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Regenerative Medicine

Regenerative Treatments to Enhance Orthopedic Surgical Outcome

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Abstract

In orthopedic surgery there has been a never-ending quest to improve surgical outcome and the patient's experience. Progression has been marked by the refinement of surgical techniques and instruments and later by enhanced diagnostic imaging capability, specifically magnetic resonance. Over time implant optimization was achieved, along with the development of innovative minimally invasive arthroscopic technical skills to leverage new versions of classic procedures and implants to improve short-term patient morbidity and initial, mid-term, and long-term patient outcomes. The use of regenerative and/or biological adjuncts to aid the healing process has followed in the drive for continual improvement, and major breakthroughs in basic science have significantly unraveled the mechanisms of key healing and regenerative pathways. A wide spectrum of primary and complementary regenerative treatments is becoming increasingly available, including blood-derived preparations, growth factors, bone marrow preparations, and stem cells. This is a new era in the application of biologically active material, and it is transforming clinical practice by providing effective supportive treatments either at the time of the index procedure or during the postoperative period. Regenerative treatments are currently in active use to enhance many areas of orthopedic surgery in an attempt to improve success and outcome. In this review we provide a comprehensive overview of the peer-reviewed evidence-based literature, highlighting the clinical outcomes in humans both with preclinical data and human clinical trials involving regenerative preparations within the areas of rotator cuff, meniscus, ligament, and articular cartilage surgical repair.

Introduction

Regenerative adjunctive treatment is the next logical step in the progression of surgical intervention. Biologically augmented or regenerative techniques are at the very forefront of modern treatment and have the potential to transform the practice of medicine and surgery significantly in a very short period. Less than 20 years ago, one of the first applications of platelet-rich growth factors was successfully used to help augment dental implantation [1]. From this starting point progressive advancements have been made, but much remains to be learned. Although the basic science remains in its infancy, especially in the areas of signaling, regulation, and mechanism, regenerative knowledge has expanded significantly in volume and across disciplines. The purpose of this review is to provide a road map of the significant developments in preclinical and clinical results involving biological solutions to improve rotator cuff, ligament, meniscus, and articular cartilage surgical repair.

Discussion

Methodology

Searches via PubMed (through August 15, 2014) and Google Scholar (through August 15, 2014) were performed to identify both scientific investigations and review articles to ensure inclusion of pertinent data. Key words used included platelet-rich plasma (PRP), mesenchymal stem cell (MSC), stem cell, growth factor, basic science, cell signaling, paracrine, autocrine, anterior cruciate ligament (ACL), rotator cuff, meniscus, and cartilage. The articles were downloaded directly from publishers or other online resources when they were not available from the local medical library and/or through interlibrary loan. The articles were then

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reviewed for additional references and originality. Primarily, the methodology and results were extracted from each pertinent scientific article and categorized as either preclinical or clinical data and presented in the respective section.

Basic Science

Background

Orthopedic surgery and therapeutics have come a long way since the time of Aristotle, when the use of bone marrow for restorative procedures was described. Connolly and others [2-4] initiated the modern era of evidence-based medicine regarding the role of bone marrow in surgical treatments such as bone fracture reconstruction. Indeed, needle aspiration of the iliac crest to obtain autologous bone and marrow, especially for spinal fusions, became a standard procedure in the 1980s [5,6]. This later understanding that marrow could stimulate or add value to osteogenic reconstruction and the popularity of bone marrow transplantation for the past 50 years [7] has resulted in further scientific exploration into the cellular basis of marrow's therapeutic properties.

Based on this research, it has been proposed that bone marrow aspirate (BMA) contains multipotent progenitors, which Caplan [8] has named MSCs, as pictured in Figure 1. It should be emphasized that the understanding in the late 1980s and early 1990s was that adults had only one stem cell in marrow, the hematopoietic stem cell. Furthermore, the scientific community maintained that individuals were born with the requisite number of specific organ-specific cells (say, cardiac myocytes) and that those cells became bigger or smaller but did not increase in number [9].

The current scientific evidence has now provided evidence that *every* tissue in the body has tissue-specific progenitors and that MSCs are derived, totally or in part, from perivascular cells called pericytes [10-12]. Thus muscle has satellite cells (myogenic progenitors) and a separate yet distinctive class of MSCs that reside as functional pericytes in uninjured muscle, and tendons have tendon progenitors and pericyte-derived MSCs. The MSCs from bone marrow, muscle, and tendon have the same general properties, but their basic chemistries are quite different as controlled by both their tissue of origin and the genome of the donor.

PRP

Platelets are small non-nucleated bodies in peripheral blood that are involved in hemostasis. Platelets contain a number of proteins, cytokines, and other bioactive factors that regulate wound healing. Plasma is the fluid portion of blood and contains clotting factors, proteins, and ions. Several authors have suggested that the definition of PRP should include preparations that have a platelet concentration of at least 1 million platelets per microliter and a 3- to 5-fold increase in growth factor concentration and cytokines. Preparations of this composition have been associated with the enhancement of healing [13,14].

