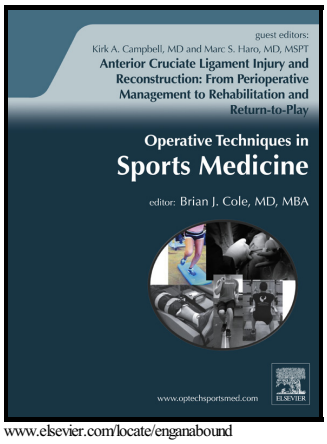

Stem and Progenitor Cells for Cartilage Repair: Source, Safety, Evidence and Efficacy

Francisco Rodriguez Fontan, Nicolas S. Piuzzi, Jorge Chahla, Karin Payne, Robert F. LaPrade, George F. Muschler, Cecilia Pascual-Garrido

PII: S1060-1872(16)30061-2
DOI: http://dx.doi.org/10.1053/j.otsm.2016.12.005
Reference: YOTSM50572

To appear in: *Operative Techniques in Sports Medicine*

Cite this article as: Francisco Rodriguez Fontan, Nicolas S. Piuzzi, Jorge Chahla, Karin Payne, Robert F. LaPrade, George F. Muschler and Cecilia Pascual-Garrido, Stem and Progenitor Cells for Cartilage Repair: Source, Safety, Evidence and Efficacy, *Operative Techniques in Sports Medicine,* http://dx.doi.org/10.1053/j.otsm.2016.12.005

Stem and Progenitor Cells for Cartilage Repair: Source, Safety, Evidence and Efficacy

Francisco Rodriguez Fontan, MD[1]; Nicolas S. Piuzzi, MD *[2,3]; Jorge Chahla, MD *[4]; Karin Payne, PhD[1, 5]; Robert F. LaPrade, MD, PhD*[4]; George F. Muschler, MD*[2]; Cecilia Pascual-Garrido, MD[1]

[1] *Department of Orthopedics,* [5] *Regenerative Orthopedics Laboratory, University of Colorado Denver, Aurora, CO, USA*

[2] *Department of Orthopedic Surgery and Bioengineering Cleveland Clinic Foundation, Cleveland, OH, USA*

[3] *Instituto Universitario del Hospital Italiano de Buenos Aires, Buenos Aires, Argentina*

[4]*Steadman Philippon Research lnstitute, Vail, CO, USA*

Corresponding Author:

Cecilia Pascual-Garrido, MD

Assistant Professor.

Department of Orthopedics, University of Colorado

University of Colorado Hospital – Anschutz Outpatient Pavilion

1365 N. Aurora Court

Aurora, CO 80045

P: 720-848-8200

Cecilia.pascual-garrido@ucdenver.edu

cpgruns@gmail.com

Author's disclosure statement: Dr. Pascual Garrido receives research support from Biomet-Zimmer. Dr. Muschler reports that he receives funding from NIH and the Department of Defense. He serves as a consultant to FDA and NIH, and receives funding from a research agreement with Fortus. Dr. LaPrade is a consultant and receives royalties from Arthrex, Ossur and Smith & Nephew. The other authors report no actual or potential conflict of interest in relation to this article.

ABSTRACT

Cartilage is a sensitive tissue prone to damage with sports and aging. Degenerative joint diseases are among the most profound in limiting quality of life and daily activities. Biological therapies have become available to potentially treat osteoarthritis and focal chondral defects. However, there remains no efficient way to regenerate native hyaline cartilage. Stem cell therapy and bioengineering constitutes a promising field which may transform our paradigms in orthopaedics. This review provides an overview of the current status and efficacy of stem and progenitor cell therapies which include cultured and non-expanded sources such as bone marrow, adipose tissue, synovium and peripheral blood. The purpose of this review is to summarize the reported potential of adult stem cells therapies focusing on focal chondral defects (FCD) and osteoarthritis (OA).

Keywords: cartilage; progenitor cells; stem cells; osteoarthritis; focal chondral lesions; biologics

INTRODUCTION

Cell based therapies are exponentially emerging as promising treatments for many musculoskeletal conditions affecting athletes and aging populations. [1-3] Stem and progenitor cell therapies provide a potential for clinical benefit through mechanisms of tissue regeneration or immunomodulation.[4-9] Fertile fields for stem cell use within orthopaedics include focal chondral lesions, osteoarthritis (OA), fracture healing, and soft tissue lesions involving tendon, muscle and ligaments.

Progenitor cells include any cell that can proliferate to form progeny and can differentiate into a derived tissue. Stem cells are a special subset of progenitor cells which have "self- renewal capacity". [10-14] Self - renewal is the process where a cell divides asymmetrically, producing two daughter cells. One daughter cell is identical to the initial cell and remains available for another asymmetrical "self-renewing" cell division. The second cell, a progenitor cell which, unlike the stem cell proceeds to divide and differentiate. Progenitor cells are far more prevalent than stem cells in any tissue. Often the term "stem cell" is used incorrectly to describe both stem and progenitor cells as a whole. [15, 16]

Use of an accurate standardized nomenclature is crucial for understanding the biological behavior of cells *in vivo* and *in vitro*, and improves science communication. Stem cells can be classified in several ways: 1) autologous or allogenic, 2) adult, embryonic or IPSCs (induced pluripotent stem cells) and, 3) native (tissue resident) or culture expanded. The purpose of this review is to assess adult stem cells utility within orthopaedics with a special focus on focal chondral lesions and OA, stating sources, safety, efficacy and subjective and objective outcomes.[17]

Embryonic stem cells (ESCs) have pluripotent differentiating potential, towards all tissues, such as: ectoderm, endoderm or mesoderm derived. [14] They are obtained from the embryo in its first stages [18, 19] and an often time involves ethical issues [18] and risk

of oncogenic transformation. [20, 21] Recent advances in genetic manipulation of adult fibroblasts, mainly from dermis, and fetal cells have generated induced pluripotent stem cells (IPSCs) through viral and non - viral gene reprogramming mechanisms. [22-26] They are also pluripotent in nature, and since they can be obtained from adult tissue, they are not associated with the ethical concerns surrounding ESCs. In contrast, adult stem cells are capable of differentiating into one or more embryonically-related tissue phenotypes; they can be easily obtained from several tissues, they do not present ethical issues and are usually not associated with the concern of malignant transformation.[27]

Adult Stem and Progenitor Cells Nomenclature

Many terms have been used to describe the same adult stem and progenitor cell populations in native tissue. In an approach to provide clarification the term Connective Tissue Progenitors (CTPs) has been proposed. CTPs include the entire heterogeneous native (tissue resident) population of stem and progenitor cells, with the potential to be activated and generate progeny that can contribute to one or more connective tissues (e.g., bone, fat, cartilage, fibrous tissue, blood and muscle).[10, 11, 28] CTPs are resident in and can be harvested from bone marrow, fat, cartilage and other tissues. However, CTPs in each tissue often have different niches, biological attributes and potential. The term CTP recognizes that these tissue-derived cells are not a uniform population, and until detailed characterization is achieved, CTPs may only be detectable by their capacity to proliferate and form colony on a 2D surface or in a 3D viscous medium colony forming unit (CFU) assay. [10, 11, 28]

Conversely, culture expanded cells differ from native and minimally manipulated cells. Culture expanded cells provide more homogenous populations and greater numbers than native tissue derived cells. However, cell attributes change quickly in culture. The most promoted and commercialized example of culture expanded cells are mesenchymal stem cells (MSCs).[29] MSCs are culture expanded and plastic adherent adult cells that to be classified as cells which retain the capacity for trilineage differentiation (cartilage, bone or adipose tissue), [30-32] they must also express the following set of surface markers CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules [33] (**Table 1**).

