Narrative Review

Clinical Challenges and Opportunities of Mesenchymal Stem Cells in Musculoskeletal Medicine

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The use of stem cells in orthopedics has been researched for many years, with robust animal data that show efficacy in cartilage healing, tendon repair, and intervertebral disk treatment. Early clinical data are also just starting to be published, and these results are encouraging. Safety data in large case series, some that lasted for many years, have also been published. The field of tissue engineering with stem cells in musculoskeletal impairments has the potential to reduce morbidity and improve clinical outcomes. The regulatory environment for this area of medicine is still developing.

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INTRODUCTION

This past decade has seen the emergence of physical medicine and rehabilitation (PM&R) specialists and sports medicine–trained family physicians as providers of minimally invasive, interventional musculoskeletal (MSK) care. Biologics, for example, platelet-rich plasma, have allowed a new generation of practitioners to treat maladies, for example, chronic lateral epicondylopathy, that were previously refractory to nonsurgical care [1]. These same groups of physicians have now begun to seek other biologic agents that may help treat a broader variety of MSK problems, for example, moderate-to-severe osteoarthritis. One of the tools that has been increasingly considered as an adjunct to platelet-rich plasma is stem cells. My clinical experience with stem cells began in 2005 with treating osteoarthritis (cartilage and bone repair) and intervertebral disk (IVD) disease. This review will focus on the published data in these areas of MSK medicine.

Stem cells fall into 3 major categories: embryonic, adult, and induced pluripotent cells. Adult stem cells can be found in almost every human tissue and can further be broken down into allogeneic and autologous, and are differentiated by the degree of processing required to obtain a therapeutic agent. Of the many adult stem cell types considered for therapy, the greatest publication volume listed in the U.S. National Library of Medicine for MSK disorders is on mesenchymal stem cells (MSC). For example, as of this writing, there are 2315 articles listed in PubMed for cartilage and MSCs [2].

WHY THE CELL SOURCE FOR CARTILAGE OR IVD REPAIR IS IMPORTANT IN MSK THERAPIES

MSCs are multipotent, adult stem cells that show clinical promise as therapeutic agents in regenerative medicine [3-7]. Does the cell source matter to promote the kinds of tissue repair that are important to the MSK practitioner? MSCs can be easily isolated from many anatomic locations, including from whole marrow aspirate, muscle biopsy, adipose liposuction aspirate, and from other tissues [3]. For orthopedic uses, these sources have been compared by many researchers for their ability to heal bone and cartilage, with differences being noted. As a rule, the closer the source tissue is to the target tissue being treated, the more effective the MSCs appear to be at differentiation into the target tissue. For example, Vidal et al [8] compared equine MSCs derived from bone marrow (bm-MSC) versus adipose tissue (a-MSC) for chondrogenic potential and found that bm-MSCs produced a

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more hyaline-like matrix and had improved glycosaminoglycan production. Additional animal studies demonstrated that bm-MSCs produced better repair of a tibial osteochondral defect when compared with a-MSCs [9]. In keeping with this trend, Yoshimura et al [10] determined that MSCs derived from the synovial tissue of the knee (closest to the target tissue of chondral cartilage) had better chondrogenesis than bm-MSCs.

Significant controversy exists about whether adipose or bone marrow is a better source for orthopedic tissue repair [11]. Although a-MSCs are more prevalent and are capable of orthopedic tissue differentiation, obtaining orthopedic tissues from this type of cell requires the use of considerably more growth factors. In addition, as stated above, the native chondrogenic potential of a-MSCs does not appear to be as robust as bm-derived MSCs [12]. To compare these 2 tissue sources further, a review of the U.S. National Library of Medicine citations for all common stem cell types in which animal models of cartilage repair are used is shown in Figure 1. Note the great disparity between bm-MSCs and a-MSCs (stromal vascular fraction [SVF]). The number of subjects who have been the topic of an indexed publication or a U.S. Food and Drug Administration trial in which cartilage is being treated based on stem cell type is shown in Figure 2. Also note the great disparity between bone marrow and adipose tissue sources (SVF). Finally, the results of in vitro research in which bone marrow stem cells versus adipose stem cells were compared for cartilage production are shown in Figure 3. Again, note the disparity between the numbers of articles that show that bone marrow is a superior cell source for this purpose.