The basic cytokines from the alpha granules of platelets include transforming growth factor−β (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor I and II, fibroblast growth factor, epidermal growth factor, vascular endothelial growth factor (VEGF), and endothelial growth factor. These growth factors have important regulatory effects on MSCs [15,16].

Bioactive factors are also found in the dense granules in platelets, including serotonin, histamine, dopamine, calcium, and adenosine. These non−growth factors affect aspects of wound healing such as inflammation proliferation and remodeling [17].

The platelets in PRP can be delivered in a clot that contains adhesion molecules such as fibronectin, fibrin, and vitronectin [18].

MSCs

Bone marrow MSCs can be isolated and expanded in culture [19,20]. These MSCs are a heterogeneous mixture of cells that have at least 2 different capabilities. Some of these cells are already committed to the osteogenic pathway and accelerate bone formation and regenerative repair [2-4,21,22], whereas other MSCs have the capacity to be immunomodulatory and trophic [23]. These MSCs are formed at broken and inflamed blood vessels where the local pericyte detaches from the vessel and becomes an activated MSC. This in situ MSC secretes a curtain of bioactive agents that locally inhibit the overaggressive immune system from sending in integrating cells. This is the body's first line of control and defense against establishing an autoimmune reaction against the antigens exposed by the injured tissue. This immunomodulatory capacity of MSCs can be harnessed to provide therapeutic effects against graft-versus-host disease, Crohn disease with its inflammation of the gastrointestinal tract, and a large array of other clinical situations (for more information, search for "mesenchymal stem cells" at clinicaltrials.gov).

The "trophic" effects of MSCs establish a regenerative microenvironment at the site of injury by (1) inhibiting ischemia-related apoptosis, (2) inhibiting scar formation, (3) stimulating angiogenesis by secreting large amounts of VEGF and by transforming some of the MSCs back into pericytes that function to stabilize the fragile, newly forming capillaries, and (4) secreting tissue progenitor-specific mitogens so that the slow process of tissue regeneration is enhanced [24]. Thus MSCs serve as "drug stores" [25] for sites of injury and/or inflammation by providing an array of bioactive molecules tailored for that site and the injury (Figure 1) [25,26].

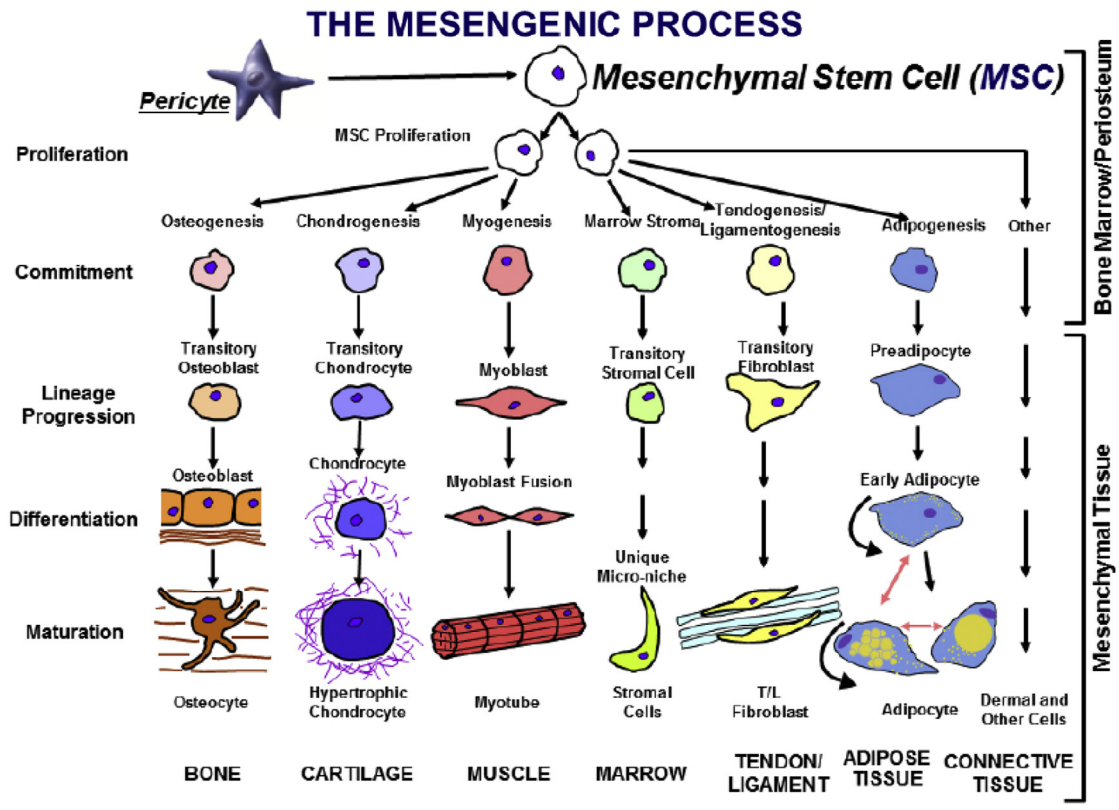

Figure 1. The mesengenic process was first envisioned in the late 1980s as a pathway for marrow mesenchymal stem cells (MSCs) to differentiate into a number of mesodermal cell types that could contribute to the fabrication of bone, cartilage, and muscle [25,26]. It is now clear that MSCs can be isolated from many tissues because they are derived from perivascular cells and pericytes.