The International Society for Cellular Therapy (ISCT) developed these criteria to define MSCs. [29, 33] Without these proven characteristics the MSC term should not be used. The MSC definition although ideal, has helped bring order to the indiscriminate use of "MSC" to describe all culture-expanded fibroblasts regardless of characterization. However recent data demonstrates that a MSC population, that meets all these criteria, can vary widely in biological potential. [34]

To date no specific set of markers identify all CTPs from native tissues. Nevertheless, the concentration, prevalence and biological potency can be estimated with in vitro colony forming unit (CFU) assays. CTPs assays have been enhanced dramatically by using, criteria incorporated into the American Society for Testing Materials (ASTM) International standard: "Automated Colony Forming Unit (CFU) Assays—Image Acquisition and Analysis Method for Enumerating and Characterizing Cells and Colonies in Culture" for use with automated system for image analysis. [35] Traditionally methods of colony counting using subjective "skilled "observer have been shown to be subject to wide variation. [36]

Freshly obtained tissue (e.g. Bone Marrow Aspirate - BMA) contains CTPs, but the prevalence and function of these CTPs are not known without CFU assay. Therefore, if unprocessed and unmeasured cells from BMA are used, they should be described on the most quantitative measured metric, for example, mixed tissue derived nucleated cells (MTDNCs) [37], or mixed bone marrow derived nucleated cells (MBMDNCs).

Since CTPs are the heterogeneous population of stem and progenitor cells resident in native tissue, they include the proliferative cells from which culture expanded MSCs are derived. However, the attributes of colony founding CTPs are distinct from the attributes in the definition of MSCs from the International Society for Cellular Therapy (ISCT). Both stem and progenitor cells are believed to be in almost every tissue in the body and have the ability to migrate towards sites of injury and neoplasm through chemokines. [38-42] These assist within tissue regeneration either directly through differentiation into adult cells or indirectly through cytokines, growth factors, chemokines for immunomodulation, stimulating angiogenesis, and recruiting tissue specific progenitor cells, in order to create a regenerative microenvironment.[43-46]

Stem Cells and Progenitor Cell Sources

Native stem and progenitor cells (CTPs) can be isolated from all connective tissues that contain CTPs, such as: bone marrow, bone, adipose, synovial membrane, peripheral blood and periosteum. [47-50] The cells obtained from each tissue source, vary including intrinsic differences in proliferation and differentiation capacity towards certain lineages.[51]

It has been reported that better outcomes on grafts survival are achieved when harvesting the cells from the same or neighboring tissue from the one they will be used to regenerate.[52] Special attention to the graft's fate should be taken into account since it may be affected depending on the harvesting site and characteristics.

When culture expansion is performed, culture supplementation with growth factors assists MSC differentiation towards any of the three lineages [53-55] (**Table 2**).

Bone Marrow derived Progenitor Cells

Bone marrow is one of the most common sources for harvesting stem and progenitor cells, usually by iliac crest aspiration. CTPs account for a small population within the bone marrow. CTPs concentration averages from 1,000 to 2,000 CTPs/ml of aspirate, with an estimated prevalence between $1x10^{-4}$ to $1x10^{-6}$ cells [56], depending on patient variables and the aspiration technique.[57-60] Bone marrow aspirate has been one of the most common sources of cells used in therapy due to its accessibility for surgeons, and the extensive studies done upon these. [61-64] Optimal technique stipulates that less than 2-4 ml aspirate is taken per site. Depending on the total volume needed, different numbers of bone perforations may be required. Inserting the trochar deep into the ilium, aspirating 2-4 ml, retracting the needle 5 mm and aspirating more, and repeating this step allows for the harvesting of more stem cells. Additional bone perforations may be required to increase the total volume of the bone marrow harvest. This increases the yield of CTPs harvested, by limiting hemodilution from peripheral blood. [57, 58, 65, 66] If aspirating 10 ml at once, this will drop CTPs concentration by

2 – 4 folds. [58] Processing can increase both the concentration and prevalence of CTPs, by removing RBC (red blood cells), serum and non-CTPs from a mixed population. [59, 67]

There are different alternatives to achieve a higher number of stem/progenitor cells from the BMA sample: 1) in vitro culture expansion to obtain BM-MSCs, or 2) processing techniques, like density separation. Currently, preparations of autologous concentrated bone marrow aspirate (BMAC) are used directly intraoperatively to process BMA for implantation with minimal manipulation.[68] A BMAC shortcoming is the heterogeneous cell population found in its preparation, including endothelial, hematopoietic and inflammatory cells. Preparations also vary widely between individuals and due to age and sex [60, 69-72], and by the site of aspiration within a same individual. Density separation methods to prepare BMAC often require at least 60 ml of BMA from the anterior or posterior iliac crest, but this is not taken from one site. The BMA is ideally aspirated as multiple 2-4 ml samples (to reduce hemodilution) through cortical perforations, going deeper into the medullary cavity using the same skin incision.[66] This is done in a perpendicular technique (to the iliac crest): having the needle in the medullary cavity, advancing every 5-10 mm using the obturator and in a fan-like projection, once or twice followed by aspiration, allows two or three aspirates through a same cortical perforation, and then move into a new cortical perforation. Another approach is the parallel technique (to inner and outer tables of the iliac crest): advancing the needle every 5-10 mm using the obturator between both tables and in a fan – like projection, allows taking 3 or more aspirates [10]. Centrifugation (density separation) is used to remove platelets, granulocytes and red blood cells. This concentrates the number of cells and CTPs, therefore the cells that can be used intraoperatively [66] (**Figure 1**).

Adipose tissue

Another common source of stem and progenitor cell therapy is adipose tissue. [73, 74] It is mainly harvested from aspirates or liposuction, or surgical removal (e.g., a recently emerging source is the infrapatellar fat pad). [75-77] It is far less cellular than bone marrow aspirate, but the CTPs prevalence is higher, averaging 1 in 4,000 cells. Some authors consider adipose to be an attractive and easily available reservoir for stem cell therapy.[78] However, adipose derived colony founding CTPs and culture expanded cells present different patterns of behavior, cell proliferation and differentiation, compared to CTPs found in bone marrow. Therefore a better characterization is needed. [73] These variables are in turn affected with the same intrinsic factors mentioned before, such as individuals, age and sex. It has been reported that ASCs (adipose stem cells) have reduced chondrogenic and osteogenic capacity under standard culture conditions [79-82], in favor of a more robust differentiation towards muscle cells or cardiomyocytes. [83] This may be in part, due to endogenous reduced expression of BMP (bone morphogenetic protein) mRNA for subtypes 2, 4 and 6, and lacking expression of TGF-β- receptor-1 (transforming growth factor). [79] BMPs promote chondrogenic differentiation and cartilage production and have autocrine stimulation on other MSCs for producing the same factors. Using pellet cultured ASCs under chondrogenic factors, chondrogenic differentiation and collagen formation takes place, with TGF- β and BMP-6 being the strongest combination.

