Review Article

Application of Biologics in the Treatment of the Rotator Cuff, Meniscus, Cartilage, and Osteoarthritis

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Abstract

Advances in our knowledge of cell signaling and biology have led to the development of products that may guide the healing/regenerative process. Therapies are emerging that involve growth factors, blood-derived products, marrow-derived products, and stem cells. Animal studies suggest that genetic modification of stem cells will be necessary; studies of cartilage and meniscus regeneration indicate that immature cells are effective and that scaffolds are not always necessary. Current preclinical animal and clinical human data and regulatory requirements are important to understand in light of public interest in these products.

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Orthopaedic surgery has made strides in the past few decades regarding outcomes evaluation and technical advancements. However, better success rates are desired in healing of the rotator cuff, meniscus, and cartilage as well as in the nonsurgical management of osteoarthritis (OA).[1-3] In the past 10 years, the medical profession has focused on ways to optimize the biology of healing. Orthopaedics has joined this movement with investigations involving preclinical animal models and clinical utilization.

In sports medicine, biologics refers to natural products that are harvested and used to augment a medical process and/or the biology of healing. Products include autograft, allograft, and xenograft and encompass a wide spectrum of tissues (Table 1). For the purposes of orthopaedic surgery, the three main categories of therapy are growth factor, cell, and tissue.

Growth factor therapies involve the harvest and delivery of growth factors to a site, such as in the use of platelet-rich plasma (PRP) to augment healing after partial tear of a tendon. Cell therapies involve the harvest and delivery of cells to a site, such as in the use of autologous chondrocyte therapy in the setting of cartilage repair. Tissue therapies involve the use of tissue to replace damaged structures or augment a repair, such as in the setting of meniscal allograft transplantation. Many factors have effects on function, the potential for success, and the regulatory concerns related to these modalities.

Regulatory Affairs

It is important to understand the regulatory affairs concerning biologics to understand their potential for clinical application. This is especially important for cell therapies because cells are living biologic products.

Table 1

Biologics: General Components and Orthopaedic Applications

Growth factor therapy
Isolated growth factor therapy
Platelet-rich plasma
Conditioned plasma preparations
Cell therapy
Bone marrow aspirate concentrate
Differentiated cell therapy
Chondrocyte implantation
Autograft
Allograft
Stem cell therapy
Autologous
Marrow-derived stem cells
Adipose-derived stem cells
Synovial-derived stem cells
Peripheral blood-derived stem cells
Allogenic
Mesenchymal adult stem cells
Amniotic-derived stem cells
Tissue therapy
Allograft musculoskeletal tissue
Xenograft tissue

In 1997, the US FDA set forth in Title 21, Part 1271 of the Code of Federal Regulations an approach to articles containing or consisting of all human cells, tissues, and cellular and tissue-based products (HCT/Ps) intended for implantation, transplantation, infusion, or transfer into a human recipient.[4,5] The FDA employed a tiered approach to the regulation of these articles based on their assessment of patient risk.

Lower-risk HCT/Ps are regulated by section 361 of the Public Health Service Act, which requires only that the products be manufactured under good tissue practices to prevent the introduction, transmission, or spread of communicable diseases.[4,5] These products, often referred to as 361 products, do not require premarket clinical studies or approval before marketing. Higher-risk products are regulated under section 351 of the Public Health Service Act, whereby they also must be manufactured according to good tissue practices as well as additional manufacturing specifications determined on a case-by-case basis by the FDA during a preclinical developmental process[4,5] (Figure 1). The preclinical developmental process involves animal and human clinical studies to prove safety and efficacy. These products, which are often referred to as 351 products, must pass a premarket approval process involving clinical studies with an active investigational new drug (IND) application in place, with clearance for clinical application and marketing after receipt of an approved Biologics License Application (Figure 1). This process is time-consuming and financially difficult, and it prevents the immediate application of many products in clinical practice.

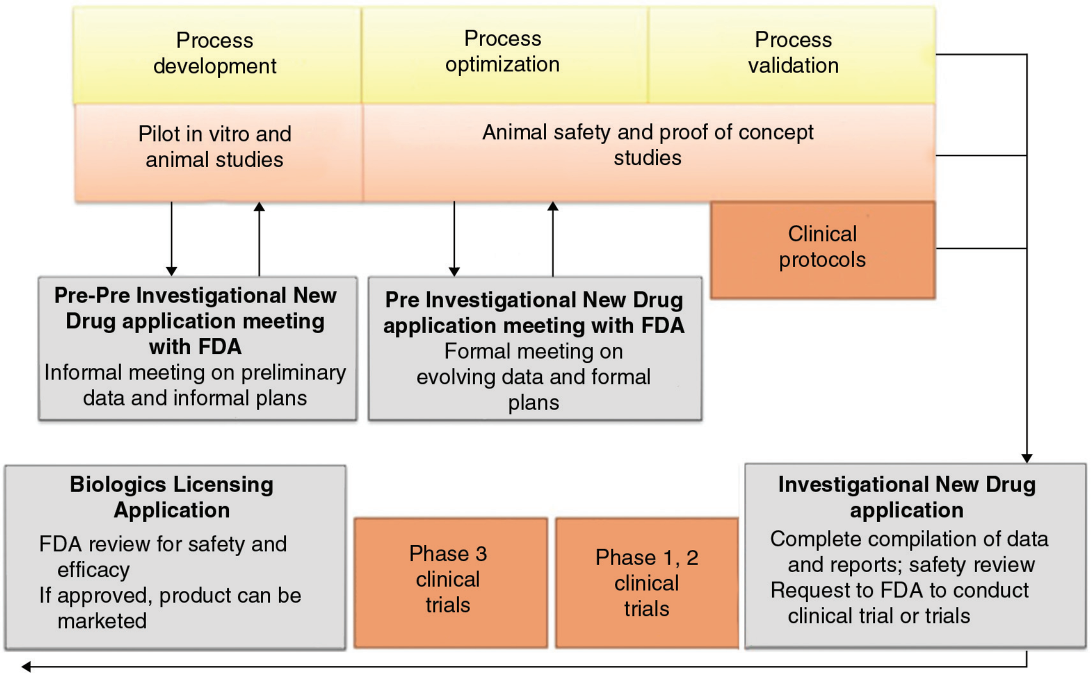
The differentiation of low-risk 361 products from high-risk 351 products is based on four criteria that help determine the risk for adverse events. The criteria are based on the principles of minimal manipulation, homologous use, noncombination products, and lack of systemic effect (Table 2). Any product that does not meet all four criteria is categorized as a 351 product and requires premarket approval, including animal and clinical studies, to demonstrate safety and efficacy.[4,5]

In the past decade, the FDA has made clarifications and rulings on HCT/Ps, including a clarification in 2005 that stated that any procedure in which human cells are manipulated for clinical use is subject to federal manufacturing standards and oversight.[4] Certain articles have been excluded by regulation from the HCT/P classification, including minimally manipulated bone marrow, xenografts, blood products, and secreted or extracted products.[4] Consequently, to date, minimally manipulated bone marrow aspirate (BMA) and PRP have not been regulated as HCT/Ps, and the FDA has not taken regulatory steps other than ensuring appropriate establishment registration and well-controlled and documented manufacturing processes.