For IVD repair, most research to date has been carried out with bone marrow—derived expanded MSCs. For example, Risbud et al [13] and Richardson et al [14] investigated the coculturing of expanded bm-MSCs with cells from the nucleus pulposus, which shows that this can partially differentiate MSCs that are capable of repopulating the nucleus pulposus in an animal model. Other studies, by Sakai et al [15] and Zhang et al [16], also used bm-MSCs as a tissue source and noted robust repair of animal IVDs. Li et al [17] and Liang et al [18] also tested a-MSCs in vitro for the purpose of mimicking conditions in the human IVD.

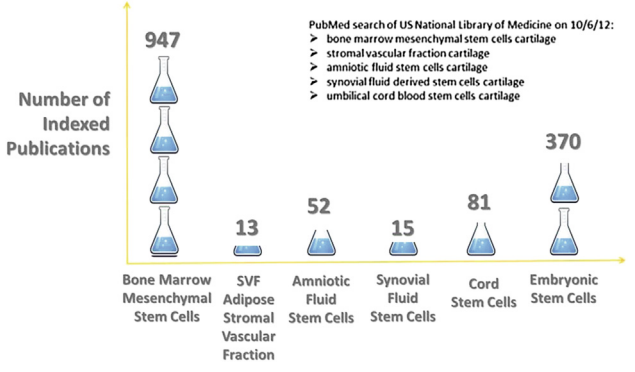

Figure 1. The number of publications listed in the U.S. National Library of Medicine for common stem cell types in the area of cartilage repair.

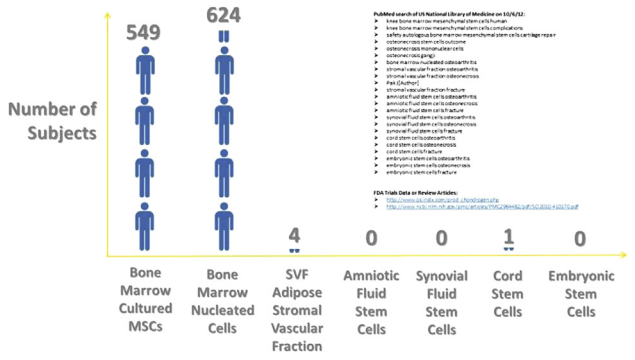

Figure 2. The number of subjects treated for cartilage repair listed in publications in the U.S. National Library of Medicine or the U.S. Food and Drug Administration clinical trials, by stem cell type.

HOW MANY CELLS DO YOU NEED? STEM CELL CULTURE VERSUS SAME-DAY PROCEDURES

Although most of the indexed publications on MSCs are based on expanded cells, because of regulatory difficulties, the vast majority of U.S. clinical experience is with stem cells that are isolated the same day. The 2 procedures are quite different, both in the number and in the purity of the stem cells obtained. A limited number of cells can be obtained from any tissue. In some instances, the numbers that can be harvested are fewer than the quantity of cells needed for tissue repair. One method of obtaining more cells is by culturing (also called "expanding") to obtain larger numbers. MSCs are usually culture expanded via monolayer culture, which involves seeding a certain density of cells onto a specialized flask and allowing the MSCs to attach to a plastic surface. In this way, MSCs are selected from the marrow nucleated cell population through adherence and form colonies on the plastic. The MSCs that are adherent are then fed

Figure 3. The number of indexed articles that show that bone marrow or adipose stem cells are superior for cartilage production when the 2 stem cell types are compared head to head in in vitro studies.

via a nutrient broth called a "culture medium," which is maintained above the growing cells. Because MSCs are contact inhibited, they will grow on this surface until they touch one another, a state known as "confluence." Once confluence occurs, MSCs will abruptly stop propagating; it is commonly believed that this "contact inhibition" property of MSCs is a key feature of their enhanced safety profile over other stem cell types that will propagate indefinitely. MSCs are then replated in a similar flask, and fresh medium is added, a process known as a "passage." Most MSCs in culture are grown to the second to fifth passage, because some studies have shown decreased differentiation if MSCs are grown for prolonged periods in culture, with a higher chance of genetic mutation (Figure 4) [12,19-21].

