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BIOLOGICS

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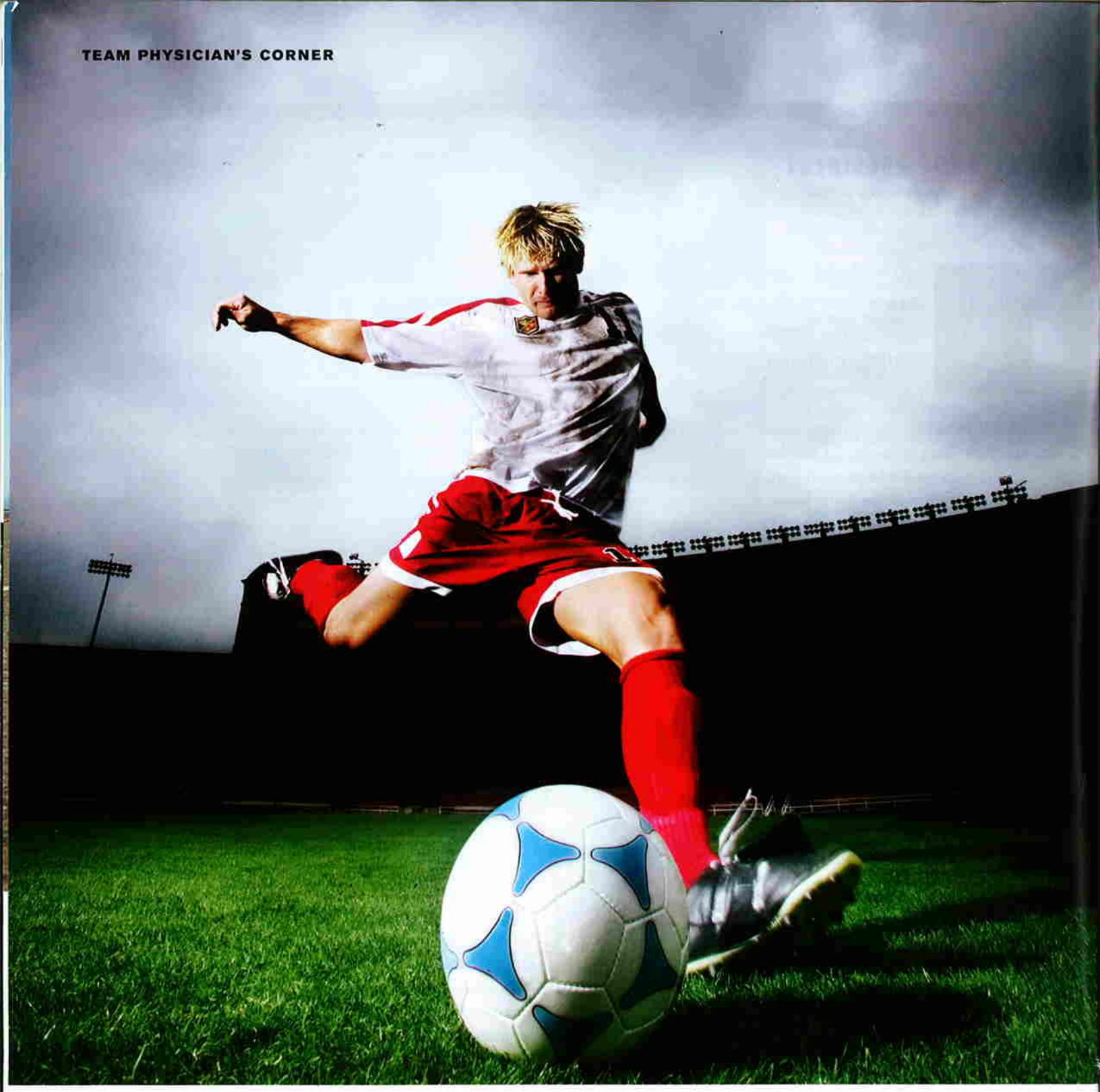

BIOLOGICS FOR SPORTS MEDICINE AND ORTHOPAEDIC HEALING

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The promise of biologics for sports medicine and orthopaedic healing is exciting to not only the physician, but has brought much interest to the public and media as well. Biological augmentation is the science of using autologous stem cells and growth factors to enhance our own body's ability to heal. Tissue engineering is the addition of cells and growth factors to a human scaffold to improve tissue healing and regeneration. The sports medicine physician needs to understand the science and principles of these biological processes to be able to offer the best treatment options for patients. Numerous products are entering the market and there is little guidance as to the indications and cost effectiveness of these treatments.