Different names lead to confusion when referring to adipose tissue derived stem cells. The International Federation for Adipose Therapeutics and Science (IFATS) stated that adipose derived stem cells (ASCs) should be the term to adopt when addressing the isolated culture expanded, plastic adherent and multipotent stem cells. [84] There are many subcutaneous white adipose tissue depots for stem cell recovery: arm, thigh, abdomen and breast. [85] The standard sequence consists upon tumescent lipoaspirate; enzymatic digestion follows using collagenase, trypsin, dispase, among other enzymes in varying combinations, under determined time (30 to 60 min) and temperature (37°C). [86] Once enzymes are neutralized, centrifugation follows, allowing the separation of the floating mature adipocytes from the stromal vascular fraction (SVF), a heterogeneous cellular population consisting of red blood cells, fibroblasts, endothelial cells, lymphocytes, pericytes, monocytes, adipose stromal cells, hematopoietic stem cells and progenitor cells. [87, 88] Finally, SVF cells are seeded into culture, and after further purification through washing and culture expansion steps in media, similar to the ones used with BM-MSCs, in order to deplete most of hematopoietic cells, ASCs can be obtained. The quantity used also varies from 5,000 to 1,500,000/ml of tissue collected. [89] Different methods were proposed for extracting ASCs. For instance ultrasound - assisted liposuction appeared to be promising as compared to standard tumescent liposuction, but further studies showed that stem cell viability and proliferative capacity seemed to be decreased with these processing methods. [90]

ASCs are similar to BM-MSCs, but exhibit different attributes and behavior. First, the differentiation potency tends towards muscle tissue. Second, the immunophenotype is slightly different with a set of markers above 90% identical [75, 84, 91-93] (**Table 1**).

Synovium

Synovium derived stem cells (SDSCs) are increasingly recognized as a viable option when aiming for cartilage repair. [94] Comparative human and animal studies have shown that between adipose, muscle, bone marrow, periosteum and synovium derived adult stem cells; although ranging from 1,000 -30,000 stem cells/ml of tissue collected [89], synovium has the highest yield. [95] In terms of differentiation potential it has been reported to have greater adipogenic and chondrogenic potential than BM-MSCs. [95-97]

After culture expansion and isolation of SDSCs, these cells present identifiable set of markers with interesting immunophenotype subpopulations that reflect their different chondrogenic potential and familiarity to BM-MSCs [97, 98] (**Table 1**).

The knee is the most common studied site for harvesting SDSCs. The standard procedure consists of obtaining synovium with subsynovial tissue through arthroscopy, followed by enzymatic digestion with a collagenase/dispase solution at 37°C for 3 hours, and finally filtering the cells through a nylon filter to yield single-cell suspensions. These are then cultured in different media depending on the desired adult tissue. [99]

Peripheral blood

Peripheral blood mononuclear cells (PBMCs) [100] or peripheral blood progenitor cells (PBPCs) [101] give new perspectives on stem cell therapy, which cannot be underestimated, as they are involved in tissue healing of many organs. [102-104]

PBMCs are a heterogeneous cell population harvested from fresh whole blood.[105] A common technique is as follows: venous blood sample is collected and centrifuged, and nucleated cells from the buffy coat layer can be frozen and stored for later use or culture expanded. When freshly collected, flow cytometry for PBMCs shows 90 % expression for hematopoietic markers CD34 and

CD45, and negative for MSC set of markers. Peripheral blood does not contain CTPs or MSC-like cells under normal circumstances. These are embedded in niches in the bone marrow, subject to low oxygen levels. CTPs can be present in the blood stream after trauma or marrow stimulation.[106]

Comparative studies have shown different growth patterns and sets of markers when culturing human PBMCs in different oxygen tension conditions [105] (**Table 1**). Under hypoxic conditions, similar to their bone marrow niches, they expressed more than 90% MSCs markers and maintained a trilineage differentiating potential for all three chondrogenic, osteogenic and adipogenic tissues. [105] This phenomenon reflects a potential use for cartilage repair, which is hypoxic by nature. On the contrary, under normoxic conditions, the PBMC rendered macrophage-like adherent cell population expressing less than 50% of MSCs markers. [105]

Another approach employed for harvesting these cells with a higher yield, involves initiating a week prior to the blood drawn a series of subcutaneous administration of human granulocyte colony-stimulating factor (G-CSF) to the patient, which regulates and promotes the release of neutrophils and monocytes from the bone marrow into the bloodstream, increasing the circulating concentration of PBMCs. These growth factors mobilized PBMCs are collected by an automated cell separator (aphaeresis) using a central or peripheral venous access. [101, 107-109] In healthy adults, depending on the protocols used, the yield averages $2 – 5 \times 10^6$ CD 34^+ cells per kg of patient's body weight.[110, 111]

When PBMCs are separated into its different cell components in an attempt to isolate the stem and progenitor cells responsible for this behavioral pattern (e.g. monocytes $CD14^+$, granulocytes, lymphocytes) through CD14 and CD105, and cultured in hypoxic and normoxic media, all differentiated into macrophage-like adherent cells, and failed on fibroblastic-like cell differentiation. [105] This supports the importance of cell signaling through direct contact and chemokines, in a heterogeneous cell population. [112-115] Co-culturing PBMCs and ASCs in a chondrogenic media show a synergic differentiating and migrating potency on ASCs populations. [113] Therefore, it reflects cell signaling as a centerpiece for cartilage repair. Apparently, hypoxia is the corner stone for triggering mononuclear migration from blood vessels (normoxic media) towards injured tissue, and differentiating into hematopoietic and non-hematopoietic cells. [116]

Comparative studies performed in animals show promising cartilage repair in vivo for osteochondral lesions, with a similar outcome as with BM-MSCs [105, 117-120] (**Table 1**).

Why use Stem and Progenitor Cell Therapy?