Adipose-Derived MSCs

Adipose-derived MSCs can be derived from the abluminal side of blood cells in fat. They lack the expression of TGF-β type 1 receptor and have reduced expression of bone morphogenetic protein (BMP)-2, BMP-4, and BMP-6 when compared with bone marrow—derived MSCs, and hence supplementation of these factors is needed for chondrogenic differentiation. BMP-6 stimulates stronger chondrogenic differentiation compared with TGF-β [27].

Peripheral Blood Stem Cells

Stem cells harvested from the peripheral blood after mobilization have garnered recent attention for orthopedic application. Immature monocytes are normally present in low numbers in the bloodstream. Their production and release into the peripheral circulation can be increased with granulocyte colony-stimulating factor analogues such as granulocyte colony-stimulating factor. Once mobilized, these cells can be harvested from the bloodstream through a process called *apheresis*. The techniques of stimulation with a mobilization agent and harvest with apheresis have been used safely by hematology oncology professionals for bone marrow transplantation for more than 20 years [28]. These cells represent a heterogenous mixture with markers indicating hematopoietic precursors and mesenchymal cell precursors [29]. The authors of some studies utilizing cells collected through mobilization and blood harvest have termed these cells *peripheral blood stem cells* (PBSCs) [29-31]. Basic science study into the properties of these cells has shown that they are similar to embryonal stem cells in that they express transcription factors specific to embryonal stem cells, have proliferative potential, have the ability to differentiate into a multitude of cell types, and are more immature than bone marrow—derived mesenchymal stem cells [30]. In addition, when injected subcutaneously into mice, these cells have been found to migrate to multiple organs, integrate, and function as the surrounding cells. Direct comparison of MSCs and PBSCs has illustrated the same potential with regard to proliferative and trophic ability [30]. PBSC harvest through apheresis can produce high numbers of cells [29]. Although the site of harvest of stem cells does not appear to effect multipotentiality, proliferative potential, or trophic ability, it does affect cell availability and regulatory constraints.

Management of Innate Regenerative Capacities

With the further understanding of the medicinal signaling properties of MSCs [32], a new concept involving the management of the patient's own intrinsic regenerative capacity has emerged. The current data

clearly indicate that almost every tissue has its own dedicated and tissue-specific progenitors and thus the capacity to regenerate. Therefore, by calculated manipulation of the local MSCs, true scarless regeneration can be achieved. For example, a torn meniscus or rotator cuff has the potential to regenerate (and probably does so in young children because of their large titers of MSCs and progenitors). By adding "extra" MSCs, it is theoretically possible to regenerate these tissues. The concept of MSC-mediated immune modulation and trophic activities are the drivers behind the more than 425 clinical trials using MSCs for a large spectrum of clinical conditions that are currently registered at clinicaltrials.gov. The strategic issue is how to deliver the MSCs in the right numbers to the right place at the right time.

Medical Management of MSCs

The titers of MSCs at various tissue locations naturally decrease with age, with rough estimates in marrow showing a 10-fold decrease between birth and the teen years and another 10-fold decrease into older ages [33]. This decrease is primarily due to decreases in vascular density, which is not only the source of local MSCs but also the cause of poor wound repair in older adults. With this decrease in vascular density comes the issue of how to medically manage local MSC levels at sites of injury.

One approach is to add autologous MSCs to the vascular circulation because MSCs naturally locate and function at sites of injury or inflammation. The MSCs will dock at these sites, sense the local environment, and react by secreting agents based on cell types at that site (eg, bone versus brain). Alternately, mobilizing and freeing MSCs from their tethers in the basement membranes surrounding all blood vessels could also be efficacious. PRP is a multicomponent cocktail that, when introduced into a vascularized tissue of a joint space, not only assists in the detachment of MSCs from their perivascular niche but also contains mitogens to cause the newly released MSCs to replicate themselves, increasing their local titers [34]. Thus, by increasing local MSCs, we would expect an enhanced regeneration of injured or surgically treated tissues.

Source of MSCs

If all MSCs are derived from pericytes [10-12], it follows that MSCs could be derived from any vascularized tissue. The best studied sources of MSCs are bone marrow [19-24,32-35] and fat [36,37], although MSCs also have been isolated from umbilical cord, placenta, skin, and muscle. In this regard, if all MSCs secrete a curtain of immune-modulatory agents, allogeneic MSCs should likewise be as useful medically as autologous MSCs. It is to be expected that MSCs from different anatomic locations or from different allogenic donors will have different intrinsic chemistries even though they may all provide efficacious medical outcomes. Indeed, several companies have reported encouraging clinical results using freshly isolated autologous MSCs (eg, Cytori Therapeutics, Inc, Tissue Genesis, and Lipogems International SRL) or culture-expanded allogeneic MSCs (eg, Mesoblast Ltd, Osiris Therapeutics, Inc, Celgene Corp, Athersys Inc, and Pluristem Therapeutics, Inc) from different donors. Clearly, the medical use of MSCs is just beginning, but based on the very successful use of marrow alone, MSC use in orthopedics will be at the forefront of new regenerative medicine protocols.