PRP

PRP has been utilized since 1950.[1,2] PRP is autologous blood with concentration of platelets above baseline value.[3] There are several products on the market. PRP is produced by a centrifugation process of an initial soft spin of 1,200 to 1,500 rpms where the plasma and platelets are separated from the blood cells and white cells. A second hard spin is done at 4,000 to 7,000 rpms. This further separates the platelet rich and platelet poor plasma components. The second phase concentration is controversial since some commercial formulations do not use this portion of the process.

The basic science of PRP is that the platelets release numerous growth factors and bioactive proteins on activation. PRP attracts mesenchymal stem cells, macrophages, and fibroblasts. PRP stimulates cell proliferation and extracellular matrix protein production.

PRP has more than 1,100 different proteins, including PDGF platelet derived growth factor; TGF-Beta transforming growth factor; IGF insulin-like growth factor; FGF fibroblast growth factor; and VEGF, vascular endothelial growth factor. There are studies that show positive results from proteins such as insulin-like growth factor 1, vascular endothelial growth factor, and basic fibroblast growth factor.[4] Other studies have shown deleterious effects with proteins such as transforming growth factor (TGF) having negative effects.[5]

PRP can be classified as leukocyte poor PRP (LP-PRP) versus leukocyte rich PRP (LR-PRP). Leukocytes are white blood cells and they play a key role in initial phases of inflammation but they also increase muscle damage and may impede healing through a release of various enzymes. White blood cells in PRP joint injections may hamper results. Although we don't know the exact reason, it could be due to inflammation. Red blood cells are not tolerated in a joint and are known to cause cartilage damage such as occurs in hemophilia and trauma. This leads to the question of the clinical response of micro fracture producing red blood cells even though the purpose is to promote stem cells into the area for some cartilage growth. Braun et al. showed that cultured synoviocytes leukocyte-rich PRP causes cell death.[6]

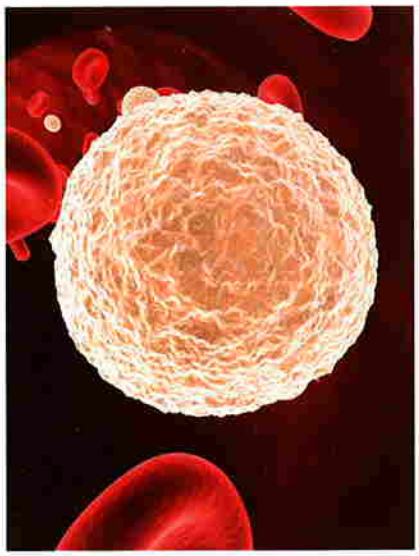
The joint is a complex and constantly changing environment. All of the structures in the joint must be considered when performing intra-articular injections. With the available data present, it is apparent that WBCs with PRP are not advantageous, probably due to an inflammatory response. RBCs are not tolerated well, as known in trauma and hemophiliac arthropathy and therefore should not be used with intra-articular PRP injections. The effect of PRP on the synovium and synoviocytes is still not well studied.

There are more than 40 commercial systems available, but many factors contribute to the content of PRP. The final platelet and growth factor concentrations depend on the amount of whole blood used, the efficacy of platelet recovery, and the final volume of plasma in which the platelets are suspended. Castillo has shown more than a 50 percent variation in platelet concentration even with the same technique.[7] Mazzocca showed that

PRP has been used in chronic tendonopathy, especially elbow epicondylitis.

there was a difference in PRP according to the preparation method and human variability.[8] He showed that the platelet concentrations for all of the PRP was greater than whole blood, but there was no superiority over single versus double spin, and even in the same system there was a high variability and a high variability with intra-individual measurements.

Having higher concentrations of platelets within PRP does not necessarily lead to a more pronounced positive effect. Giusti suggested that the most effective platelet concentration for tissue healing was 1.5 x 106 per microliter.[9] Though the response curve was not linear, there



was even a saturation effect in which an inhibitory effect was noted once a high concentration of platelets reached. He also noted that platelets exhibited the greatest influence on healing immediately after the inflammatory phase of the injuries, and this may mean that the timing of administration of PRP may be important.