Articular cartilage is a weight and friction bearing tissue, composed of extracellular matrix (ECM), mainly collagen type 2, proteoglycans, aggrecans and chondrocytes. Its only vascular supply is the subchondral bone. [121] Its low cellularity and avascularity, makes for a limited regeneration and cartilage restoration capacity. [122]

A cartilage defect can be: 1) chondral or partial thickness, when confined to articular cartilage, or 2) osteochondral, or full thickness, when the defect is deep enough to affect the subchondral bone. These lesions can be classified under the Outerbridge classification going from 0 to 4 depending on how severe and deep the lesion is. [123, 124] Generally while no repair takes place in chondral defects, a repairing attempt is seen in osteochondral defects on account of the subchondral blood supply, rendering a

suboptimal tissue by stem and progenitor cells migrating from the bone marrow. [125] Small full thickness lesions are repaired with hyaline cartilage, but large ones are usually repaired with fibrocartilage formation. [41, 126] Multiple treatments are currently used for cartilage defects. These include microfracture, arthroscopic lavage and debridement, autologous or allogenic osteochondral grafting, and autologous chondrocyte implantation (ACI) [127] among others. Although promising with midterm results and pain relief, the main disadvantage is that some of them are a two - step procedure where harvesting and expanding of chondrocytes is needed. In addition, many of these therapies often lead to the formation of fibrocartilage. [128] This last is a hyaline like tissue, composed mainly by collagen 1, and with far less load and shear stress resistance, leading to eventual breakdown and secondary OA in the long term. [129]

OA is a highly prevalent joint disease affecting athletes following trauma and aging people. [130] Multiple factors have a role in OA pathophysiology, such as sex, age, injury, obesity, joint misalignment and genetic predisposition. Common features include chronic low grade inflammation, with subchondral lesions and progressive joint degeneration. The progression leads to loss of function in final stages due to increasing pain, swelling and loss of range of motion (ROM). [131] OA may affect multiple joints, the most prevalent and incapacitating is the knee and hip. [1] The current treatments for early to moderate OA include non-steroid anti-inflammatory drugs (NSAIDS), corticosteroids, hyaluronic acid, and physiotherapy; however none seems to stop the degenerative progression, and in the best scenario provide some pain relief and improved function. This provides an opportunity to step in with stem cell therapy research and innovation, due to their hypoimmunogenic profile [132], immunosuppressive activity, as well as their proliferation and differentiation capacity into adult tissues.

Currently, BMAC, using density separation (centrifuge) is one of the few “cell therapies” that is allowed by FDA regulations to deliver progenitor cells. All other further manipulation, are under 361 and 351 sections of the Public Health Safety (PHS) Act. [133] The FDA categorized stem cell therapies as human cells, tissues, and cellular and tissue based products (HCT/Ps). Section 361 mandates the FDA to regulate low-risk HCT/Ps and provides safety without requiring preclinical studies. Four principles must be fulfilled to be categorized as low risk: 1- minimal manipulation, 2- autologous or non-systemic effect, 3-non-combination product, and 4- homologous use. If a product does not meet all of these 4 principles, it must be regulated under section 351, which demands preclinical studies, clinical studies, and premarket review. Little to no stem cell therapy is under section 361.[134]

Clinical Evidence and Efficacy for Focal Chondral Lesions and OA

Multiple clinical investigators have reported on the efficacy and safety of stem cell therapy in cartilage repair for OA and focal chondral lesions. The following clinical trials: Vega et al. [135], Koh and Choi [136], Koh et al.[37], Wong et al. [137], Saw et al. [107], Skowronski and Rutka [138] and Lee et al. [139] have reported outcomes on cell based therapies. Although showing significant heterogeneity in the cell-therapies used, a common denominator was that the majority of them showed positive outcomes, with minimum post surgical adverse events.

Conversely, it might be premature to generalize that cell based therapies provide benefit for the treatment of patients compared to other available treatments.

The efficacy must be tested with rigorous randomized and blinded trials, large sample sizes and longer term follow up. The outcomes assessed with thorough standardized metrics as the ones used above [140], the inclusion of imaging, and second look arthroscopy with biopsy should be the mainstay. High quality clinical studies will be the answer to an active patient population seeking higher levels of improvement [141], and the importance of blinded trials in future studies should surpass the high level expectation of the patients enrolled which constitutes a source of bias. [142, 143]

CONCLUSION

Stem and progenitor cells hold a promising future. There has been significant advance in cell therapy options for OA and focal chondral lesions. Overall these therapies using minimally manipulated autogenous cells appear to be safe. However, a rigorous approach must be made to provide better characterization of the identity, concentration, prevalence and biological potential of the stem and progenitor cells being transplanted. There is a need for standardization beginning with the stem cell nomenclature, cell processing and outcome measurement.

In the upcoming years stem cell therapy might become a first line therapy within orthopaedics. This will require a parallel growth of stem cell adjuncts such as scaffolds, PRP (platelet - rich plasma), soluble growth factors, among other bioengineering techniques. All of these have potential for a substantial synergic effort.

References

1. Gupta, P.K., et al., *Mesenchymal stem cells for cartilage repair in osteoarthritis.* Stem Cell Res Ther, 2012. **3**(4): p. 25.
2. Mithoefer, K., et al., *Clinical efficacy of the microfracture technique for articular cartilage repair in the knee: an evidence-based systematic analysis.* Am J Sports Med, 2009. **37**(10): p. 2053-63.
3. Jorgensen, C., et al., *Multipotent mesenchymal stromal cells in articular diseases.* Best Pract Res Clin Rheumatol, 2008. **22**(2): p. 269-84.
4. Ross, R., N.B. Everett, and R. Tyler, *Wound healing and collagen formation. VI. The origin of the wound fibroblast studied in parabiosis.* J Cell Biol, 1970. **44**(3): p. 645-54.
5. Sugaya, K., *Potential use of stem cells in neuroreplacement therapies for neurodegenerative diseases.* Int Rev Cytol, 2003. **228**: p. 1-30.
6. Chapel, A., et al., *Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome.* J Gene Med, 2003. **5**(12): p. 1028-38.
7. Di Nicola, M., et al., *Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli.* Blood, 2002. **99**(10): p. 3838-43.
8. Le Blanc, K., et al., *Mesenchymal stem cells inhibit the expression of CD25 (interleukin-2 receptor) and CD38 on phytohaemagglutinin-activated lymphocytes.* Scand J Immunol, 2004. **60**(3): p. 307-15.
9. Jorgensen, C. and D. Noel, *Mesenchymal stem cells in osteoarticular diseases.* Regen Med, 2011. **6**(6 Suppl): p. 44-51.
10. Muschler, G.F., C. Nakamoto, and L.G. Griffith, *Engineering principles of clinical cell-based tissue engineering.* J Bone Joint Surg Am, 2004. **86-A**(7): p. 1541-58.
11. Muschler, G.F., R.J. Midura, and C. Nakamoto, *Practical Modeling Concepts for Connective Tissue Stem Cell and Progenitor Compartment Kinetics.* J Biomed Biotechnol, 2003. **2003**(3): p. 170-193.
12. Patel, J., et al., *Self-Renewal and High Proliferative Colony Forming Capacity of Late-Outgrowth Endothelial Progenitors Is Regulated by Cyclin-Dependent Kinase Inhibitors Driven by Notch Signaling.* Stem Cells, 2016. **34**(4): p. 902-12.