Rotator Cuff Repair

Rotator cuff tears, both partial and full thickness, can be a debilitating source of pain and cause significant shoulder dysfunction. Interestingly, in the 1990s, decompression and not repair was the gold standard. It is logical that surgically reattached tendons provided for better function of the shoulder. Furthermore, as repair of torn tendons has become the gold standard, one would assume that all repairs heal. However, a review of the literature shows that the failure rate of rotator cuff repair as reported in some publications exceeds 50%.

It has become clear that the biology of the healing needs to be addressed to augment the healing of tendon to bone. The tendons attach to bone through a transitional area of fibrocartilage and then Sharpey fibers. If a substance could be added to the repair site to help facilitate the reattachment, this technique could potentially result in higher healing rates. In other sections we have discussed the action of PRP and stem cells on healing in general; in this section we will focus on preclinical and clinical studies and the application of PRP and/or stem cell augmentation to rotator cuff repairs.

Preclinical Studies

PRP has been shown to stimulate tendon stem cells to differentiate into tenocytes and initiate collagen extracellular matrix production [38,39]. Tenocyte proliferation was shown to be significantly increased by the addition of leukocyte-poor PRP compared with control subjects, who received 2% phosphate-buffered saline solution [PBS] or 10% PBS. No difference in the proliferation of tenocytes was found when comparing all 3 PRP types [40]. Platelets are released at an injury site by activation of collagen in a more sustained fashion compared with activation by thrombin [41].

Rat rotator cuff tendons treated with PRP demonstrated better collagen linear alignment than did tendons of control subjects. Non-PRP groups had higher failure strain at 7 days, PRP had higher failure strain at 21 days, PRP had higher fibroblastic response and vascular response at each time interval, and PRP had more linear-aligned fibers at 21 days (+). These data illustrate that with use of PRP, cautious progression of rehabilitation is probably warranted during the first 3 weeks after surgery [42]. Beck et al [42] suggest that

PRP can have a negative impact on healing at 7 days, but one could also interpret that a cautious rehabilitation process for 3 weeks after surgery is prudent and that PRP has positive effects beyond 3 weeks.

The application of bone marrow—derived cells to augment rotator cuff healing has also been studied. Gulotta et al [43] used a rat model to compare cultured MSCs contained in a fibrin carrier versus fibrin carrier alone versus cultured MSCs alone. Results did not demonstrate any difference between the 3 groups in terms of collagen formation or strength of repair [43].

Clinical Studies

Meta-analysis regarding the efficacy of PRP in augmenting rotator cuff tear repair was carried out before consensus of the definition of PRP was reached [44]. The analysis highlighted lack of standardization in study methodology, PRP preparation, and outcome measures. Unfortunately, and as noted by the authors of the review, this lack of standardization would likely result in a comparison of heterogenous PRP type products and would make any conclusion difficult to ascertain with any level of certainty.

As part of orthopedic surgical integration, early pioneers set out to incorporate PRP into the repair of rotator cuffs. Many of the early adopters used platelet-rich fibrin matrix (PRFM). PRFM was naturally chosen because the processing technique allowed for creation of a clot of the PRFM, analogous to the fibrin clot process used in meniscal repair in the 1980s, and sutures could then be run through the material to help hold it in place. This approach was helpful when arthroscopic techniques were used because the PRFM clot could be run down the suture into the rotator space between the bone and the rotator cuff tendon (Figure 2).

Standardization of the surgical technique with PRFM never evolved, although it was recommended that the clot be placed between the tendon and the bone. The number of clots, size of clots per torn area, and other parameters differed among the early studies. The peer-reviewed publications in orthopedic journals have consisted of a high number of PRFM repairs. Of the 5 studies, 80% showed no benefit to healing of the rotator cuff tear [45-48]. Only a study by Barber et al [49] showed a lower retear rate when PRFM was added to rotator cuff repairs. The white pages from gravitational platelet separation (GPS) studies suggest that the platelet concentrations in PRFM may be close to baseline blood, although the spinning algorithm may push concentrations closer to 2 times baseline. All of the aforementioned studies used PRFM, which may not have had platelet concentrations much above baseline (Figure 3). Antuna et al [50] prospectively evaluated the application of platelet-rich fibrin in massive rotator cuff tears with a minimum of 24 months follow-up and noted that there was no difference in retear rate or subjective outcome between groups. Recently, thrombin-activated PRP obtained by apheresis that was used to augment arthroscopic rotator cuff repair in a randomized, controlled trial (RCT) of 2 groups of 27 patients did not show any difference in ultimate clinical improvement or the retear rate [51].