As clinicians have sought to use stem cell therapies, the FDA has demonstrated the determination and ability to regulate this emerging technology, with strict rulings on the concepts of homologous use and minimal manipulation.[6,7] Consequently, most scenarios of orthopaedic implementation of stem cell therapy will require passage through the 351 regulatory pathway (Figure 1). Specifically, the FDA has ruled that a cell cannot be harvested from bone marrow and expanded in culture for injection into the knee because this is

Dr. Anz or an immediate family member serves as a paid consultant to Ceterix Orthopaedics, Arthrex, and MicroAire and has received institutional support from Celling Biosciences and Ceterix Orthopaedics. Dr. Nilssen or an immediate family member is a member of a speakers' bureau or has made paid presentations on behalf of Arthrex, ETEX, and Medartis; is an employee of ASE Medical; serves as a paid consultant to ETEX and Medartis; and has stock or stock options held in Tenex Health, CorMatrix, ETEX, and Fuse Medical. Dr. Andrews or an immediate family member has received royalties from Biomet Sports Medicine; serves as a paid consultant to Biomet Sports Medicine, Bauerfeind, Theralase Technologies, and MiMedx; is an employee of Physiotherapy Associates; has stock or stock options held in Patient Connection and Connective Orthopaedics; and serves as a board member, owner, officer, or committee member of FastHealth Corporation and Physiotherapy Associates. Neither Dr. Hackel nor any immediate family member has received anything of value from or has stock or stock options held in a commercial company or institution related directly or indirectly to the subject of this article.

Figure 1



Flowchart of typical steps in the US FDA 351 regulatory pathway, which is the premarket approval process for human cells, tissues, and cellular and tissue-based products deemed to be high risk. The gray boxes represent communication milestones with the FDA. The yellow boxes represent steps typically involving preclinical animal study. The orange boxes represent clinical process development and study.

Table 2

The Four Criteria Required for Human Cells, Tissues, and Cellular and Tissue-Based Products to Qualify as Low Risk[a]

1. Minimal manipulation: Manufacturing is limited to simple procedures.
2. Advertised/labeled for homologous use only: Product must carry out the same biologic function as it normally would.
3. Noncombination product: Combining products increases complexity, so the product cannot be combined with another product, with the exception of simple electrolyte solutions and preservation agents.
4. Nonsystemic effect or is autologous: If the product may have a systemic effect, it must be autologous or from a close blood relative, in order to reduce the risk of an immune reaction.

[a] Products that meet all four criteria are regulated under section 361 of the Public Health Service Act and are sometimes referred to as 361 products.

considered to be more than minimal manipulation.[6] Additionally, although some adipose-derived stem cells (ASCs) may be harvested in a manner that involves minimal manipulation, the FDA does not consider injection of a subcutaneous-procured ASC into a knee joint to be homologous use.[7] The FDA has also stated that such products may be used developmentally in humans only if an investigational new drug application is in effect; stem cell products that do not meet the 361 criteria cannot be lawfully offered or marketed without an approved biologics license.[7]

Basic Science

Platelet-rich Plasma and Bone Marrow Aspirate

Chemokines and cytokines are bioactive proteins that can be found in multiple tissues within the human body, including blood plasma and platelet granules. Some of these proteins have been identified as growth factors related to documented functions. Platelets are instrumental in healing processes because they release a number of growth factors and additional bioactive proteins upon activation. Multiple techniques exist to concentrate platelets and/or

Figure 2

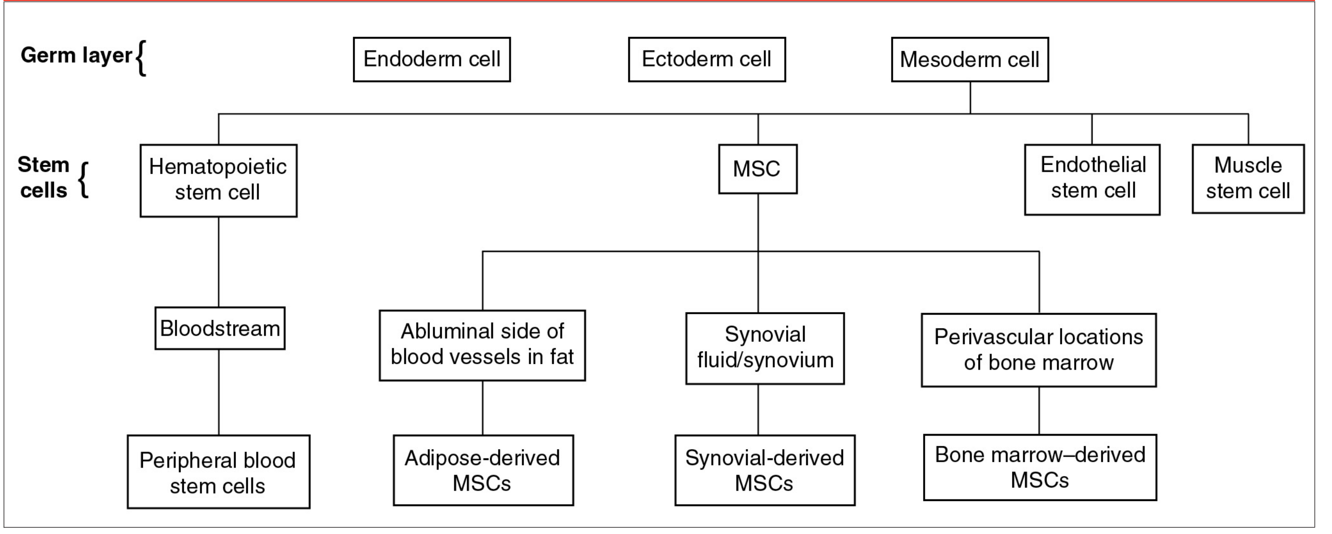


Illustration of stem cell locations and level of maturation. The germ layer consists of endoderm, ectoderm, and mesoderm cells. Stem cells are one generation matured from cells of the germ layer. Mesenchymal stem cells (MSCs) originate from cells of mesoderm origin. Peripheral blood stem cells are hematopoietic stem cells found within the bloodstream. Adipose-derived MSCs are those that can be isolated from the abluminal side of blood vessels in fat. Synovial-derived MSCs can be isolated from synovial tissue or fluid. Bone marrow–derived MSCs can be obtained through bone marrow aspirate.

growth factors from blood serum and can be classified as growth factor therapies. PRP is a growth factor therapy in which platelets are concentrated from serum; in vitro data support the ability of PRP to attract mesenchymal stem cells (MSCs), macrophages, and fibroblasts as well as to stimulate cell proliferation and extracellular matrix protein production, which can improve healing.[8,9]

Bone marrow contains a complex mixture of platelets, red blood cells, white blood cells, hematopoietic precursors, and nonhematopoietic precursors. Platelets and nonhematopoietic precursors can be isolated through the process of bone marrow aspiration and centrifugation. Based on basic science study, nonhematopoietic precursors were initially thought to be feeder cells of hematopoietic precursors alone. Later, these cells were found to have the ability to propagate and differentiate, at which point they came to be called MSCs. MSCs were first isolated through their ability to adhere to tissue culture surfaces, and MSCs isolated from BMA represent a heterogeneous mixture of cells.[10] Available BMA concentration techniques and devices isolate platelets and MSCs from BMA, providing the potential for growth factor therapy and cell therapy from a single source.