Same-day procedures are quite different. In this process, the tissue is harvested and then processed either to release cells and then isolate, or to simply isolate a fraction of the tissue that contains stem cells. For example, for adipose tissue, the MSCs are locked in a collagen matrix that needs to be broken down before they can be isolated via centrifugation [11]. Once this fraction is isolated, it is called the SVF. For bone marrow, the MSCs exist in suspension, so simple centrifugation steps without digestion can isolate a fraction of nucleated cells described as the "buffy coat," which contains stem cells [22]. In both instances, the cell population obtained is not just an isolated population of MSCs but contains many other cell types.

HOW DOES THIS WORK? DIFFERENTIATION VERSUS PARACRINE EFFECTS

Animal studies have demonstrated the multipotency of MSCs and how they can differentiate into muscle, bone, cartilage, tendon, and various cells of internal organs. This process was once believed to be the only way that MSCs could act to repair tissue. However, it is now known that these cells also act via paracrine mechanisms to assist in tissue repair. For cell therapy, "paracrine" means the production of certain growth factors and cytokines by the MSCs, which then facilitate tissue repair [23]. These growth factors include transforming growth factor—β (TGF-β), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and other signaling factors that can help recruit other cells to the local area [24]. In this way, MSCs can act as coordinator of the repair response without having to act directly through differentiation to repair tissue [12].

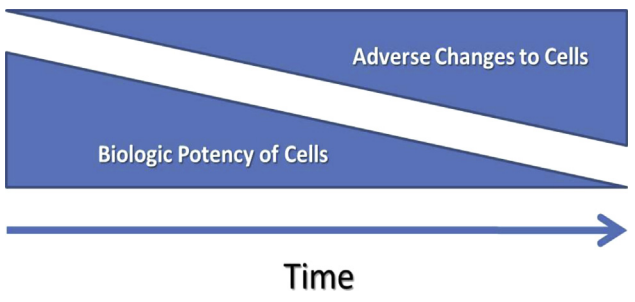

Figure 4. Adverse changes in cells increase with time in a culture as biologic potency decreases.

To use osteoarthritis as an example, the MSCs could either differentiate into new cartilage tissue to replace worn-out areas or to coordinate a repair response through other cells. In addition, recent research has shown a third possible mechanism of action in cartilage. Van Lent et al [25] recently demonstrated that MSCs can detect activated macrophages in an animal model of osteoarthritis. These macrophages usually become activated by cartilage breakdown products and thus participate in further catabolism. These researchers noted that MSCs deactivated the macrophages and triggered them to secrete anabolic cytokines (Figure 5).

YOUR CELLS OR SOMEONE ELSE'S: AUTOLOGOUS VERSUS ALLOGENEIC

Autologous stem cells obviously do not have the same communicable disease—risk profile as donor allogeneic cells. However, there may be practical reasons why donor cells may be attractive. For example, results of some studies have shown a decreased differentiation potential for MSCs obtained from older patients [26]. In addition, somatic genetic variants (ie, trisomy V and VII) have been demonstrated in the MSCs and osteoprogenitors of some patients with osteoarthritis [27]. Allogeneic cells could also be able to be mass produced in bioreactors and provide a ready supply of new cells for therapy. However, some concerns have been raised about the use of allogeneic stem cells. As an example, Ueda et al [28] observed that stem cells transplanted from the bone marrow of elderly mice bred to have osteoporosis were able to induce osteoporosis in young healthy mice, which indicates that the stem cells themselves may be a new and previously unknown genetic disease vector. In addition, many researchers have argued that allogeneic MSCs are immune privileged because they lack major histocompatibility complexes; however, several researchers have noted that allogeneic MSCs can activate various parts of the host's immune system [29-31]. Recently, another entry in this debate demonstrated that this loss of immunoprivilege with differentiation may be mediated by a downregulation of interleukin-6 [32]. In summary, although allogeneic MSCs may solve some issues, such as easy cell availability and mass distribution, they also present significant hurdles that have to be overcome to allow laboratory-to-bedside translation [12].