13. Gerson, S.L., *Mesenchymal stem cells: no longer second class marrow citizens.* Nat Med, 1999. **5**(3): p. 262-4.
14. Itskovitz-Eldor, J., et al., *Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers.* Mol Med, 2000. **6**(2): p. 88-95.
15. Marcucio, R.S., et al., *Stem Cell Therapies in Orthopaedic Trauma.* J Orthop Trauma, 2015. **29 Suppl 12**: p. S24-7.
16. Chahla, J., et al., *Intra-Articular Cellular Therapy Injection for Knee Osteoarthritis and Focal Cartilage Defects.* The Journal of Bone and Joint Surgery, 2016.
17. Ashton, B.A., et al., *Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo.* Clin Orthop Relat Res, 1980(151): p. 294-307.
18. Thomson, J.A., et al., *Embryonic stem cell lines derived from human blastocysts.* Science, 1998. **282**(5391): p. 1145-7.
19. Shamblott, M.J., et al., *Derivation of pluripotent stem cells from cultured human primordial germ cells.* Proc Natl Acad Sci U S A, 1998. **95**(23): p. 13726-31.
20. Hirzinger, C., et al., *ACL injuries and stem cell therapy.* Arch Orthop Trauma Surg, 2014. **134**(11): p. 1573-8.
21. Lodi, D., T. Iannitti, and B. Palmieri, *Stem cells in clinical practice: applications and warnings.* J Exp Clin Cancer Res, 2011. **30**: p. 9.
22. Hynes, K., et al., *Generation of functional mesenchymal stem cells from different induced pluripotent stem cell lines.* Stem Cells Dev, 2014. **23**(10): p. 1084-96.
23. Cui, P., et al., *Biological characterization and pluripotent identification of sheep dermis-derived mesenchymal stem/progenitor cells.* Biomed Res Int, 2014. **2014**: p. 786234.
24. Takahashi, K., et al., *Induction of pluripotent stem cells from fibroblast cultures.* Nat Protoc, 2007. **2**(12): p. 3081-9.
25. Takahashi, K., et al., *Induction of pluripotent stem cells from adult human fibroblasts by defined factors.* Cell, 2007. **131**(5): p. 861-72.
26. Gonzalez, F., S. Boue, and J.C. Izpisua Belmonte, *Methods for making induced pluripotent stem cells: reprogramming a la carte.* Nat Rev Genet, 2011. **12**(4): p. 231-42.
27. Centeno, C.J., et al., *Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique.* Curr Stem Cell Res Ther, 2011. **6**(4): p. 368-78.
28. Muschler, G.F. and R.J. Midura, *Connective tissue progenitors: practical concepts for clinical applications.* Clin Orthop Relat Res, 2002(395): p. 66-80.
29. Caplan, A.I., *Mesenchymal stem cells.* J Orthop Res, 1991. **9**(5): p. 641-50.
30. Pittenger, M.F., et al., *Multilineage potential of adult human mesenchymal stem cells.* Science, 1999. **284**(5411): p. 143-7.
31. Liu, H., et al., *A subpopulation of mesenchymal stromal cells with high osteogenic potential.* J Cell Mol Med, 2009. **13**(8b): p. 2436-47.
32. Wakitani, S., et al., *Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage.* J Bone Joint Surg Am, 1994. **76**(4): p. 579-92.
33. Dominici, M., et al., *Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement.* Cytotherapy, 2006. **8**(4): p. 315-7.
34. Lo Surdo, J.L., B.A. Millis, and S.R. Bauer, *Automated microscopy as a quantitative method to measure differences in adipogenic differentiation in preparations of human mesenchymal stromal cells.* Cytotherapy, 2013. **15**(12): p. 1527-40.
35. International, A., *ASTM F2944-12, standard test method for automated colony forming unit (CFU) assays - image acquisition and analysis method for enumerating and characterizing cells and colonies in culture.* ASTM International, West Conshohocken, PA, 2012.
36. Powell, K.A., et al., *Quantitative image analysis of connective tissue progenitors.* Anal Quant Cytol Histol, 2007. **29**(2): p. 112-21.
37. Koh, Y.G., et al., *Comparative outcomes of open-wedge high tibial osteotomy with platelet-rich plasma alone or in combination with mesenchymal stem cell treatment: a prospective study.* Arthroscopy, 2014. **30**(11): p. 1453-60.
38. Granero-Molto, F., et al., *Regenerative effects of transplanted mesenchymal stem cells in fracture healing.* Stem Cells, 2009. **27**(8): p. 1887-98.
39. Mosna, F., L. Sensebe, and M. Krampera, *Human bone marrow and adipose tissue mesenchymal stem cells: a user's guide.* Stem Cells Dev, 2010. **19**(10): p. 1449-70.

40. Nakamizo, A., et al., *Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas.* Cancer Res, 2005. **65**(8): p. 3307-18.
41. Baghaban Eslaminejad, M. and E. Malakooty Poor, *Mesenchymal stem cells as a potent cell source for articular cartilage regeneration.* World J Stem Cells, 2014. **6**(3): p. 344-54.
42. Honczarenko, M., et al., *Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors.* Stem Cells, 2006. **24**(4): p. 1030-41.
43. LaPrade, R.F., et al., *Biologic Treatments for Sports Injuries II Think Tank-Current Concepts, Future Research, and Barriers to Advancement, Part 1: Biologics Overview, Ligament Injury, Tendinopathy.* Am J Sports Med, 2016.
44. Liu, Z.J., Y. Zhuge, and O.C. Velazquez, *Trafficking and differentiation of mesenchymal stem cells.* J Cell Biochem, 2009. **106**(6): p. 984-91.
45. Chamberlain, G., et al., *Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing.* Stem Cells, 2007. **25**(11): p. 2739-49.
46. Phinney, D.G. and D.J. Prockop, *Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair--current views.* Stem Cells, 2007. **25**(11): p. 2896-902.
47. Parker, A.M. and A.J. Katz, *Adipose-derived stem cells for the regeneration of damaged tissues.* Expert Opin Biol Ther, 2006. **6**(6): p. 567-78.
48. Bakopoulou, A., et al., *Comparative analysis of in vitro osteo/odontogenic differentiation potential of human dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP).* Arch Oral Biol, 2011. **56**(7): p. 709-21.
49. Jankowski, R.J., B.M. Deasy, and J. Huard, *Muscle-derived stem cells.* Gene Ther, 2002. **9**(10): p. 642-7.
50. Wickham, M.Q., et al., *Multipotent stromal cells derived from the infrapatellar fat pad of the knee.* Clin Orthop Relat Res, 2003(412): p. 196-212.
51. Toh, W.S., et al., *Advances in mesenchymal stem cell-based strategies for cartilage repair and regeneration.* Stem Cell Rev, 2014. **10**(5): p. 686-96.
52. Dragoo, J.L., et al., *Healing full-thickness cartilage defects using adipose-derived stem cells.* Tissue Eng, 2007. **13**(7): p. 1615-21.
53. Murray, I.R., et al., *Natural history of mesenchymal stem cells, from vessel walls to culture vessels.* Cell Mol Life Sci, 2014. **71**(8): p. 1353-74.
54. Murray, I.R., et al., *Recent insights into the identity of mesenchymal stem cells: Implications for orthopaedic applications.* Bone Joint J, 2014. **96-b**(3): p. 291-8.
55. Johnstone, B., et al., *In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells.* Exp Cell Res, 1998. **238**(1): p. 265-72.
56. Connolly, J.F., *Injectable bone marrow preparations to stimulate osteogenic repair.* Clin Orthop Relat Res, 1995(313): p. 8-18.
57. Hernigou, P., et al., *Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells.* Int Orthop, 2013. **37**(11): p. 2279-87.
58. Muschler, G.F., C. Boehm, and K. Easley, *Aspiration to obtain osteoblast progenitor cells from human bone marrow: the influence of aspiration volume.* J Bone Joint Surg Am, 1997. **79**(11): p. 1699-709.
59. Patterson, T.E., et al., *Cellular strategies for enhancement of fracture repair.* J Bone Joint Surg Am, 2008. **90 Suppl 1**: p. 111-9.
60. Muschler, G.F., et al., *Age- and gender-related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors.* J Orthop Res, 2001. **19**(1): p. 117-25.
61. Afizah, H., et al., *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**(4): p. 659-66.
62. Friedenstein, A.J., S. Piatetzky, II, and K.V. Petrakova, *Osteogenesis in transplants of bone marrow cells.* J Embryol Exp Morphol, 1966. **16**(3): p. 381-90.
63. Petrakova, K.V., A.A. Tolmacheva, and F. Ala, *[BONE FORMATION OCCURRING IN BONE MARROW TRANSPLANTATION IN DIFFUSION CHAMBERS].* Biull Eksp Biol Med, 1963. **56**: p. 87-91.
64. Marmotti, A., et al., *Bone marrow derived stem cells in joint and bone diseases: a concise review.* Int Orthop, 2014. **38**(9): p. 1787-801.
65. Adams, M.K., et al., *Equine bone marrow-derived mesenchymal stromal cells (BMDMSCs) from the ilium and sternum: are there differences?* Equine Vet J, 2013. **45**(3): p. 372-5.
66. Hernigou, P., et al., *Percutaneous autologous bone-marrow grafting for nonunions. Surgical technique.* J Bone Joint Surg Am, 2006. **88 Suppl 1 Pt 2**: p. 322-7.