Stem Cells

Stem cells are one generation in maturation from germ layer cells (Figure 2). The four defining qualities of stem cells are the ability to reproduce (proliferative potential), the ability to differentiate and mature into a different number of cell lines (multipotentiality), the ability to mobilize in situations of angiogenesis, and the ability to activate and control cells within their environment (paracrine functions)[11] (Figure 3). Although all four of these functions can be used to the advantage of regenerative medicine, most investigators have sought to use two of these functions—the ability to differentiate a given cell and the ability to release growth factors and trophic immune regulators.[12,13] In orthopaedics, the MSC has garnered the most interest because of its direct lineage regarding tissues important in orthopaedic interventions.

MSCs can be isolated from the bone marrow, synovial tissue, periosteum, and fat. As they mature in distinct microenvironments, cells obtained from different sites exhibit unique phenotypes and cell markers. The peripheral blood stem cell (PBSC), also known as the peripheral blood progenitor cell, recently has garnered attention for orthopaedic application (Figure 2). The PBSC is an immature monocyte that is present in the bloodstream and originates from the bone marrow.[13] It is normally present in low numbers in the bloodstream, but production and peripheral circulation can be increased with granulocyte colony–stimulating factor analogues such as filgrastim. Once mobilized, these cells can be harvested from the bloodstream

Figure 3

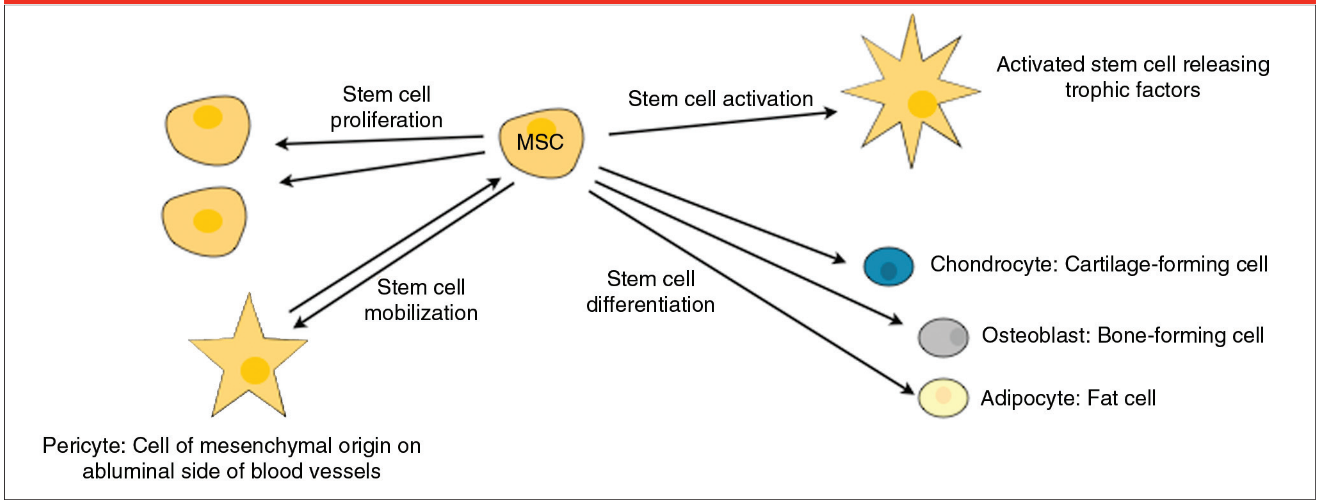


Illustration of mesenchymal stem cell (MSC) functions. The four defining functions of stem cells are the ability to proliferate, to differentiate, to monitor/respond to circulating signaling molecules as pericytes on the surface of blood vessels, and to enter a so-called activated state whereby they release trophic, paracrine, and immune modulators that enhance the regenerative potential of their environment.

through the process of apheresis. PBSCs have been harvested and used safely in the field of hematology oncology for bone marrow transplant, with documented 12-year safety data in healthy volunteers.[14]

Bone marrow–, adipose-, periosteum-, and synovial-derived MSCs as well as PBSCs have demonstrated the capacity to differentiate into cells of the osteocyte, chondrocyte, and adipocyte lineage.[10,13,15-17] Additionally, bone marrow–derived MSCs and PBSCs have illustrated the ability to differentiate into cells from the remaining two germ layers, including cells of the brain, heart, and liver.[10,13] Direct comparison of MSCs and PBSCs has shown that they have the same potential with regard to proliferative and trophic ability.[13]

Quantifying bone marrow–derived MSCs is difficult; historically, this has been based on the number of colony-forming units that emerge from in vitro culture of samples of bone marrow, with studies estimating between 109 and 664 colony-forming units per milliliter of BMA.[18,19] In animal studies, investigators using bone marrow–derived cells have cultured the cells isolated in this fashion for certain orthopaedic applications, such as cartilage regeneration.[20,21] Investigators seeking to use cells from synovial tissue have also used culture processes to increase cell numbers.[22] PBSC can be harvested in higher numbers through a process involving stimulation with a mobilization drug and harvest through apheresis.[23] The site of harvest of stem cells has a greater effect on the number of cells available and the regulatory constraints imposed on the application of said stem cells than do multipotentiality, proliferative potential, or trophic ability.

Orthopaedic Applications

Rotator Cuff: Preclinical and Clinical Evidence

Five randomized controlled trials and three nonrandomized comparative studies have been done on the use of PRP to augment rotator cuff repair, and a review of the most current studies has been published.[24-32] Five studies intercalated a platelet-rich fibrin matrix between the osseous bed of repair and the tendon.[25,28-31] All five of these studies showed no functional benefit with the addition of a platelet-rich fibrin matrix, and two of them[29,30] illustrated a detrimental effect (ie, decreased healing rates on postoperative imaging analysis of the healing tendon).