In contrast, other studies have showed benefits of PRP in augmentation of rotator cuff repair. Randelli et al [52] concluded that the addition of PRP decreased pain and positively affected rotator cuff healing. Gumina et al [53], who reported on 80 consecutive patients with large tears that were treated arthroscopically with single row repair, concluded that platelet leukocyte membrane significantly improved repair integrity on the basis of postoperative magnetic resonance imaging (MRI) but did not demonstrate a superior functional outcome.

Theoretically, techniques that may result in an increase in bone marrow—derived MSCs at the rotator cuff repair site could include performing a microfracture around the rotator cuff repair footprint and/or the addition of MSCs from exogenous sources, such as BMA (locally or remotely). Recently, investigators have used bone marrow aspirate concentrate (BMAC) and demonstrated beneficial results. Ellera Gomes et al [54] reported on traditional mini-open rotator cuff repair augmented with BMAC in 14 patients; at 12 months,

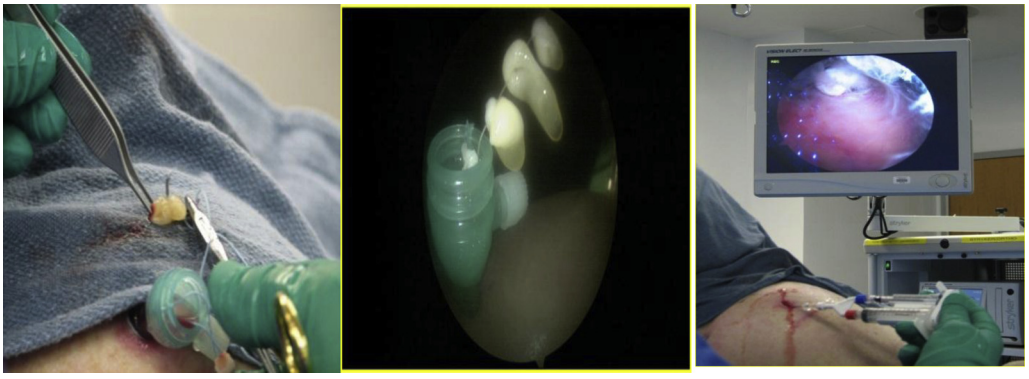

Figure 2. Arthroscopic photos showing platelet-rich fibrin matrix clots being sutured and run down the arthroscopic cannula. The final photo shows the clot just prior to placement between the tendon and the bone.

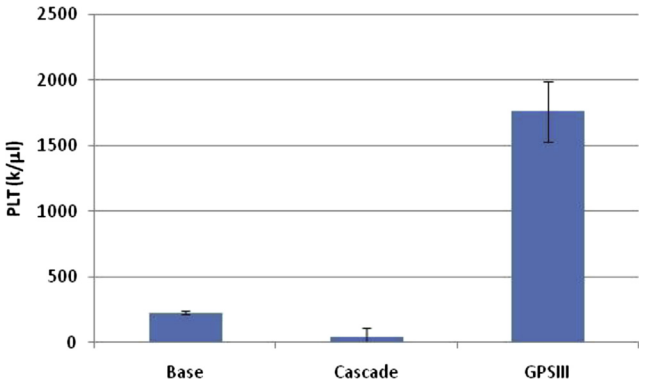

Figure 3. Early studies that showed the benefit of adding platelet-rich plasma to rotator cuff tears. Only 25% were platelet-rich fibrin matrix studies; the remainder had admixtures higher than 2.5 times baseline platelet concentrations. PLT = platelet; GSPIII = gravitational platelet separation III.

tendons were intact in 100% of patients (14/14) as determined by MRI [54]. Hernigou et al [55] compared single-row rotator cuff tear with and without BMAC adjuvant treatment with known quantitative dosing with grading of repair by monthly ultrasound for 24 months and confirmed by MRI with a minimum 10-year follow-up. The authors found that the rotator cuff healed within 6 months in 100% of patients in the BMAC group (45/45) and in only 67% of the control group (30/45). The retear rate was lower in the BMAC group, with 87% of repairs still intact (39/45), versus only 44% in the control group (20/45) at a minimum of 10-year follow-up [55].

Ligament Repair/Reconstruction

ACL reconstruction (ACLR) and other ligament surgeries are increasing in the United States and are now numbering more than 120,000 reconstructions per year [56]. Although ACLR is able to improve symptoms and function compared with preoperative function, when comparisons are made with persons who have never had an ACL injury, the results of surgery are not that impressive [57]. Poor rate of return to play [58], repeat rupture or contralateral rupture [59], and premature progression of osteoarthritis (OA) are all legitimate concerns [60] and have not been optimized with the current level of treatment despite great improvements in technique.

Regenerative therapies have the potential to improve current surgical interventions in the area of ligament reconstruction and/or repair in the following area(s): improved graft incorporation and strengthening, gene therapy, trophic induction, tissue engineering, and microenvironment facilitation with cells or bioactive factors to optimize, delay, or obviate premature progression of OA [61]. In this section, ACL repair and reconstruction and techniques to augment surgical repair without engineered materials will be discussed.