TRANSLATIONAL STUDIES IN OSTEOARTHRITIS AND IVD REPAIR

The pace of clinical translation of MSC use for orthopedic care is accelerating. Centeno et al [33-35] published case studies in which positive magnetic resonance imaging (MRI)

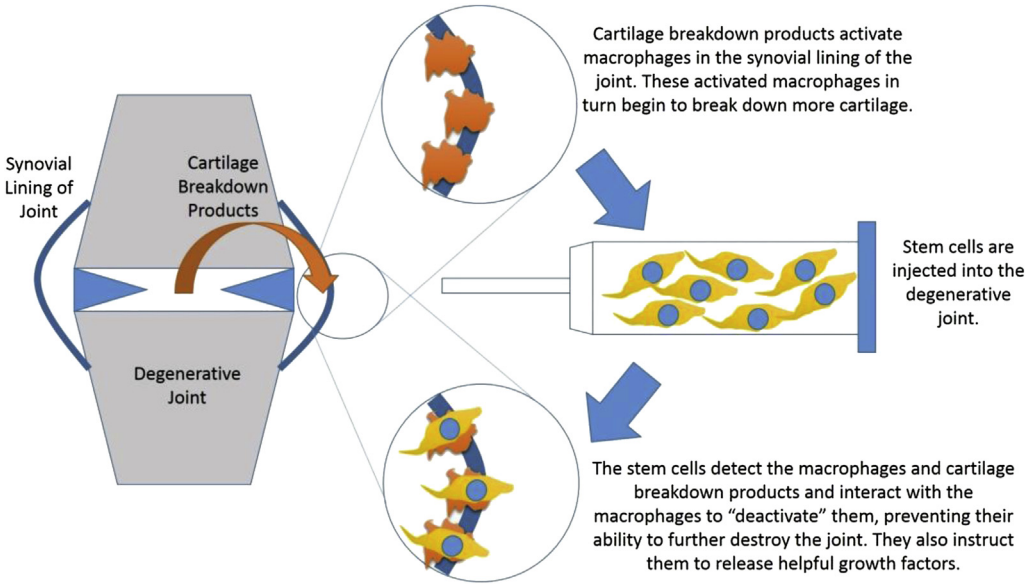

Figure 5. Mesenchymal stem cells can deactivate macrophages that have been previously activated by joint cartilage breakdown products.

changes in cartilage and meniscus were observed in knees treated with culture-expanded MSCs and in hips treated with nucleated cell concentrates, which corresponded with symptomatic improvement. The researchers also noted that the complication rate of expanded MSC injection procedures is no longer than that of other needle-based interventional techniques directed at peripheral joints [36]. In published data about 339 patients, this safety profile continued up to 3 years after MSC reimplantation, with more than 200 MRIs of the reimplant sites showing no evidence of ectopic tissue or tumor formation [12,37]. In addition, the researchers observed clinical outcomes of 135 patients with moderate-to-severe knee arthritis in which approximately 2 of 3 were candidates for knee replacement. More than 75% improvement was reported in 41.4%; more than 50% improvement was noted in 63.2%, and, at 11.3 months from the first procedure, the average reported relief was 53.1%.

Osiris Therapeutics (Columbia, MD), a company that pursues U.S. Food and Drug Administration (FDA) clinical trials, reported similar results when using injections of allogeneic cultured MSCs with hyaluronic acid used for patients with moderate osteoarthritis. In this smaller ($n = 55$) but randomized controlled trial, the average improvement in the visual analogue scale pain score was approximately 30 mm better than with placebo injections. The researchers also noted decreased degenerative changes in the stem cell—treated knees [38]. Other researchers have described similar safety profiles with the use of more-invasive surgical implantation techniques. Wakatani et al [39] described effective treatment of cartilage defects in 9 knees with culture-expanded MSCs. They also published an 11-year prospective study of 45 knees (of 41 patients) treated with autologous bm-MSCs, with results that indicated both safety and efficacy [40]. Nejadnik et al [41] recently described a comparison between surgically implanted chondrocytes versus MSCs placed by needle in 72 knees of older patients, which demonstrated safety and efficacy over those of an autologous chondrocyte procedure [12]. Haleem et al [42], in a case series, noted similar cartilage healing.