Three studies have investigated PRP application after completion of repair. Results include no difference,[32] improved pain scores within the first 30 days and clinical scores at 3 months alone,[26] and an improvement in tendon integrity noted on MRI evaluation.[27] The findings of Randelli et al[26] were unique in that they demonstrated improved pain scores and clinical scores with the application of an injectable form of PRP in combination with an autologous thrombin component. Synthesis of all the studies illustrates that there is no clear advantage to using PRP as a surgical adjunct to rotator cuff repair.[24-32] However, these clinical studies illustrate the importance of mechanism of application in biologics and the need to refine the methods of application and investigation. Currently, there are no clinical or preclinical data regarding the use of BMA in the setting of rotator cuff repair.

Animal data are available regarding the use of MSCs to augment rotator cuff healing.[33-38] In rat models, Gulotta and colleagues[33-35,37] have investigated the use of MSCs in a fibrin carrier placed at the tendon-bone interface at the time of repair, including methods guiding cell differentiation. Initial study focused on immature bone marrow–derived, cultured MSCs.[33] Although the MSCs survived and remained metabolically active at the site of repair, there was no difference in structure, composition, or strength between the MSC group and the control animals. Genetically modified MSCs were assessed in three follow-up studies.[34,35,37] Modifying cells with gene transfer of human bone morphogenetic protein-13 did not improve healing at either 2- or 4-week follow-up.[35] Modification of cells with a gene up-regulated in embryos at sites of tendon-bone interface development (ie, membrane type 1 matrix metalloproteinase) resulted in more fibrocartilage at the site of insertion and improved strength at 4-week follow-up.[34] Inducing cells with the transcription factor scleraxis to direct tendon development resulted in improved biomechanics at 2 weeks and improved biomechanics and histology at 4 weeks.[37]

Although these results regarding genetic modification are encouraging, they represent modalities that involve more than minimal manipulation of cells, which places cells used in this fashion into the category of high-risk HCT/Ps. Thus, considerable work remains to be done before these methods can be applied in clinical practice. Combining cells with a growth factor is another technique that has shown promise. In a rabbit repair model, Chen et al[36] created a hydrogel with periosteal-derived MSCs, polyethylene glycol diacrylate, and bone morphogenetic protein-2 and applied it at the tendon-bone interface. Histologic and biomechanical improvement was noted at 4- and 8-week follow-up. Similar success has been seen in a rotator cuff defect model in rabbits.[38] MSCs seeded onto a polyglycolic acid sheet produced improved type I collagen scores, tendon maturation, and tensile strength compared with use of a polyglycolic acid sheet alone.

Meniscus Repair: Preclinical and Clinical Evidence

Animal study results are mixed regarding the use of PRP and BMA to augment meniscal repair.[39-41] Two studies have investigated the effects of PRP on defect healing in a rabbit model.[39,40] In each study, punch defects were created and scaffolds were used for PRP deployment.

Ishida et al[39] constructed gelatin hydrogel scaffolds to elute PRP in a time-release fashion and reported improved histologic scores at 12-week follow-up. Zellner et al[40] used hyaluronan-collagen scaffolds but did not design the scaffolds for timed release of the PRP. There was no improvement in the study group compared with the control group, nor was there improvement in fill tissue in rabbits treated with BMA loaded onto a hyaluronan-collagen scaffold. In a sheep model in which BMA was used to aid in healing of longitudinal tears of the red-white zone, histologic evaluation revealed no difference in collagen fibril formation.[41] However, improvement was noted in neovascularization, cell count, and formation of cartilage plaques. This study did not involve a scaffold for the BMA application nor repair of the meniscus.

Four studies have illustrated the ability of MSCs to enhance meniscal repair.[22,40,42,43] In the first study, large defects equivalent to resection of the body of the meniscus were created in both knees in rabbits.[42] In six rabbits, these defects were filled with a hyaluronan-gelatin scaffold alone in the treated knee, with the contralateral defect left untreated. In 12 rabbits, the defects in one knee were filled with a scaffold loaded with cultured autologous marrow-derived MSCs, and the defects in the other were filled with an empty scaffold. The MSC-loaded scaffold produced integration with meniscus-like fibrocartilage in 8 of 11 rabbits, whereas use of the empty scaffold alone produced similar integration in 2 of 11 rabbits. The width of the regenerated tissue was significantly greater than that of the control knees ($P < 0.004$).

A follow-up study investigated smaller punch defects using a similar scaffold.[40] Scaffolds were either loaded with MSCs and precultured for 14 days or loaded with MSCs and implanted. A novel scoring system was used 3 months after implantation. The uncultured scaffold scored highest, with near complete integration of a meniscus-like repair tissue.

The use of allogenic synovial MSCs has been investigated in a meniscal punch defect model[22] and in a meniscectomy model in rats.[43] In the punch defect model, the quantity and quality of repair tissue illustrated significant improvement when defects were loaded with cells in a phosphate-buffered saline (PBS) solution.[22] In the meniscectomy model, the anterior half of the medial meniscus was excised in two groups. An intra-articular injection of MSCs and PBS was placed after wound closure in one group, whereas the control group received an injection of PBS. Meniscal defects exhibited a statistically significant improvement in tissue regeneration at 2-, 4-, and 8-week follow-ups in the MSC group, but not at 12 weeks. Neither of these studies relied on a scaffold, and both illustrated labeled cells at the site of repair tissue 12 weeks after implantation. Rats have a higher tendency of meniscal regeneration than do humans and larger animals, so larger animal studies would help further progress the application of MSCs to meniscal repair in humans.

These animal studies not only illustrate the usefulness of the addition of MSC in the setting of meniscal defects but also guide the development of methods for application of stem cells. Although larger defects require a scaffold loaded with stem cells, smaller defects or situations involving augmentation of repair may not require a scaffold because cells appear to be able to localize and remain at a site of repair.[20,22,43] A scaffold loaded with stem cells represents a product that must pass through the high-risk HCT/P regulatory pathway.

Cartilage Repair

Investigation into cartilage repair has generated the largest collection of biologic data. BMA has proved effective as an adjunct to marrow stimulation in two animal studies.[44,45] The first study involved subchondral drilling of a cartilage defect in a goat model to investigate postoperative injections of BMA.[44] Histologic scoring was best in a group treated with three postoperative injections of BMA in combination with sodium hyaluronate at weekly intervals. Two additional groups included one with no postoperative injections and one with sodium hyaluronate injections alone. A similar study compared microfracture and BMA placed at the site of microfracture in an equine model.[45] The BMA group had higher gross morphologic and histologic scores, as well as MRI data indicating increased fill of the defects and improved integration of repair tissue into surrounding normal cartilage.

Cartilage regeneration using isolated stem cells has long been studied in animals.[20,21,46-49] In 1994, Wakitani et al[46] published an investigation involving MSCs in a rabbit model. These cells were embedded into a type I collagen gel and placed into full-thickness cartilage defects. Serial histologic evaluation revealed that the cells differentiated into chondrocytes in a uniform fashion as soon as 2 weeks and that at 24 weeks, a subchondral bone layer was reestablished. Subsequent investigators have sought to determine whether maturation and differentiation of cells is important.