67. Luangphakdy, V., et al., *Assessment of Methods for Rapid Intraoperative Concentration and Selection of Marrow-Derived Connective Tissue Progenitors for Bone Regeneration Using the Canine Femoral Multidefect Model.* Tissue Eng Part A, 2016. **22**(1-2): p. 17-30.
68. Chahla, J., et al., *Concentrated Bone Marrow Aspirate for the Treatment of Chondral Injuries and Osteoarthritis of the Knee: A Systematic Review of Outcomes.* Orthop J Sports Med, 2016. **4**(1): p. 2325967115625481.
69. D'Ippolito, G., et al., *Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow.* J Bone Miner Res, 1999. **14**(7): p. 1115-22.
70. Majors, A.K., et al., *Characterization of human bone marrow stromal cells with respect to osteoblastic differentiation.* J Orthop Res, 1997. **15**(4): p. 546-57.
71. Lavasani, M., et al., *Muscle-derived stem/progenitor cell dysfunction limits healthspan and lifespan in a murine progeria model.* Nat Commun, 2012. **3**: p. 608.
72. Payne, K.A., D.M. Didiano, and C.R. Chu, *Donor sex and age influence the chondrogenic potential of human femoral bone marrow stem cells.* Osteoarthritis Cartilage, 2010. **18**(5): p. 705-13.
73. Gimble, J.M., et al., *The function of adipocytes in the bone marrow stroma: an update.* Bone, 1996. **19**(5): p. 421-8.
74. Zuk, P.A., et al., *Human adipose tissue is a source of multipotent stem cells.* Mol Biol Cell, 2002. **13**(12): p. 4279-95.
75. Aust, L., et al., *Yield of human adipose-derived adult stem cells from liposuction aspirates.* Cytotherapy, 2004. **6**(1): p. 7-14.
76. Zuk, P.A., et al., *Multilineage cells from human adipose tissue: implications for cell-based therapies.* Tissue Eng, 2001. **7**(2): p. 211-28.
77. Dragoo, J.L., et al., *Tissue-engineered cartilage and bone using stem cells from human infrapatellar fat pads.* J Bone Joint Surg Br, 2003. **85**(5): p. 740-7.
78. Gimble, J. and F. Guilak, *Adipose-derived adult stem cells: isolation, characterization, and differentiation potential.* Cytotherapy, 2003. **5**(5): p. 362-9.
79. Hennig, T., et al., *Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6.* J Cell Physiol, 2007. **211**(3): p. 682-91.
80. Im, G.I., Y.W. Shin, and K.B. Lee, *Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells?* Osteoarthritis Cartilage, 2005. **13**(10): p. 845-53.
81. Huang, J.I., et al., *Chondrogenic potential of progenitor cells derived from human bone marrow and adipose tissue: a patient-matched comparison.* J Orthop Res, 2005. **23**(6): p. 1383-9.
82. Winter, A., et al., *Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells.* Arthritis Rheum, 2003. **48**(2): p. 418-29.
83. Choi, Y.S., et al., *Mechanical derivation of functional myotubes from adipose-derived stem cells.* Biomaterials, 2012. **33**(8): p. 2482-91.
84. Gimble, J.M., A.J. Katz, and B.A. Bunnell, *Adipose-derived stem cells for regenerative medicine.* Circ Res, 2007. **100**(9): p. 1249-60.
85. Schipper, B.M., et al., *Regional anatomic and age effects on cell function of human adipose-derived stem cells.* Ann Plast Surg, 2008. **60**(5): p. 538-44.
86. Bourin, P., et al., *Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT).* Cytotherapy, 2013. **15**(6): p. 641-8.
87. Cawthorn, W.P., E.L. Scheller, and O.A. MacDougald, *Adipose tissue stem cells meet preadipocyte commitment: going back to the future.* J Lipid Res, 2012. **53**(2): p. 227-46.
88. Han, J., et al., *Adipose tissue is an extramedullary reservoir for functional hematopoietic stem and progenitor cells.* Blood, 2010. **115**(5): p. 957-64.
89. Vangsness, C.T., Jr., H. Sternberg, and L. Harris, *Umbilical Cord Tissue Offers the Greatest Number of Harvestable Mesenchymal Stem Cells for Research and Clinical Application: A Literature Review of Different Harvest Sites.* Arthroscopy, 2015. **31**(9): p. 1836-43.
90. Oedayrajsingh-Varma, M.J., et al., *Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure.* Cytotherapy, 2006. **8**(2): p. 166-77.
91. Yoshimura, K., et al., *Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates.* J Cell Physiol, 2006. **208**(1): p. 64-76.