In a recent meta-analysis that includes many of the articles noted above, Peeters et al [43] reviewed 8 published studies that encompassed 844 procedures in human patients for whom MSCs were used in peripheral joints. They then compared the safety of intra-articular expanded MSC therapy to hyaluronic acid injections for knee osteoarthritis and found the published safety profile to be better than that of hyaluronic acid therapy. Several early clinical trials that used same-day stem cell isolate procedures have also been published. Varma et al [44] reported on 50 patients who were treated with either bone marrow buffy coat cells plus arthroscopic debridement or debridement alone, and showed improved osteoarthritis outcome scores and quality-of-life measures in the stem cell group. Giannini et al [45] reported on a case series of 48 patients with osteochondral lesions of the talar dome treated surgically with debridement and bone marrow—nucleated cells, which demonstrated functional improvement. Hernigou and Beaujean [46] reported on a prospective study of 189 hips in 116 patients treated with core decompression and marrow-nucleated cells. At 5-10 year follow-up, only 9 of 145 hips with Assocation Research Circulation Osseous (ARCO) grade 1-2

lesions and 25 of 44 hips with ARCO grade 3-4 lesions needed total hip arthroplasty despite the therapy. Finally, Pak [47] published 2 cases of bone and cartilage repair in hip avascular necrosis and knee osteoarthritis using adipose SVF.

Traditional spinal surgery for degenerative disk disease (DDD) continues to show mixed results [48]. In particular, no disease-modifying agent for DDD has been demonstrated. Stem cells hold promise that it may be possible to someday effectively treat DDD or its components. Several animal models of disk repair using MSCs have been published. For example, Sakai et al [15] validated animal models in which MSCs are usually combined with atelocollagen and inserted into an experimentally created degenerative disk. This research group observed encouraging improvements in MRI disk hydration, height, and morphology. Zhang et al [16] showed that MSCs injected into disks without preconditioning or coculture can help to increase proteoglycan production in the nucleus pulposus. Finally, Miyamoto et al [49] recently demonstrated that intradiskal transplantation of synovial-derived MSCs prevented IVD degeneration through suppression of catabolic genes and possibly new proteoglycan production [12].

Although very little has been published on IVD repair in humans, sparse clinical data are available. Yoshikawa et al [50] recently published on 2 patients who were treated with surgically implanted MSCs, with both patients showing modest improvements in vacuum phenomena on follow-up MRI. A recent phase 2 FDA clinical trial of cultured expanded, allogeneic MSCs injected intradiskal for DDD in 100 patients showed promising pain and/or functional results at 6 months [51]. Regrettably, the structural endpoint for the study, reversal of DDD, was not achieved.

To date, no indexed publications have described a viable model of using same-day isolated buffy coat cells in IVDs. In addition, one serious complication has been reported from early clinical use of this new technology. Subach et al [52] reported on a case of epidural abscess and cauda eqiuna syndrome after adipose stem cells, bone marrow aspirate, and platelet-rich plasma were injected into the IVD. My own experience with the use of expanded and same-day isolates for IVD repair is pending publication.

REGULATION

The regulatory environment in the United States and Europe for stem cells is still developing. In the United States, the general regulatory concept is that cells are to be classified the same as mass-produced drugs. This initial schema was worked out through a series of hearings in the late 1990s, when many vocal institutions were opposed to this concept. For example, diverse groups such as The American Red Cross, The American Society of Clinical Oncology, and Northwestern University all voiced opposition to the principle that cells should be regulated in the same way as drugs [53]. Despite that opposition, in the United States, the FDA currently considers stem cells that are more than minimally manipulated in the same regulatory category as mass-produced drugs. This "drug" category includes cultured cells using any cell for a nonhomologous use (eg, an a-MSC for an orthopedic indication) and cells that have significant processing beyond simple steps such centrifugation, mechanical cutting, or freezing. Legal challenges to this regulatory schema are in process [54].

The regulatory situation is Europe is similar but also quite different. For example, although there are similar European Medicines Agency regulatory rules in place to determine which therapies need drug-like approvals, Europe also has allowed a hospital exemption for advanced therapies [55], which means that culturing of stem cells is currently allowed as long as that use is for the patients served by that hospital. Some researchers have argued that this hospital exemption for advanced therapies allows physicians to innovate at a faster pace than the United States. Others researchers involved in cell drug development have argued that it reduces investment [56].

