR. Chondrogenic differentiation of induced pluripotent stem cells from osteoarthritic chondrocytes in alginate matrix. *European cells & materials*. 2012; 23:1–12. [PubMed: 22241609]
161. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997; 385:810–813. [PubMed: 9039911]
162. Witkowska-Zimny M, Wrobel E. Perinatal sources of mesenchymal stem cells: Wharton's jelly, amnion and chorion. *Cellular & molecular biology letters*. 2011; 16:493–514. [PubMed: 21786036]
163. Wu X, Wang S, Chen B, An X. Muscle-derived stem cells: isolation, characterization, differentiation, and application in cell and gene therapy. *Cell and tissue research*. 2010; 340:549–567. [PubMed: 20495827]
164. Yang Z, Sui L, Toh WS, Lee EH, Cao T. Stage-dependent effect of TGF-beta1 on chondrogenic differentiation of human embryonic stem cells. *Stem cells and development*. 2009; 18:929–940. [PubMed: 18855519]
165. Yang Z, Wu Y, Li C, Zhang T, Zou Y, Hui JH, Ge Z, Lee EH. Improved mesenchymal stem cells attachment and in vitro cartilage tissue formation on chitosan-modified poly(L-lactide-co-epsilon-caprolactone) scaffold. *Tissue engineering. Part A*. 2012; 18:242–251. [PubMed: 21902611]
166. Ye C, Li J, He Z, Nin X, Zhang Y, Shang X, Liu R, Duan Y. Multilineage differentiation of muscle-derived stem cells from GFP transgenic mice. *Biotechnology letters*. 2010; 32:1745–1752. [PubMed: 20632072]
167. Ye ZQ, Burkholder JK, Qiu P, Schultz JC, Shahidi NT, Yang NS. Establishment of an adherent cell feeder layer from human umbilical cord blood for support of long-term hematopoietic progenitor cell growth. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:12140–12144. [PubMed: 7527553]
168. Yoon IS, Chung W, Sung JH, Cho HJ, Kim JS, Shim WS, Shim CK, Chung SJ, Kim DD. Proliferation and chondrogenic differentiation of human adipose-derived mesenchymal stem cells in porous hyaluronic acid scaffold. *J Biosci Bioeng*. 2011; 112:402–408. [PubMed: 21802988]
169. Yoshimura K, Shigeura T, Matsumoto D, Sato T, Takaki Y, Aiba-Kojima E, Sato K, Inoue K, Nagase T, Koshima I, Gonda K. Characterization of freshly isolated and cultured cells derived

- from the fatty and fluid portions of liposuction aspirates. *Journal of cellular physiology*. 2006; 208:64–76. [PubMed: 16557516]
170. You M, Peng G, Li J, Ma P, Wang Z, Shu W, Peng S, Chen GQ. Chondrogenic differentiation of human bone marrow mesenchymal stem cells on polyhydroxyalkanoate (PHA) scaffolds coated with PHA granule binding protein PhaP fused with RGD peptide. *Biomaterials*. 2011; 32:2305–2313. [PubMed: 21190731]
 171. Young HE, Steele TA, Bray RA, Detmer K, Blake LW, Lucas PW, Black AC Jr. Human pluripotent and progenitor cells display cell surface cluster differentiation markers CD10, CD13, CD56, and MHC class-I. *Proceedings of the Society for Experimental Biology and Medicine*. Society for Experimental Biology and Medicine. 1999; 221:63–71.
 172. Yu HY, Tay CY, Leong WS, Tan SCW, Liao K, Tan LP. Mechanical behavior of human mesenchymal stem cells during adipogenic and osteogenic differentiation. *Biochem Bioph Res Co*. 2010; 393:150–155.
 173. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318:1917–1920. [PubMed: 18029452]
 174. Zhang X, Hirai M, Cantero S, Ciubotariu R, Dobrila L, Hirsh A, Igura K, Satoh H, Yokomi I, Nishimura T, Yamaguchi S, Yoshimura K, Rubinstein P, Takahashi TA. Isolation and characterization of mesenchymal stem cells from human umbilical cord blood: reevaluation of critical factors for successful isolation and high ability to proliferate and differentiate to chondrocytes as compared to mesenchymal stem cells from bone marrow and adipose tissue. *Journal of cellular biochemistry*. 2011; 112:1206–1218. [PubMed: 21312238]
 175. Zhang XH, Nakaoka T, Nishishita T, Watanabe N, Igura K, Shinomiya KI, Takahashi TA, Yamashita N. Efficient adeno-associated virus-mediated gene expression in human placenta-derived mesenchymal cells. *Microbiol Immunol*. 2003; 47:109–116. [PubMed: 12636261]
 176. Zhou J, Xu C, Wu G, Cao X, Zhang L, Zhai Z, Zheng Z, Chen X, Wang Y. In vitro generation of osteochondral differentiation of human marrow mesenchymal stem cells in novel collagen-hydroxyapatite layered scaffolds. *Acta Biomater*. 2011; 7:3999–4006. [PubMed: 21757035]
 177. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002; 13:4279–4295. [PubMed: 12475952]
 178. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng*. 2001; 7:211–228. [PubMed: 11304456]
 179. Zvaifler NJ, Marinova-Mutafchieva L, Adams G, Edwards CJ, Moss J, Burger JA, Maini RN. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res*. 2000; 2:477–488. [PubMed: 11056678]

