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

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.

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 non-surgical 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.
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 process4,5 (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).

Table 1

Biologics: General Components and Orthopaedic Applications

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considered to be more than minimal manipulation. 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. 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.

### 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
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 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.

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**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.

Figure 2

<table>
<thead>
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<th>Germ layer</th>
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<td>Synovial fluid/synovium</td>
<td>Bone marrow–derived MSCs</td>
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<tr>
<td>Abluminal side of blood vessels in fat</td>
<td></td>
<td>Perivascular locations of bone marrow</td>
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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.

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**Stem Cells**

Stem cells are one generation matured 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.14 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.
through the process of apheresis. PBSCs have been harvested and used safely in the field of hematology onco-logy for bone marrow transplant, with documented 12-year safety data in healthy volunteers.\textsuperscript{14}

Bone marrow-, adipose-, perios-teum-, and synovial-derived MSCs as well as PBSCs have demonstrated the capacity to differentiate into cells of the osteocyte, chondrocyte, and adipo-cyte lineage.\textsuperscript{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.\textsuperscript{10,13} Direct comparison of MSCs and PBSCs has shown that they have the same potential with regard to proliferative and trophic ability.\textsuperscript{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.\textsuperscript{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.\textsuperscript{20,21} Investigators seeking to use cells from synovial tissue have also used culture processes to increase cell numbers.\textsuperscript{22} PBSC can be harvested in higher numbers through a process involving stimulation with a mobilization drug and harvest through apheresis.\textsuperscript{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.\textsuperscript{11}

Three studies have investigated PRP application after completion of repair. Results include no difference,\textsuperscript{22} improved pain scores within the first 30 days and clinical scores at 3 months alone,\textsuperscript{26} and an improvement in tendon integrity noted on MRI evaluation.\textsuperscript{27} The findings of Randelli et al\textsuperscript{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

**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.\textsuperscript{24-32} Five studies intercalated a platelet-rich fibrin matrix between the osseous bed of repair and the tendon.\textsuperscript{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\textsuperscript{29,30} illustrated a detrimental effect (ie, decreased healing rates on postoperative imaging analysis of the healing tendon).

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to rotator cuff repair. 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. In rat models, Gulotta and colleagues 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. 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. Modifying cells with gene transfer of human bone morphogenetic protein-13 did not improve healing at either 2- or 4-week follow-up. 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. 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.

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 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. 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. Two studies have investigated the effects of PRP on defect healing in a rabbit model. In each study, punch defects were created and scaffolds were used for PRP deployment. Ishida et al 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 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. 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. In the first study, large defects equivalent to resection of the body of the meniscus were created in both knees in rabbits. 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. 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 and in a meniscectomy model in rats. 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. 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. 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. The first study involved subchondral drilling of a cartilage defect in a goat model to investigate postoperative injections of BMA. 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. 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. In 1994, Wakitani et al 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 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. 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. Lee et al 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. 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. In a rabbit model, ASCs applied in a fibrin glue scaffold illustrated excellent rates of subchondral bone healing. 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.

The clinical application of stem cells in cartilage regeneration has been studied in an observational cohort study, a case series with histology, and a randomized controlled trial. The observational cohort study compared autologous chondrocyte implantation in 36 patients with bone marrow–derived MSC implantation in 36 patients. 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...
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\(^2\) (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.\(^3\) The intervention group underwent postoperative injections of PBSC and hyaluronic acid, and the control group underwent injections of hyaluronic acid alone. Repair tissue as evaluated with the International Cartilage Repair Society II histologic

<table>
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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.)
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. 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.

**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.

**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.

![Image of one evolving theory of stem cell–augmented chondrogenesis. Illustrations involving use of a mesenchymal stem cell (MSC) implant to repair a defect.](image-url)
Acknowledgment

We would like to extend a special thank you to Kevin Johnson, PhD, MBA, for his assistance with the preparation of the regulatory affairs aspect of this manuscript and with Figure 1.

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.


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