Over these past few years, the FDA has made it clear that even some preparations of stem cells that are made at the bedside in a physician's office are drugs that require an Investigational New Drug Application (IND). In 2011, several letters from the FDA Tissue Reference Group sent to medical providers clarified that adipose tissue processed at the patient's bedside during the same surgical procedure to obtain SVF, qualifies as a drug style 351 biologic that must have FDA approval before clinical use (per letter from EF Lazarus, U.S. PHS, written communication, November 2011) [57]. At this point, same-day buffy coat isolation from bone marrow aspirate falls under a physician processing exemption (21 CFR 1271.15[b]), so this is not considered to be the production of a drug [58]. The rationale for why processed adipose tissue is a drug and processed bone marrow is not appears to be that adipose SVF requires digestion or breakdown of the collagen matrix of the tissue and hence alters the relevant biologic characteristics of that tissue. However, for bone marrow, the tissue exists in a liquid suspension, so that cellular digestion of the collagen matrix is not required. In summary, because this field is a delicate balance between physician-driven innovation through surgical or injection procedures, and pharmaceutical company development, ultimately the pace of innovation will be defined more by the regulatory structure that prevails than by the possibilities of the cells themselves.

WHAT IS ON THE HORIZON?

Although the clinical potential of stem cell therapy for treating osteoarthritis and IVD disease is great, much remains to be done. A review of the clinicaltrials.gov database under the search terms "osteoarthritis" or "intervertebral disk" and "stem cells" yielded 35 clinical studies ongoing in osteoarthritis and 2 in degenerative disk disease. Our

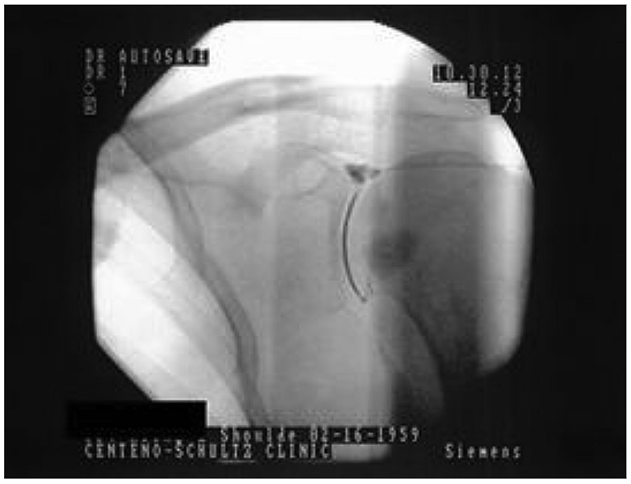

Figure 6. The placement of stem cells into a superior labral tear by using ultrasound (not shown) and fluoroscopy to guide the needle along the final delivery path (courtesy of Ron Hanson, MD, The Centeno-Schultz Clinic).

research group at The Centeno-Schultz Clinic has 3 randomized controlled trials underway in the areas of the use of biologics to replace epidural steroid injections and the use of stem cells to treat nonretracted anterior cruciate ligament (ACL) and rotator cuff tears.

Over the next 10 years, however, I believe that biologic therapies will create a new medical specialty that I call "interventional orthopedics," which will be made up of physicians who specialize in the precise percutaneous delivery of biologics to repair tissue. New medical devices and technologies will be developed to enhance what can be treated without the need for surgery, as well as to optimize the accuracy of placement. In some ways, the rapid explosion of the use of fluoroscopy during the past decade and the use of MSK ultrasound during this one is an example of the early changes that will form the foundation of this specialty. PM&R is ideally suited for this new specialization. For PM&R residency programs, this means that residents will need not only to be taught accurate MSK diagnostic examination but also advanced needle placement skills and basic cell biology. For example, I envision the placement of stem cells into a superior labrum and biceps anchor by using both MSK ultrasound and fluoroscopy to heal a superior labrum from anterior to posterior (SLAP) tear rather than surgically excising or anchoring torn tissue. To accomplish this, PM&R residents will need to be expert in both MSK ultrasound and fluoroscopy, because the needle cannot be well visualized through the course of the entire procedure by using only ultrasound (Figure 6).