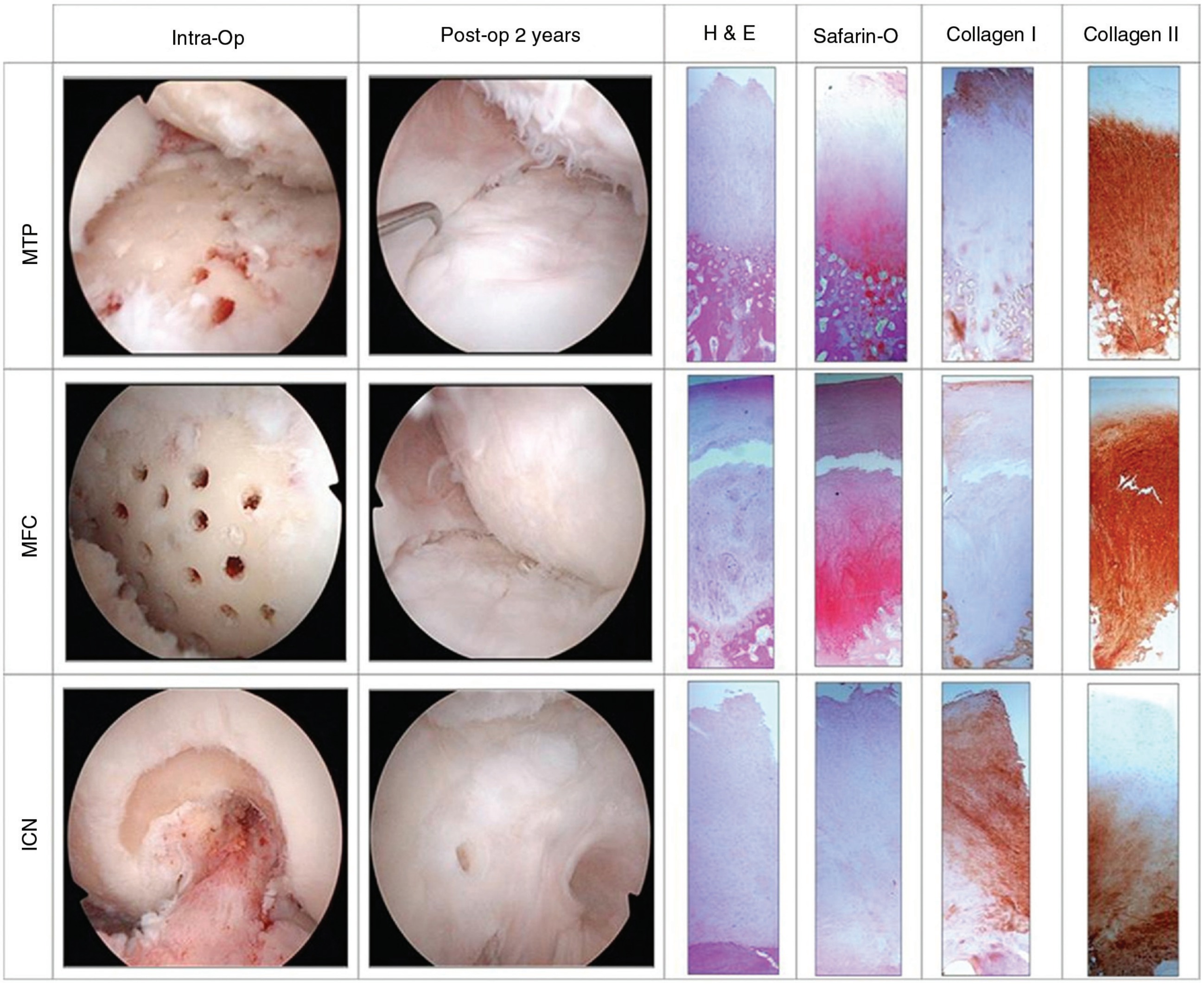
Chang et al[48] compared immature MSCs with transforming growth factor-β–induced differentiated MSCs. Superior histologic results were noted in the group with immature MSCs. A rabbit study in which allogenic MSCs were compared with cultured autologous chondrocytes demonstrated similar histologic outcomes between the two methods and a higher morphologic score with the allogenic MSCs.[49] These two studies suggest that differentiating a cell to the chondrocyte lineage is not advantageous and that for the purposes of cartilage integration and differentiation, use of a more immature cell may be more effective.

Implantation at the time of surgery with a scaffold is not the only method of cell application. Three studies, including the aforementioned goat study, have demonstrated improvement with application of stem cells following marrow stimulation.[20,21,44] Lee et al[20] investigated the effects of three once-weekly injections of MSCs suspended in 2 mL of hyaluronan after the creation of a cartilage defect in minipigs. The cell-treated group had improved histologic and morphologic scores. Additionally, carboxyfluorescein-labeled MSCs were found at the base of the repair cartilage, which suggests that the cells have an innate, functional homing mechanism. A similar study in horses evaluated the effectiveness of one injection of bone marrow–derived MSCs 1 month after microfracture.[21] This study illustrated a trend toward overall improvement, with significance achieved in repair tissue firmness and aggrecan content. These three studies suggest that postoperative injections are effective in the application of stem cells and that the timing of injections and the number of cells applied is important.

ASCs also have potential application in cartilage regeneration and have been shown to have proliferative potential superior to that of MSC.[50] In a rabbit model, ASCs applied in a fibrin glue scaffold illustrated excellent rates of subchondral bone healing.[47] However, direct comparison of the chondrogenic potential of adipose and bone marrow–derived cells has shown greater efficiency and quality of chondrogenesis with bone marrow–derived cells.[50,51]

The clinical application of stem cells in cartilage regeneration has been studied in an observational cohort study,[52] a case series with histology,[23] and a randomized controlled trial.[53] The observational cohort study compared autologous chondrocyte implantation in 36 patients with bone marrow–derived MSC implantation in 36 patients.[52] In all patients, a periosteal patch was used to retain cells at the cartilage defect site. There was no clinical difference between the two groups at a follow-up of 24 months. However, within the autologous chondrocyte implantation group, patients aged 45 years and younger did significantly better than patients older than 45 years. No age stratification was seen in the

Figure 4



Intraoperative (Intra-Op) and postoperative (Post-op 2 years) arthroscopic images and postoperative biopsy specimens with staining from patients treated with cartilage repair involving subchondral drilling and postoperative injections of peripheral blood stem cells and hyaluronic acid. Twenty-two–month postoperative hematoxylin-eosin (H & E) staining of biopsy specimens from the medial tibial plateau (MTP) and the medial femoral condyle (MFC) demonstrating columnar morphology of cells with a pale background. Safranin O (Safarin-O) staining highlights an abundance of proteoglycans throughout the regenerated cartilage layer. Collagen type I staining (Collagen I) was limited to the superficial layer except in the non–weight-bearing intercondylar notch (ICN) biopsy specimen, which shows a higher percentage of collagen type I and a disorganized pattern of healing. Collagen type II (Collagen II) was concentrated in the deep layers. (Reproduced with permission from Saw KY, Anz A, Merican S, 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]:493-506.)

group treated with MSC. In a case series in which PBSCs were used to augment subchondral drilling, morphologic and staining properties were seen on histology that approached those of natural cartilage[23] (Figure 4). This method has subsequently been investigated in a randomized controlled trial comparing clinical outcomes based on International Knee Documentation Committee scores at 24 months, morphology of repair on MRI, and repair tissue quality on histology biopsy.[53] The intervention group underwent postoperative injections of PBSC and hyaluronan, and the control group underwent injections of hyaluronan alone. Repair tissue as evaluated with the International Cartilage Repair Society II histologic score and an MRI morphologic score illustrated statistical superiority in the PBSC group. Clinical outcome scores at 24 months were not statistically different.