Figure 1.

Stem cells can be isolated from multiple anatomical locations, encompassing adult and extra-embryonic tissues. The cell sources shown above have all been investigated for cartilage regeneration, although mesenchymal sources have been studied much more than other tissues.

Figure 2.

Comparison of reported GAG synthesis amounts per DNA after chondrogenic induction of human-derived cells using box-and-whisker plots. Results were calculated from recent literature by averaging the largest GAG value reported from each study. Cell types and their respective citations include Bone Marrow Stem Cells (BMSC, n=16),^{2, 3, 37, 55, 72, 75, 76, 78, 113, 115, 135, 154, 159, 165, 170, 176} Adipose-Derived Stem Cells (ASC, n=11),^{2, 21, 23, 37, 54, 63, 68, 76, 77, 113, 168} Synovial Membrane-Derived Stem Cells (SDSC, n=5),^{51, 82, 90, 97, 104} and Embryonic Stem Cells (ESC, n=6).^{87, 107, 145, 149, 150, 164}

Table 1

Overview of stem cell characteristics.

Cell Type	Method of Purification	Cartilage Synthesis Potential	Preferred Growth Factors	Quality of Regeneration <i>in vivo</i>
BMSC	FACS Plastic Adherence Density Gradient	Mass: 0.25 - 9 GAG: 0.8 - 122 Type II Collagen: 0.04 - 50	TGF- β 3	Scaffolds are generally well-integrated and phenotypically similar to cartilage, though mechanically inferior.
ASC	FACS Plastic Adherence	Mass: 0.1 - 1 GAG: 0.3 - 120 Type II Collagen: 0.02 - 3	TGF- β 1,3	Matrix secretion observed, as well as cartilage-like appearance. However, degree of integration inferior to that of BMSC scaffolds.
SDSC	FACS	Mass: 1 - 4.5 GAG: 2 - 45 Type II Collagen: 1 - 2.25	TGF- β 3, BMP-2	Successful matrix secretion observed. Degree of integration both similar and inferior to BMSCs.
MDSC	Plastic Adherence	Mass: 0.1 - 0.5	BMP-4	Appearance similar to native cartilage, though integration reported as inferior to BMSCs.
PBMSC	Plastic Adherence Density Gradient	-	TGF- β 1,3	Successful integration and regeneration reported.
WJSC	Plastic Adherence	-	TGF- β 1,3	-
UCBSC	Density Gradient	GAG: 2.5	BMP-2	Cartilage-like matrix secretion and surface architecture reported.
AFSC	Plastic Adherence	GAG: 3.5 - 4	TGF- β 1	Matrix secretion determined to be inferior to BMSCs.
PMSC	Plastic Adherence	Type II Collagen: 0.5-15	TGF- β 1	-
ESC	Mechanical Isolation	Mass: 0.6-3.75 GAG: 0.5-42 Type II Collagen: 2.5-100	TGF- β 1, BMP-7	Cartilage-like matrix secretion and surface architecture, as well as successful integration reported.
iPSC	-	-	TGF- β 1	Matrix secretion reported.