Preclinical Studies

Although past efforts to repair torn ACLs failed, interest has resurfaced in understanding the mechanisms of this failure, healing in the synovial environment, and unique biomechanical requirements for repairs to succeed. Experiments comparing application of different growth factors in animals with partial ACL tears and ACL explant models demonstrated that TGF-β1 might promote initial healing and overall healing both histologically and biomechanically [62,63]. In 18 sheep undergoing ACL reconstruction, a VEGF-soaked graft was compared with a control group. Although the VEGF group demonstrated improved vascularization, increased graft laxity and a weakened graft were found at 12 weeks [64].

Partial ACL tears were treated in 2 separate rat models by injecting MSCs (cultured and/or BMAC) into the joints after surgical lesions were created. Both studies demonstrated nearly normal strength and ligament healing compared with control subjects [65,66].

For ACL reconstruction, comparable healing of autograft and allograft ACLs was seen along with improved strength with concomitant use of MSCs in a rabbit model in 2 separate studies [67,68]. Lui et al [69] applied tendon-derived stem cell sheets formed without a scaffold in 97 rats undergoing ACL reconstruction; this application resulted in higher tunnel bone mineral density and bone volume, better graft osteointegration, and higher intra-articular graft integrity with lower cellularity, vascularity, and cell alignment compared with the control group, but biomechanically no difference was found between groups [69]. Application of cultured autologous synovium-derived cells with and without fibrin-sealed TGF-β was performed at the time of hamstring ACL reconstruction in 50 sheep divided equally into 5 groups. This application resulted in no necrotic deterioration in the mid-substance in the 3 intervention groups as was seen in the 2 control groups, and biomechanically superior maximum load and stiffness was found in the 2 groups treated with synovium-derived cells and fibrin-sealed TGF-β [70].

Clinical Studies

Seijas et al [71] completed a retrospective review of the rate of return to play in 19 professional soccer players with partial ACL tears. All players were treated with arthroscopically controlled 4-mL and 6-mL injections of calcium chloride—activated PRP in the intact posterolateral bundle and intra-articularly, respectively. This treatment resulted in KT-1000 normalization in all cases. Eighteen of 19 players were able to return to their previous level of play at mean of 16.20 weeks [71].

Radice et al [72], in a prospective study of 100 ACL reconstructions, divided subjects into 2 groups: group A was treated with PRP gel (PRPG), and group B was a control group. MRI demonstrated that it took 179 days to complete graft homogeneity in group A compared with

369 days in group B without PRPG treatment [72]. In a double-blind RCT in 100 patients undergoing allograft patella ACL reconstruction, the intervention group was given activated PRP; at 24-month follow-up, no difference was found in terms of subjective outcome, biomechanical integration, or graft integration [73]. In a prospective study of hamstring ACL reconstruction that used grafts soaked with calcium chloride—activated PRP in 36 subjects in the intervention group versus no PRP treatment in 27 control subjects, when an evaluation was performed by second-look arthroscopy and histology, no difference in appearance was found. However, histologically, newly formed connective tissue enveloping the graft was found in 77.3% of intervention group versus 40% of control subjects [74]. In an RCT of 50 patients in 2 equal groups undergoing hamstring ACL reconstruction, the intervention group received thrombin-activated PRP-soaked grafts and demonstrated improved anterior-posterior instrumented knee stability via a KT 2000 arthrometer at 6 months [75]. Silva and Sampaio [76] prospectively evaluated graft-tunnel healing in an anatomic ACL reconstruction study in 40 patients sequentially divided into 4 groups: group A, without PRP; group B, with PRP in the femoral tunnels; group C, with PRP in the femoral tunnels and intra-articularly; and group D, with thrombin-activated PRP in the femoral tunnels. MRI completed at 3 months showed no difference between groups in terms of bone-tunnel healing [76]. A randomized single-blind prospective study compared the use of autologous platelet concentrate in 30 patients (group A) versus a control group of 20 patients (group B) undergoing ACL reconstruction with hamstring grafts. At 6 months, MRI evaluation was performed and no difference was found in graft integration, bone tunnel healing, or maturation [77].

A single study has investigated the use of BMSCs in ACL reconstruction in humans. A prospective randomized study was performed with 43 patients divided into a control group of 23 patients and an intervention group of 20 patients who received BMAC. All 43 patients underwent ACL reconstruction with hamstring grafts, and in the intervention group, BMAC was injected within and around the femoral side of the graft and inside the femoral tunnels with the graft in place [78]. Unfortunately, no characterization of the aspirate, viability, or numeration was completed on any sample. MRI did not detect any differences between groups, but no postoperative time frame was reported.