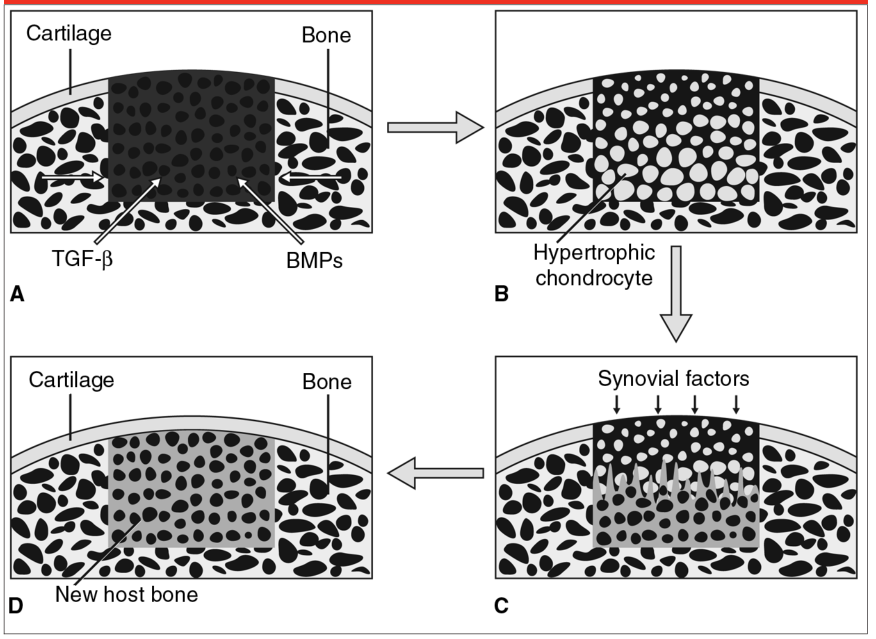
Osteoarthritis: Clinical Evidence

In the setting of OA, repairing damaged tissue is often not possible, and the goals are to improve function and reduce pain, typically by reducing inflammation. Clinical study of the application of PRP in the setting of knee OA has illustrated pain reduction and improved clinical scores at up to 12-month follow-up; however, its superiority has not been clearly established compared with viscosupplementation in all cases.[54-57] In younger patients with lesser degrees of degeneration, comparison studies illustrate a clear benefit with PRP over hyaluronan in terms of pain reduction and clinical scores; however, in middle-aged patients with moderate OA, improvement was similar between PRP and hyaluronan.[54,55]

Authors' Experience

Because of regulatory limitations and unclear clinical data concerning PRP and BMA, we have not yet implemented biologics into our regular practice in cases involving rotator cuff repair, meniscus repair, or cartilage repair. We have, however, implemented PRP and BMA into our clinical practice in the setting of OA, and we have noted positive anecdotal results in the setting of knee OA. Although both an anti-inflammatory effect and pain reduction have been illustrated in our patients, we have seen longer-lasting pain reduction with BMA. Long-term results have not been established, and we counsel our patients regarding realistic expectations. We recognize the strong preclinical data regarding the use of stem cells for rotator cuff healing, meniscus regeneration, and cartilage repair, and we look forward to the clinical availability of these products after further appropriate regulatory steps, including well-designed clinical trials.

Figure 5



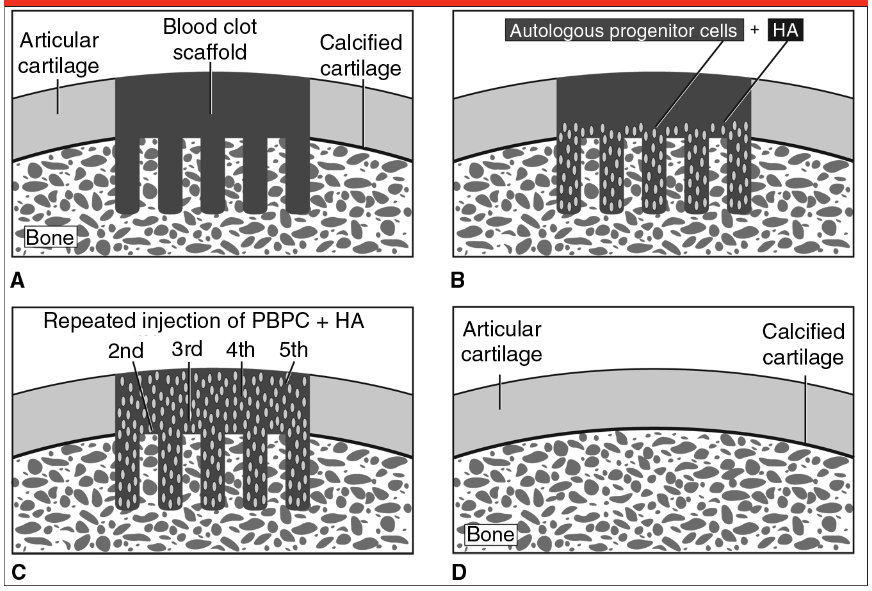
One evolving theory of stem cell–augmented chondrogenesis. Illustrations involving use of a mesenchymal stem cell (MSC) implant to repair a defect. **A,** MSC loaded on a collagen gel is implanted into a prepared cartilage defect. Host bone–derived bioactive factors are released (eg, transforming growth factor-β [TGF-β], bone morphogenetic proteins [BMPs]). **B,** Hypertrophic chondrocytes develop. **C,** Chondrocytes begin to mature and remodel subchondral bone as well as articular cartilage. **D,** The repair tissue matures and integrates to surrounding cartilage. (Adapted with permission from Wakitani S, Goto T, Pineda SJ, et al: Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1994;76[4]:579-592.)

Summary

The implementation of biologics in orthopaedics has clear benefit. Collection of growth factors and stem cells is possible from multiple tissues, with regulatory and functional ramifications based on anatomic harvest location. Animal studies of the rotator cuff suggest that genetic modification of stem cells will be necessary, whereas studies involving cartilage and meniscus regeneration suggest that immature cells are effective and scaffolds are not always necessary (Figures 5 and 6).