This table compiles general characteristics and procedures for all stem cell types concluded in this review. The extent and quality of engineered cartilage tissue varies based on reported values for pellet mass (mg), secreted sulfated glycosaminoglycans ($\mu\text{g GAG}/\mu\text{g DNA}$), and type II collagen gene expression (normalized to GAPDH). Other parameters were assessed based on literature cited in their respective sections. Stem cell types included are Bone Marrow Stem Cells (BMSC), Adipose-Derived Stem Cells (ASC), Synovial-Derived Stem Cells (SDSC), Muscle-Derived Stem Cells (MDSC), Peripheral Blood Mesenchymal Stem Cells (PBMSC), Wharton's Jelly Stem Cells (WJSC), Umbilical Cord Blood Stem Cells (UCBSC), Amniotic Fluid Stem Cells (AFSC), Placenta-Derived Mesenchymal Stem Cells (PMSC), Embryonic Stem Cells (ESC), and induced Pluripotent Stem Cells (iPSC).

Table 2

Surface marker expression profiles of adult stem cells.

Markers	BMSC	ASC	SDSC	MDSC	PBMSC
CD10	+	+	+	+	-
CD13	+	+	+	+	-
CD14	-	-	*	-	-
CD20	-	-	-	-	-
CD29	+	+	+	+	-
CD31	-	-	-	-	-
CD34	-	+	-	+/-	+/-
CD44	+	+	+	+	+
CD45	-	-	-	-	-
CD49a	+	+	+	+	+
CD49d	-	+	+	+	+
CD54	+	+	+	+	+
CD56	+	-	-	+	+
CD61	-	-	-	-	-
CD62E	-	-	-	-	-
CD71	+	+	+	+	+
CD73	+	+	+	+	+
CD80	-	-	-	-	-
CD90	+	+	+	+	+
CD104	+	-	-	-	-
CD105	+	+	+	+	+/-
CD106	+	+/-	-	+/-	+
CD117	-	+/-	-	+/-	+
CD133	+	+	+	+	+
CD144	+/-	-	-	+/-	-
CD146	+	LOW	+	+	+
CD166	+	+	+	+	+

Markers	BMSC	ASC	SDSC	MDSC	PBMSC
CD271	+	*	+	+	
HLA-ABC	+	+			
HLA-DR	-	-			+
STRO-1	+	*	+		-
SCA-1	+			+	
ALP	+	+		-	
NANOG	+	+			
OCT4	+	+			+
SOX2	+/-	+			
SSEA-4	+	+			

Markers are displayed in comparison with Bone Marrow Stem Cells (BMSC), 4, 29, 52, 118, 126, 151, 156 Other adult stem cell types include Adipose-Derived Stem Cells (ASC), 4, 52, 126, 147, 177 Synovial-Derived Stem Cells (SDSC), 4, 43, 102 Muscle-Derived Stem Cells (MDSC), 36, 67, 163 and Peripheral Blood Mesenchymal Stem Cells (PBMSC), 53, 58, 151 Bordered symbols indicate those that have been used for stem cell enrichment.

* Denotes markers found to enhance chondrogenesis.

Table 3

Surface marker expression profiles for extra-embryonic stem cells.