Meniscal Repair/Regeneration

The anatomy and vascular supply of the meniscus cartilage of the knee has been well studied and documented [79]. Biomechanical and clinical data have demonstrated the importance of the meniscus and of meniscal preservation for protection of the articular cartilage, distribution of forces, and as a secondary stabilizer [80]. Techniques for repair have evolved and include inside-out, outside-in, and all-inside techniques. Although fixation methods have improved, there has been an increasing interest in biologic augmentation of these repairs to enhance healing given the limited blood supply. Based on data demonstrating increased rates of meniscal healing when an ACL reconstruction was performed (versus a repair in isolation) [81], which potentially is thought to be due to the release of marrow elements, marrow stimulation techniques have been described [82]. Recent studies in a rabbit model have shown improvements in the quality and quantity of the reparative tissue bridging a meniscal repair when using these marrow stimulation techniques [83]. This improvement potentially is thought to be due to increased release of growth factors, including platelet-derived growth factor (PDGF) [84]. These advances have resulted in a proliferation of research in the biologic realm of the augmentation of meniscal repair.

Preclinical Studies

Multiple animal studies have examined the use of biologic augmentation on meniscal repair. Initial data from Arnoczky and Warren in dogs demonstrated the ability to heal tears in the central portion of the meniscus by creating vascular channels [85]. In addition to increasing the vascular supply, growth factors (including PDGF) have shown promise in both in vitro and in vivo studies [86,87]. These data led to the theory that PRP may be of utility in the augmentation of meniscal healing. The scientific basis for this theory is largely based on an article pertaining to a rabbit model, which used a gelatin hydrogel scaffold that resulted in a time-release elution of PRP over 4 weeks [88]. This technique resulted in improved histologic scores at 12 weeks, leading to the hypothesis that PRP may be useful in healing meniscal defects. A second study in a rabbit model used a hyaluronan-collagen scaffold to deliver PRP [89]. The results demonstrated no improvement compared with the control group, which was noted both in trials of PRP and BMA.

One study did investigate the use of BMA in a sheep model for defects in the red-white zone of the meniscus [90]. A significantly increased cell count, formation of plaques, and neovascularization were found in the BMA group without any difference seen in collagen fibril formation. Autologous, marrow-derived stem cells have been studied and were found to enhance repair in a rabbit model [91]. Large meniscal defects were created and filled with a hyaluronan-gelatin scaffold in one knee; the other knee was used as a control. In the other subgroup the defect was treated with autologous marrow-derived MSCs loaded on a scaffold, with the contralateral knee receiving a scaffold only. The width of the regenerated tissue was significantly higher in the knees treated with MSC, and integration with

meniscus-like fibrocartilage was found in 73% of the 11 specimens in the MSC group versus 18% of the control subjects that received a scaffold alone. Another study using a 2-mm punch defect in a rabbit model examined the use of MSCs that were precultured versus uncultured cells [92]. The rabbits that received precultured MSCs were found to have fibrocartilage-like repair tissue that was partially integrated. The uncultured MSCs stimulated the development of completely integrated meniscus-like reparative tissue at 3 months, suggesting that minimally manipulated cells lines may perform better for this application. Allogenic MSCs have also been tested in a massive meniscectomy model, created by excising the anterior half of the medial meniscus [92]. Two weeks later MSCs were injected in PBS and compared with control subjects. The size of the meniscus was larger at 4 and 12 weeks in the MSC group, but no difference was seen at 16 and 24 months. However, histological scores were better in the MSC group than in the control group at 1, 3, 4, and 6 months. A punch defect model (1.5 mm in the avascular zone) has also been tested with allogenic MSCs [93]. This study also used a PBS and demonstrated that the quality of the repair tissue was improved at all end points with MSCs (reaching significance at 12 and 24 weeks), as was the quantity (reaching significance at 4 and 12 weeks).

Clinical Studies

Currently, no controlled human studies have been performed to evaluate the use of PRP, BMA, or MSCs in the treatment of meniscal tears, and therefore the human clinical evidence remains largely anecdotal. A single double-blind RCT has been completed using cultured allogenic MSC injections after partial meniscectomy; it showed that 24% of patients in the intervention group had increased meniscal volume after the procedure [94]. Work in the area of meniscal repair/regeneration is in its infancy clinically, but the results of many current trials are forthcoming.

Cartilage Repair

Preclinical Studies

Cartilage repair has long been an area of difficulty within orthopedics. Animal studies investigating stem cell therapy to improve methods of cartilage repair have illustrated benefit and guided clinical development [95-101]. A report of the initial investigation of stem cells and cartilage repair was published in 1994 [101]. Investigators created a full-thickness defect in a rabbit model, into which MSCs embedded in a collagen I gel were placed. Serial histologic evaluation revealed that the cells differentiated into chondrocytes in a uniform fashion as soon as 2 weeks, and at 24 weeks a subchondral bone layer was re-established [101]. Subsequent investigators have sought to further optimize the implantation of cells for cartilage repair [95,101]. These studies have suggested that allograft cells may be as effective as autograft cells [100], differentiating a cell to the chondrocyte lineage may not be advantageous, and for cartilage integration and differentiation purposes, a more immature cell is more effective [95].