Clear regulatory and application hurdles remain, but clinical progress has been made based on animal study. We strongly believe that clinical trials that follow the appropriate regulatory pathways will result in the incorporation of biologics into our daily practice in the coming years. Work is needed to determine appropriate mechanisms of application, confirm the efficacy of established techniques, and advance products appropriately through regulatory pathways.

Figure 6



One evolving theory of stem cell–augmented chondrogenesis. **A,** Subchondral drilling creates an autologous blood scaffold at the site of cartilage defect. **B,** Initial intra-articular injection of peripheral blood progenitor cell (PBPC) in combination with hyaluronic acid (HA) at 1 week. **C,** Additional injections of PBPC and HA at weekly intervals. **D,** Repair tissue matures, redeveloping subchondral bone and articular cartilage. (Adapted with permission from Saw KY, Anz A, Merican S, 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]:493-506.)

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References

Evidence-based Medicine: Levels of evidence are described in the table of contents. In this article, references 33, 34, 39, and 40 are level I studies. References 8, 36, and 38 are level II studies. References 3, 5, 32, 35, and 37 are level III studies. References 1, 2, 4, 6, 9, 22, 26, 27, and 31 are level IV studies. References 7, 10, 14, 15, 19, and 20 are level V expert opinion.

References printed in **bold type** are those published within the past 5 years.

1. Boileau P, Brassart N, Watkinson DJ, Carles M, Hatzidakis AM, Krishnan SG: Arthroscopic repair of full-thickness tears of the supraspinatus: Does the tendon really heal? *J Bone Joint Surg Am* 2005;87(6):1229-1240.
2. Stärke C, Kopf S, Petersen W, Becker R: Meniscal repair. *Arthroscopy* 2009; 25(9):1033-1044.
3. **Harris JD, Brophy RH, Siston RA, Flanigan DC: Treatment of chondral defects in the athlete's knee. *Arthroscopy* 2010;26(6):841-852.**
4. Barone SB: "361" Human cells, tissues, and cellular and tissue-based products (HCT/Ps). The Office of Cellular, Tissue, and Gene Therapies Web Seminar Series. Available at: www.fda.gov/downloads/BiologicsBloodVaccines/NewsEvents/UCM251330.ppt. Accessed November 11, 2013.
5. US Department of Health and Human Services: Human cells, tissues, and cellular and tissue-based products. 21 CFR Part 1271. Available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271&showFR=1. Accessed November 11, 2013.
6. United States District Court for the District of Columbia Civil Action No: 10-1327: United States of America versus Regenerative Sciences, LLC. Available at: https://ecf.dcd.uscourts.gov/cgi-bin/show_public_doc?2010cv1327-47. Accessed November 11, 2013.
7. Malarkey MA: Warning Letter to IntelliCell Biosciences, Inc: 3/13/12. Available at: http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2012/ucm297245.htm. Accessed November 11, 2013.
8. Hall MP, Band PA, Meislin RJ, Jazrawi LM, Cardone DA: Platelet-rich plasma: Current concepts and application in sports medicine. *J Am Acad Orthop Surg* 2009;17(10):602-608.
9. **Boswell SG, Cole BJ, Sundman EA, Karas V, Fortier LA: Platelet-rich plasma: A milieu of bioactive factors. *Arthroscopy* 2012;28(3):429-439.**
10. Hung SC, Chen NJ, Hsieh SL, Li H, Ma HL, Lo WH: Isolation and characterization of size-sieved stem cells from human bone marrow. *Stem Cells* 2002;20(3):249-258.
11. **Caplan AI, Correa D: PDGF in bone formation and regeneration: New insights into a novel mechanism involving MSCs. *J Orthop Res* 2011; 29(12):1795-1803.**
12. Caplan AI: New era of cell-based orthopedic therapies. *Tissue Eng Part B Rev* 2009;15(2):195-200.
13. Cesselli D, Beltrami AP, Rigo S, et al: Multipotent progenitor cells are present in human peripheral blood. *Circ Res* 2009;104(10):1225-1234.
14. Hölig K, Kramer M, Kroschinsky F, et al: Safety and efficacy of hematopoietic stem cell collection from mobilized peripheral blood in unrelated volunteers: 12 years of single-center experience in 3928 donors. *Blood* 2009; 114(18):3757-3763.
15. De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP: Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum* 2001;44(8): 1928-1942.
16. Zuk PA, Zhu M, Ashjian P, et al: Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002;13(12):4279-4295.
17. De Bari C, Dell'Accio F, Vanlauwe J, et al: Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. *Arthritis Rheum* 2006;54(4): 1209-1221.

18. **Murphy MB, Moncivais K, Caplan AI: Mesenchymal stem cells: Environmentally responsive therapeutics for regenerative medicine. *Exp Mol Med* 2013;45:e45.**

19. Kevy SV, Jacobson MS: Comparison of Bone Marrow Concentrates Prepared by the Harvest/Terumo BMAC® System and the Thermogenesis Res-Q™ 60 system. Austin, Texas, Progenicare, 2012. Available at: http://www.progenicare.com/wp-content/uploads/2012/10/BMAC-v-ResQ-Comparison-Aug-2012.pdf. Accessed November 11, 2013.

20. Lee KB, Hui JH, Song IC, Ardany L, Lee EH: Injectable mesenchymal stem cell therapy for large cartilage defects: A porcine model. *Stem Cells* 2007;25(11):2964-2971.

21. **McIlwraith CW, Frisbie DD, Rodkey WG, et al: Evaluation of intra-articular mesenchymal stem cells to augment healing of microfractured chondral defects. *Arthroscopy* 2011;27(11):1552-1561.**

22. **Horie M, Driscoll MD, Sampson HW, et al: Implantation of allogenic synovial stem cells promotes meniscal regeneration in a rabbit meniscal defect model. *J Bone Joint Surg Am* 2012;94(8):701-712.**

23. **Saw KY, Anz A, Merican S, 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):493-506.**

24. **Chahal J, Van Thiel GS, Mall N, et al: The role of platelet-rich plasma in arthroscopic rotator cuff repair: A systematic review with quantitative synthesis. *Arthroscopy* 2012;28(11):1718-1727.**

25. **Castricini R, Longo UG, De Benedetto M, et al: Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: A randomized controlled trial. *Am J Sports Med* 2011;39(2):258-265.**

26. **Randelli P, Arrigoni P, Ragone V, Aliprandi A, Cabitza P: Platelet rich plasma in arthroscopic rotator cuff repair: A prospective RCT study, 2-year follow-up. *J Shoulder Elbow Surg* 2011;20(4):518-528.**

27. **Barber FA, Hrnack SA, Snyder SJ, Hapa O: Rotator cuff repair healing influenced by platelet-rich plasma construct augmentation. *Arthroscopy* 2011;27(8):1029-1035.**