Markers	BMSC	WJSC	UCBSC	AFSC	PMSC
CD9	+	+			
CD10	+	+	+	+	
CD11b	-	-			-
CD13	+	+			
CD14	-	-	-	-	-
CD18	+	-			
CD19	-	-	-		-
CD29	+	+	+	+	
CD31	-	-		-	
CD34	-	-	-	-	-
CD44	+	+	+	+	+
CD45	-	-	-	-	-
CD49b	+		+		
CD49d	-		+		
CD49e	+			+	
CD51	+	+	+		
CD54	+			+	
CD58	-		+	+	
CD73	+	+		+	+
CD90	+	+	-	+	+
CD105	+	+	+	+	+
CD117	-			+	-
CD133	+		-	-	+
CD144	+/-	-			
CD166	+	+		+	+
CD349	+				+
HLA-ABC	+	+/-	+	+	+
HLA-DR	-	-	-	-	-
STRO-1	+				-

Markers	BMSC	WJSC	UCBSC	AFSC	PMSC
NANOG	+	LOW		+	
OCT4	+	+	+	+	
SSEA-4	+		+	+	-

Markers are displayed in comparison with Bone Marrow Stem Cells (BMSC), **4, 15, 29, 52, 118, 126, 151, 156** Extraembryonic cells include Wharton's Jelly Stem Cells (WJSC), **44, 59, 86, 112, 157** Umbilical Cord Blood Stem Cells (UCBSC), **89, 100** Amniotic Fluid Stem Cells (AFSC), **4, 8, 112** and Placenta-Derived Mesenchymal Stem Cells (PMSC), **52, 134** Bordered symbols indicate those that have been used for stem cell enrichment.

Table 4

Surface marker expression profiles for embryonic and embryonic-like stem cells.

Markers	BMSC	ESC	iPSC
CD9	+	+	
Alkaline Phosphatase	+	+	
NANOG	+	+	+
OCT4	+	+	+
SOX2	+/-	+	+
SSEA-4	+	+	

Markers are displayed in comparison with Bone Marrow Stem Cells (BMSC).^{4, 15, 118, 126, 151} Embryonic and embryonic-like cells include Embryonic Stem Cells (ESC)^{35, 38} and induced Pluripotent Stem Cells (iPSC).¹⁷³

Table 5

Standard and alternative growth factors used for the induction of chondrogenic differentiation.

Stem Cell Type	Standard Growth Factors	Alternative Growth Factors
BMSC	TGF- β 3 ^{13, 119, 176} TGF- β 1 ^{11, 13, 111, 119, 138, 176}	TGF- β 3, BMP-6 ^{132, 133} TGF- β 3, BMP-4 ¹³³ TGF- β 3, BMP-2 ¹³³ TGF- β 2 ^{13, 77} BMP-7 ¹¹⁵
ASC	TGF- β 1 ^{10, 22, 101, 116, 178} TGF- β 3, BMP-6 ⁵⁴ TGF- β 3 ^{54, 113} BMP-6 ⁷⁷	TGF- β 2, BMP-7 ^{77, 88} TGF- β 2, BMP-6 ⁷⁷ TGF- β 2, BMP-2 ⁷⁷ TGF- β 1, BMP-6 ⁹⁵ TGF- β 1, BMP-2 ¹⁶⁸ TGF- β 2 ^{76, 77} BMP-7 ⁷⁷ BMP-2 ⁷⁷
SDSC	TGF- β 3, BMP-2 ^{120, 137} TGF- β 1 ^{14, 32, 42, 104}	TGF- β 1, BMP-2 ⁵¹ TGF- β 3, BMP-7 ¹³⁶ TGF- β 3 ^{42, 97}
ESC	TGF- β 1 ^{60, 87, 107, 145, 164} BMP-2 ^{48, 108, 149, 150}	TGF- β 1, BMP-2 ¹⁴⁹ TGF- β 1, BMP-7 ^{48, 107, 149} BMP-7 ^{107, 149}

Description of growth factors commonly used, as well as alternative options, for chondrogenic differentiation of stem cells derived from human and animal sources as reported in recent literature. Information relates to Bone Marrow Stem Cells (BMSC), Adipose-Derived Stem Cells (ASC), Synovial-Derived Stem Cells (SDSC), and Embryonic Stem Cells (ESC).