92. Williams, S.K., et al., *Liposuction-derived human fat used for vascular graft sodding contains endothelial cells and not mesothelial cells as the major cell type.* J Vasc Surg, 1994. **19**(5): p. 916-23.
93. Katz, A.J., et al., *Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells.* Stem Cells, 2005. **23**(3): p. 412-23.
94. Jones, B.A. and M. Pei, *Synovium-derived stem cells: a tissue-specific stem cell for cartilage engineering and regeneration.* Tissue Eng Part B Rev, 2012. **18**(4): p. 301-11.
95. Shirasawa, S., et al., *In vitro chondrogenesis of human synovium-derived mesenchymal stem cells: optimal condition and comparison with bone marrow-derived cells.* J Cell Biochem, 2006. **97**(1): p. 84-97.
96. Sakaguchi, Y., et al., *Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source.* Arthritis Rheum, 2005. **52**(8): p. 2521-9.
97. Yoshimura, H., et al., *Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle.* Cell Tissue Res, 2007. **327**(3): p. 449-62.
98. Arufe, M.C., et al., *Chondrogenic potential of subpopulations of cells expressing mesenchymal stem cell markers derived from human synovial membranes.* J Cell Biochem, 2010. **111**(4): p. 834-45.
99. Sekiya, I., et al., *Arthroscopic Transplantation of Synovial Stem Cells Improves Clinical Outcomes in Knees With Cartilage Defects.* Clin Orthop Relat Res, 2015. **473**(7): p. 2316-26.
100. Kuwana, M., et al., *Human circulating CD14+ monocytes as a source of progenitors that exhibit mesenchymal cell differentiation.* J Leukoc Biol, 2003. **74**(5): p. 833-45.
101. Saw, K.Y., et al., *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**(4): p. 493-506.
102. Bucala, R., et al., *Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair.* Mol Med, 1994. **1**(1): p. 71-81.
103. Zhao, Y., D. Glesne, and E. Huberman, *A human peripheral blood monocyte-derived subset acts as pluripotent stem cells.* Proc Natl Acad Sci U S A, 2003. **100**(5): p. 2426-31.
104. Kodama, H., et al., *Cardiomyogenic potential of mesenchymal progenitors derived from human circulating CD14+ monocytes.* Stem Cells Dev, 2005. **14**(6): p. 676-86.
105. Hopper, N., et al., *Peripheral Blood Mononuclear Cells Enhance Cartilage Repair in in vivo Osteochondral Defect Model.* PLoS One, 2015. **10**(8): p. e0133937.
106. Kuznetsov, S.A., et al., *Circulating connective tissue precursors: extreme rarity in humans and chondrogenic potential in guinea pigs.* Stem Cells, 2007. **25**(7): p. 1830-9.
107. Saw, K.Y., et al., *Articular cartilage regeneration with autologous peripheral blood stem cells versus hyaluronic acid: a randomized controlled trial.* Arthroscopy, 2013. **29**(4): p. 684-94.
108. Saw, K.Y., et al., *High Tibial Osteotomy in Combination With Chondrogenesis After Stem Cell Therapy: A Histologic Report of 8 Cases.* Arthroscopy, 2015. **31**(10): p. 1909-20.
109. Fatorova, I., et al., *Timing of peripheral blood stem cell yield: comparison of alternative methods with the classic method for CD34+ cell determination.* Biomed Res Int, 2014. **2014**: p. 575368.
110. Jobin, C., et al., *Heterogeneity of in vitro-cultured CD34+ cells isolated from peripheral blood.* Cytotherapy, 2015. **17**(10): p. 1472-84.
111. Hosing, C., et al., *Peripheral blood stem cell yield calculated using preapheresis absolute CD34+ cell count, peripheral blood volume processed, and donor body weight accurately predicts actual yield at multiple centers.* Transfusion, 2014. **54**(4): p. 1081-7.
112. Gordon, S. and P.R. Taylor, *Monocyte and macrophage heterogeneity.* Nat Rev Immunol, 2005. **5**(12): p. 953-64.
113. Hopper, N., et al., *Peripheral blood derived mononuclear cells enhance the migration and chondrogenic differentiation of multipotent mesenchymal stromal cells.* Stem Cells Int, 2015. **2015**: p. 323454.
114. Krenning, G., et al., *Combined implantation of CD34+ and CD14+ cells increases neovascularization through amplified paracrine signalling.* J Tissue Eng Regen Med, 2013. **7**(2): p. 118-28.
115. Geissmann, F., et al., *Unravelling mononuclear phagocyte heterogeneity.* Nat Rev Immunol, 2010. **10**(6): p. 453-60.
116. Reilkoff, R.A., R. Bucala, and E.L. Herzog, *Fibrocytes: emerging effector cells in chronic inflammation.* Nat Rev Immunol, 2011. **11**(6): p. 427-35.
117. Fu, W.L., C.Y. Zhou, and J.K. Yu, *A new source of mesenchymal stem cells for articular cartilage repair: MSCs derived from mobilized peripheral blood share similar biological characteristics in vitro and chondrogenesis in vivo as MSCs from bone marrow in a rabbit model.* Am J Sports Med, 2014. **42**(3): p. 592-601.