28. **Jo CH, Kim JE, Yoon KS, et al: Does platelet-rich plasma accelerate recovery after rotator cuff repair? A prospective cohort study. *Am J Sports Med* 2011;39(10):2082-2090.**

29. **Bergeson AG, Tashjian RZ, Greis PE, Crim J, Stoddard GJ, Burks RT: Effects of platelet-rich fibrin matrix on repair integrity of at-risk rotator cuff tears. *Am J Sports Med* 2012;40(2):286-293.**

30. **Rodeo SA, Delos D, Williams RJ, Adler RS, Pearle A, Warren RF: The effect of platelet-rich fibrin matrix on rotator cuff tendon healing: A prospective, randomized clinical study. *Am J Sports Med* 2012;40(6):1234-1241.**

31. **Gumina S, Campagna V, Ferrazza G, et al: Use of platelet-leukocyte membrane in arthroscopic repair of large rotator cuff tears: A prospective randomized study. *J Bone Joint Surg Am* 2012;94(15):1345-1352.**

32. **Weber SC, Kauffman JI, Parise C, Weber SJ, Katz SD: Platelet-rich fibrin matrix in the management of arthroscopic repair of the rotator cuff: A prospective, randomized, double-blinded study. *Am J Sports Med* 2013;41(2):263-270.**

33. Gulotta LV, Kovacevic D, Ehteshami JR, Dagher E, Packer JD, Rodeo SA: Application of bone marrow-derived mesenchymal stem cells in a rotator cuff repair model. *Am J Sports Med* 2009;37(11):2126-2133.

34. **Gulotta LV, Kovacevic D, Montgomery S, Ehteshami JR, Packer JD, Rodeo SA: Stem cells genetically modified with the developmental gene MT1-MMP improve regeneration of the supraspinatus tendon-to-bone insertion site. *Am J Sports Med* 2010;38(7):1429-1437.**

35. **Gulotta LV, Kovacevic D, Packer JD, Ehteshami JR, Rodeo SA: Adenoviral-mediated gene transfer of human bone morphogenetic protein-13 does not improve rotator cuff healing in a rat model. *Am J Sports Med* 2011;39(1):180-187.**

36. **Chen CH, Chang CH, Wang KC, et al: Enhancement of rotator cuff tendon-bone healing with injectable periosteum progenitor cells-BMP-2 hydrogel in vivo. *Knee Surg Sports Traumatol Arthrosc* 2011;19(9):1597-1607.**

37. **Gulotta LV, Kovacevic D, Packer JD, Deng XH, Rodeo SA: Bone marrow-derived mesenchymal stem cells transduced with scleraxis improve rotator cuff healing in a rat model. *Am J Sports Med* 2011;39(6):1282-1289.**

38. **Yokoya S, Mochizuki Y, Natsu K, Omae H, Nagata Y, Ochi M: Rotator cuff regeneration using a bioabsorbable material with bone marrow-derived mesenchymal stem cells in a rabbit model. *Am J Sports Med* 2012;40(6):1259-1268.**

39. Ishida K, Kuroda R, Miwa M, et al: The regenerative effects of platelet-rich plasma on meniscal cells in vitro and its in vivo application with biodegradable gelatin hydrogel. *Tissue Eng* 2007;13(5):1103-1112.

40. **Zellner J, Mueller M, Berner A, et al: Role of mesenchymal stem cells in tissue engineering of meniscus. *J Biomed Mater Res A* 2010;94(4):1150-1161.**

41. **Duygulu F, Demirel M, Atalan G, et al: Effects of intra-articular administration of autologous bone marrow aspirate on healing of full-thickness meniscal tear: An experimental study on sheep. *Acta Orthop Traumatol Turc* 2012;46(1):61-67.**

42. Angele P, Johnstone B, Kujat R, et al: Stem cell based tissue engineering for meniscus repair. *J Biomed Mater Res A* 2008;85(2):445-455.

43. Horie M, Sekiya I, Muneta T, et al: Intra-articular injected synovial stem cells differentiate into meniscal cells directly and promote meniscal regeneration without mobilization to distant organs in rat massive meniscal defect. *Stem Cells* 2009;27(4):878-887.

44. Saw KY, Hussin P, Loke SC, et al: Articular cartilage regeneration with autologous marrow aspirate and hyaluronic acid: An experimental study in a goat model. *Arthroscopy* 2009;25(12):1391-1400.

45. **Fortier LA, Potter HG, Rickey EJ, et al: Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. *J Bone Joint Surg Am* 2010;92(10):1927-1937.**

46. Wakitani S, Goto T, Pineda SJ, et al: Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1994;76(4):579-592.

47. Dragoo JL, Carlson G, McCormick F, et al: Healing full-thickness cartilage defects using adipose-derived stem cells. *Tissue Eng* 2007;13(7):1615-1621.

48. **Chang CH, Kuo TF, Lin FH, et al: Tissue engineering-based cartilage repair with mesenchymal stem cells in a porcine model. *J Orthop Res* 2011;29(12):1874-1880.**

49. **Tay LX, Ahmad RE, Dashtdar H, et al: Treatment outcomes of alginate-embedded allogenic mesenchymal stem cells versus autologous chondrocytes for the repair of focal articular cartilage defects in a rabbit model. *Am J Sports Med* 2012;40(1):83-90.**

50. **Reich CM, Raabe O, Wenisch S, Bridger PS, Kramer M, Arnhold S: Isolation, culture and chondrogenic differentiation of canine adipose tissue- and bone marrow-derived mesenchymal stem cells: A comparative study. *Vet Res Commun* 2012;36(2):139-148.**

51. **Jakobsen RB, Shahdadfar A, Reinholt**

FP, Brinchmann JE: Chondrogenesis in a hyaluronic acid scaffold: Comparison between chondrocytes and MSC from bone marrow and adipose tissue. *Knee Surg Sports Traumatol Arthrosc* 2010; 18(10):1407-1416.

52. Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH: Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: An observational cohort study. *Am J Sports Med* 2010;38(6): 1110-1116.

53. Saw KY, Anz AW, Siew-Yoke Jee C, et al: Articular cartilage regeneration with autologous peripheral blood stem cells versus hyaluronic acid: A randomized controlled trial. *Arthroscopy* 2013;29(4):684-694.

54. Kon E, Mandelbaum B, Buda R, et al: Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: From early degeneration to osteoarthritis. *Arthroscopy* 2011;27(11):1490-1501.

55. Filardo G, Kon E, Di Martino A, et al: Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: Study design and preliminary results of a randomized controlled trial. *BMC Musculoskelet Disord* 2012;13:229.

56. Halpern B, Chaudhury S, Rodeo SA, et al: Clinical and MRI outcomes after platelet-rich plasma treatment for knee osteoarthritis. *Clin J Sport Med* 2013; 23(3):238-239.

57. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A: Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: A prospective, double-blind, randomized trial. *Am J Sports Med* 2013;41(2):356-364.