For IVD repair, PM&R residents will not find ultrasound helpful, so advanced interventional spine skills using fluoroscopy will be needed. For example, imagine the placement of specific cells into the posterior disk annulus fissure by using a novel catheter to enhance fibrous tissue repair. Again, to be performed safely (Figure 7), this procedure requires many years of spinal injection experience. In addition, to achieve highly accurate placement into a high-intensity zone, I can envision fluoroscopy systems that

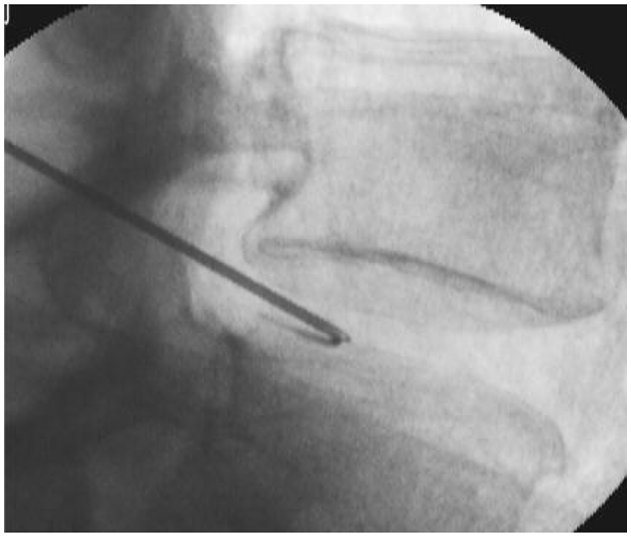

Figure 7. The delivery of stem cells to the lumbar posterior disk annulus via a novel nitinol catheter (courtesy of John Schultz, MD, and The Centeno-Schultz Clinic).

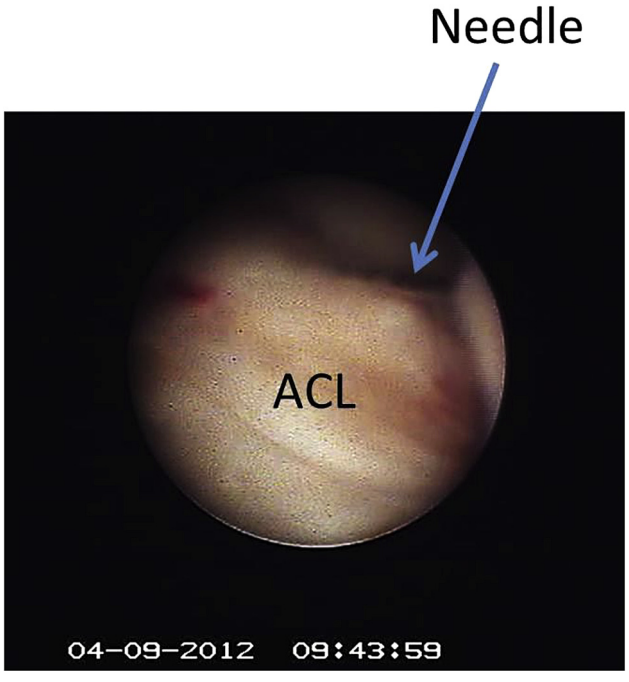

Figure 8. The placement of stem cells into a nonretracted knee anterior cruciate ligament (ACL) tear, targeting the ACL ligament sheath under direct visualization of the needle by using needle arthroscopy through a second injection site (courtesy of Ron Hanson, MD, and The Centeno-Schultz Clinic).

would overlay the MRI target on the screen, which would allow the interventionalist to guide a catheter or specialized needle to the exact region of the tear.

In the future, think about how orthopedic care would change if PM&R physicians were routinely placing stem cells into completely torn and retracted ACL ligaments by using new tools to approximate the torn ligament via needle placement. What new skills might residents need to be able to "see" the approximated tissue to accurately place cells at the correct interface? As an example, "needle arthroscopes" will allow the physician to visualize tissue at the end of the needle (Figure 8). The use of this type of technology by PM&R physicians would require a ground-up reworking of even the most sophisticated residency programs now offering interventional training.

I believe that new biologics such as stem cells will change how orthopedic care is delivered, moving much of that care to interventionalists in the next 20 years. Just as interventional cardiology replaced much of the need for coronary artery bypass grafting via sternotomy in the 1980s to 1990s, I expect that, by 2020, the transformation of orthopedics from a surgical to a percutaneous specialty will be well underway. Finally, PM&R physicians will be well positioned to help patients enjoy the benefits of these much-less-invasive therapies that will repair rather than excise damaged tissue.

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