118. Fu, W.L., et al., *Comparative study of the biological characteristics of mesenchymal stem cells from bone marrow and peripheral blood of rats.* Tissue Eng Part A, 2012. **18**(17-18): p. 1793-803.
119. Song, F., et al., *Comparison of the efficacy of bone marrow mononuclear cells and bone mesenchymal stem cells in the treatment of osteoarthritis in a sheep model.* Int J Clin Exp Pathol, 2014. **7**(4): p. 1415-26.
120. Zheng, R.C., et al., *Bone Regeneration of Blood-derived Stem Cells within Dental Implants.* J Dent Res, 2015. **94**(9): p. 1318-25.
121. Hunziker, E.B., *Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects.* Osteoarthritis Cartilage, 2002. **10**(6): p. 432-63.
122. Kim, H.K., M.E. Moran, and R.B. Salter, *The potential for regeneration of articular cartilage in defects created by chondral shaving and subchondral abrasion. An experimental investigation in rabbits.* J Bone Joint Surg Am, 1991. **73**(9): p. 1301-15.
123. Outerbridge, R.E., *The etiology of chondromalacia patellae. 1961.* Clin Orthop Relat Res, 2001(389): p. 5-8.
124. Widuchowski, W., J. Widuchowski, and T. Trzaska, *Articular cartilage defects: study of 25,124 knee arthroscopies.* Knee, 2007. **14**(3): p. 177-82.
125. Frenkel, S.R. and P.E. Di Cesare, *Degradation and repair of articular cartilage.* Front Biosci, 1999. **4**: p. D671-85.
126. Shelbourne, K.D., S. Jari, and T. Gray, *Outcome of untreated traumatic articular cartilage defects of the knee: a natural history study.* J Bone Joint Surg Am, 2003. **85-A Suppl 2**: p. 8-16.
127. Viste, A., et al., *Autologous chondrocyte implantation for traumatic full-thickness cartilage defects of the knee in 14 patients: 6-year functional outcomes.* Orthop Traumatol Surg Res, 2012. **98**(7): p. 737-43.
128. Jiang, Y.Z., et al., *Cell transplantation for articular cartilage defects: principles of past, present, and future practice.* Cell Transplant, 2011. **20**(5): p. 593-607.
129. Vijayan, S., et al., *Cartilage repair: A review of Stanmore experience in the treatment of osteochondral defects in the knee with various surgical techniques.* Indian J Orthop, 2010. **44**(3): p. 238-45.
130. Arden, N. and M.C. Nevitt, *Osteoarthritis: epidemiology.* Best Pract Res Clin Rheumatol, 2006. **20**(1): p. 3-25.
131. Loeser, R.F., et al., *Osteoarthritis: a disease of the joint as an organ.* Arthritis Rheum, 2012. **64**(6): p. 1697-707.
132. Le Blanc, K., et al., *HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells.* Exp Hematol, 2003. **31**(10): p. 890-6.
133. Chirba, M.A., et al., *FDA regulation of adult stem cell therapies as used in sports medicine.* J Knee Surg, 2015. **28**(1): p. 55-62.
134. Anz, A., *Current and Future Stem Cell Regulation: A Call to Action.* The American Journal of Orthopedics, 2016. **45**(5): p. 274-278, 318.
135. Vega, A., et al., *Treatment of Knee Osteoarthritis With Allogeneic Bone Marrow Mesenchymal Stem Cells: A Randomized Controlled Trial.* Transplantation, 2015. **99**(8): p. 1681-90.
136. Koh, Y.G. and Y.J. Choi, *Infrapatellar fat pad-derived mesenchymal stem cell therapy for knee osteoarthritis.* Knee, 2012. **19**(6): p. 902-7.
137. Wong, K.L., et al., *Injectable cultured bone marrow-derived mesenchymal stem cells in varus knees with cartilage defects undergoing high tibial osteotomy: a prospective, randomized controlled clinical trial with 2 years' follow-up.* Arthroscopy, 2013. **29**(12): p. 2020-8.
138. Skowronski, J. and M. Rutka, *Osteochondral lesions of the knee reconstructed with mesenchymal stem cells - results.* Ortop Traumatol Rehabil, 2013. **15**(3): p. 195-204.
139. Lee, K.B., et al., *A novel, minimally-invasive technique of cartilage repair in the human knee using arthroscopic microfracture and injections of mesenchymal stem cells and hyaluronic acid--a prospective comparative study on safety and short-term efficacy.* Ann Acad Med Singapore, 2012. **41**(11): p. 511-7.
140. LaPrade, C.M., et al., *How should we evaluate outcomes for use of biologics in the knee?* J Knee Surg, 2015. **28**(1): p. 35-44.
141. Kopka, M. and J.P. Bradley, *The Use of Biologic Agents in Athletes with Knee Injuries.* J Knee Surg, 2016. **29**(5): p. 379-86.
142. McAlindon, T.E., et al., *OARSI Clinical Trials Recommendations: Design, conduct, and reporting of clinical trials for knee osteoarthritis.* Osteoarthritis Cartilage, 2015. **23**(5): p. 747-60.
143. Losina, E., et al., *OARSI Clinical Trials Recommendations: Key analytic considerations in design, analysis, and reporting of randomized controlled trials in osteoarthritis.* Osteoarthritis Cartilage, 2015. **23**(5): p. 677-85.
144. Jin, D., et al., *Time-gated flow cytometry: an ultra-high selectivity method to recover ultra-rare-event mu-targets in high-background biosamples.* J Biomed Opt, 2009. **14**(2): p. 024023.
145. Arufe, M.C., et al., *Differentiation of synovial CD-105(+) human mesenchymal stem cells into chondrocyte-like cells through spheroid formation.* J Cell Biochem, 2009. **108**(1): p. 145-55.

146. Tew, S.R., et al., *Cellular methods in cartilage research: primary human chondrocytes in culture and chondrogenesis in human bone marrow stem cells.* Methods, 2008. **45**(1): p. 2-9.
147. Stashower, M., et al., *Stromal progenitor cells present within liposuction and reduction abdominoplasty fat for autologous transfer to aged skin.* Dermatol Surg, 1999. **25**(12): p. 945-9.
148. Gronthos, S., et al., *Surface protein characterization of human adipose tissue-derived stromal cells.* J Cell Physiol, 2001. **189**(1): p. 54-63.
149. McIntosh, K., et al., *The immunogenicity of human adipose-derived cells: temporal changes in vitro.* Stem Cells, 2006. **24**(5): p. 1246-53.
150. Mitchell, J.B., et al., *Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers.* Stem Cells, 2006. **24**(2): p. 376-85.
151. Estes, B.T., A.W. Wu, and F. Guilak, *Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6.* Arthritis Rheum, 2006. **54**(4): p. 1222-32.

Legend

Figure 1. A) Picture demonstrating harvesting technique of bone marrow sample followed by B) of the sample (BMAC) with three defined layers. RBC: Red blood cell.

Table 1 Comparative CD expression and chondrogenic potential [33]

	Harvesting sequenced process	Positive CD markers	Negative CD Markers	CP	Stem cells concentration
SDSCs [97, 98, 144-146]	Synovium + subsynovial tissue, enzymatic digestion, filter, culture (approx 2 wks)	44, 69, 73, 90, 105, 106, 166, 271 is the most chondrogenic subpopulation	11b, 34, 45	***	1,000 -30,000/ml
BM-MSCs [58, 89]	BMA, centrifugation, culture (approx 2-4 wks)	73, 90, 105	14, 34, 45, or 11b, 79alpha, or 19 and HLA DR	**	1-300,000/ml(depending on patient's age, site, gender and health; and harvesting technique)
PBMCs [105, 110] or PBPCs [101, 111]	Fresh whole blood in EDTA, centrifugation, hypoxic or normoxic culture (approx 2-4 wks); or subcutaneous series of G-CSF, followed by aphaeresis	34, 45, 133 (freshly isolated / normoxic conditions) among others 44, 90, 105, 106, 146, 166 and Stro-1	MSCs panel 34, 45,133 (Hypoxic conditions)	**	2 - 5 x 10^6/kg of patient's weight (heterogeneous stem cell population)
ASCs [86, 89, 147-150]	Lipoaspirate or infrapatellar fat pad, enzymatic digestion, centrifugation, culture (approx 2-4 wks)	13, 29, 44, 73, 90 and 105(>80%), 34 (initially), 36, 10, HLA ABC among others	11b, 31, 45, 106, HLA DR among others	*	5,000 -1,500,000/ml

Table 1: SDSCs: Synovial derived stem cells; BM-MSCs: Bone marrow derived mesenchymal stem cells; PBMCs: Peripheral blood mononuclear cells; PBPCs: Peripheral blood progenitor cells; G-CSF: Granulocyte colony stimulating factor; ASCs: Adipose stem cells; BMA: Bone marrow aspirate; EDTA: ethylenediaminetetraacetic acid; CP: Chondrogenic potential: ***= Most, **= Moderate, *= Least

Table 2 Growth factors for trilineage differentiation[55, 76, 84, 151]		
Desired adult cell	Driving culture media	Staining
Chondrocyte	Ascorbic acid, BMP-6-7, TGF-β3, dexamethasone, insulin, IGF-1, FGF	Alcian blue, Safranin O/Fast green
Osteoblast	Ascorbic acid, BMP-2, dexamethasone, 1,25 OH vitamin D3	Alizarin red / Picosiruis Red
Adipocyte	Dexamethasone, isobutyl methylxanthine, indomethacin, insulin, thiazolidinedione	Oil red O

Table 2: BMP= bone morphogenic protein, TGF= tumoral growth factor, IGF= insulin like growth factor, FGF= fibroblastic growth factor.