Implantation at the time of surgery with a scaffold is not the only method for cell application. Three studies have illustrated improvement with application of stem cells at a time point after marrow stimulation [97-99]. Lee et al [97] investigated 3 weekly injections of MSCs suspended in 2 mL of hyaluronan after creation of a cartilage defect in mini-pigs [97]. The cell-treated group demonstrated improvement in histologic and morphologic scores. Additionally, carboxyfluorescein-labeled MSCs were found at the base of the repair cartilage, suggesting an innate, functional homing mechanism of the cells [97]. A similar study evaluated the effectiveness of one injection of bone marrow—derived MSCs 1 month after microfracture [98]. This study illustrated a trend toward overall improvement, with significance achieved in repair tissue firmness and aggrecan content [98]. These 3 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.

Adipose-derived MSCs also have potential application in cartilage regeneration and have been shown to have superior proliferative potential compared with other types of MSCs [102]. Within a rabbit model, adipose-derived MSC applied in a fibrin glue scaffold illustrated excellent rates of subchondral bone healing [96]. However, direct comparison of the chondrogenic potential of adipose-derived and bone marrow—derived cells has illustrated a greater efficiency and quality of chondrogenesis with bone marrow—derived cells [102,103].

Because of regulatory restraints with isolated stem cell populations, clinicians have also sought to augment current cartilage repair techniques with bone marrow aspirate. Authors of 2 animal studies have investigated the addition of bone marrow aspirate to a common, simple cartilage repair technique and found effective results [98,99]. The first study involved augmentation of subchondral drilling with postoperative injections of BMA. Histologic scoring was best in the group that received 3 postoperative injections of BMA and sodium hyaluronate at week intervals compared with the group with no postoperative injections and a group with sodium hyaluronate injections alone [99]. A similar study compared microfracture and BMA placed at the site of microfracture in a horse model. The BMA group scored higher in MRI, gross morphologic, and histologic scores [98].

Clinical Studies

The clinical application of stem cells to cartilage regeneration has 4 studies for evaluation, including 2 prospective observational cohort studies, a case series

with histology, and a randomized controlled trial [29,31,104,105]. The first observational cohort study compared autologous chondrocyte implantation (ACI) in 36 patients with bone marrow—derived MSC implantation in 36 patients [104]. No clinical difference was found between the 2 groups at the 24-month time point. A follow-up study investigated MSC implantation in an open surgical fashion to postoperative injection of MSCs after an arthroscopic surgery. The arthroscopic cohort scored higher in subjective outcome scores [105]. PBSCs have also been investigated clinically. An initial case series utilizing PBSCs to augment arthroscopic subchondral drilling illustrated morphologic and staining properties on histology that approached natural cartilage [29]. This method has subsequently been investigated in an RCT comparing clinical outcomes with International Knee Documentation Committee scores at 18 months, morphology of repair with MRI, and repair tissue quality with histologic biopsy [31]. The intervention group underwent postoperative injections of PBSCs and hyaluronan, whereas 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 did not illustrate a statistical difference [31].

Conclusion

It is clear from the known basic science that many of the therapeutic benefits seen in the augmentation of current surgical procedures are mediated by the activation or addition of MSCs or pericytes. The use of bone marrow—derived MSCs are most commonly reported in the literature and in some investigations are demonstrating promising early clinical results in the augmentation of current surgical procedures. Use of stem cells derived from adipose tissue, peripheral blood, and umbilical blood are also being reported clinically.

Additional study of the use of PRP to augment rotator cuff surgery is required at the basic science and clinical levels. Additionally, standardization of preparations and consensus among practitioners regarding the correct constitution of PRP for specific uses requires elucidation. A long-term clinical report has demonstrated the effectiveness of BMAC augmentation to decrease failure rate compared with control subjects. The administration of BMAC should be considered as initial treatment after known rotator cuff injury and could possibly reduce the use of corticosteroids in rotator cuff syndrome/tendinopathy/impingement management in the future.

In both preclinical and clinical studies, ACL injury/reconstruction with biologic augmentation has shown positive results in limited investigations in the treatment of partial ACL tear alone. Both PRP and BMA have sufficient evidence to support their use in further clinical investigations for treatment of partial ACL tears. To date, biologic augmentation of ACL reconstruction has not shown any positive or harmful effect, and further basic science investigation is warranted.

Based on existing data using PRP, BMAC, and MSCs, early evidence is available for biologic augmentation of meniscal repair for use in clinical investigation. Fibrin clots have been used successfully for many years for meniscal repair, and use of PRP is an improvement of the technique. Additional research is necessary to fully validate these treatments for meniscal disorders.

The use of biological augmentation of cartilage repair techniques has good and evolving evidence regarding the use of peripheral blood, BMAC, MSCs, and allogenic cord blood to improve currently performed marrow stimulating techniques with documented safety and efficacy.

The use of biologics in the augmentation of current surgical procedures has a promising future. Many investigators are hopeful that biologics or biologically induced regeneration will some day supplant many of the surgical reconstructive procedures currently being performed today.

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Disclosure

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