PRP has been used clinically for tendinopathy, soft tissue injuries, arthritis, surgical repair enhancement, and bone healing. An excellent review article by Hsu can be found in the *Journal of American Academy of Orthopaedic Surgeons.*[10]

PRP and Bone Healing

The effect of PRP on bone healing has been studied. There are osteogenic properties of PRP in vitro[11,12] but there is limited clinical evidence demonstrating any beneficial effects. It has been used in spine fusion trials but there is no evidence that PRP is helpful in these cases.[13,14] Current evidence indicates that PRP is not effective either alone or as an adjunct to a local bone graft.

PRP and Tendonopathy

PRP has been used in chronic tendonopathy, especially elbow epicondylitis. A study by Peerbooms compared injection of PRP with corticosteroids for a lateral epicondylitis.[15] Comparison of the outcomes at one and two-year follow-ups show that the clinical improvements in corticosteroid groups tend to decline, whereas the improvements in PRP groups were maintained. Studies suggest that PRP formulations containing WBCs improved patient's outcomes, compared to either a local injection of anesthetic or corticosteroid usage. This points to the fact that WBCs may be advantageous in PRP use in tendonopathy.

Studies on Achilles tendinitis and patellar tendinitis or jumper's knees have not been as successful with no difference in clinical outcomes on several studies.[16,17,18]

PRP and ACL Reconstruction

ACL reconstruction studies have shown possible increased faster graft maturation of the ACL when studied with MRI.[19] However, no difference in clinical outcomes has been reported. It should be noted that the clinical outcomes' variability can be attributed to many factors, including PRP preparation, graft choice, rehabilitation, and application techniques.

PRP and Rotator Cuff and Achilles Tendon Repairs

Rotator cuff repair use of PRP has mixed data and results. Although there are some studies showing possible benefit,[20] there is no convincing data that shows better clinical outcomes or decreased re-tear rates.[21]

Using PRP as part of the treatment of Achilles tendon ruptures has had variable results. One study shows Achilles tendon repair with PRP having a faster recovery of range of motion and time to running.[22] However, another study showed no difference between the PRP group and the control and, in fact, the Achilles tendon rupture score was lower in the PRP group. The author suggested that PRP may be detrimental when used intra-operatively.[23]

PRP and Knee Osteoarthritis

Knee osteoarthritis has been studied and compared to other treatment modalities such as visco-supplementation. Sanchez, in 2012 in the *Journal of Arthroscopic and Related Surgery,* showed superior results of PRP in mild to moderate osteoarthritis.[24] Patel et al., in 2013 in the *American Journal of Sports Medicine,* found PRP was superior to placebo but the results declined after six months and there was no advantage of two PRP injections over one.[25]

Cerza showed better clinical outcomes in PRP group.[26] Compared to hyaluronic acid injections, PRP was effective for grade III osteoarthritis and hyaluronic acid supplementation was not. Filarado, in the *BMC Musculoskeletal Disorders* in 2012, compared three weekly PRP injections versus hyaluronic acid injections and found no significant difference.[27]

The AAOS Clinical Practice Guidelines are unable to recommend for or against growth factor-PRP injections for patients with symptomatic osteoarthritis of the knee. A recent article by Riboh in the *American Journal of Sports Medicine* in 2015 showed that leukocyte poor PRP resulted in improved functional outcome scores when compared with hyaluronic acid and placebo for osteoarthritis of the knee.[34]

The increase in investigational studies regarding PRP has fueled the demand for increased clinical use. The market for PRP has gone from $45 million in 2009 to an expected $126 million by 2016.[28] A cost-benefit analysis has not been proven. Since PRP is listed as experimental by most insurance companies, it is not covered and reimbursed by most insurance plans. The questions that need to be answered in the future concern scenarios where the immediate cost of PRP might be greater, but if there is decrease in further treatments such as surgery and reinjection, then there indeed may be a cost savings.

In summary, PRP injections have been shown to be detrimental or not helpful in bone healing. There are some possible benefits from knee osteoarthritis, tennis elbow, and ACL reconstruction. There have been indeterminate results with Achilles tendinitis, rotator cuff repair, and Achilles tendon repairs.

Stem Cells

Stem cell usage has also exploded in sports medicine and orthopaedics as a form of treatment for injuries and recovery. Stem cells are undifferentiated cells that can mature and differentiate into several cell lines. Stem cells can reproduce, differentiate, and activate other cells in the environment for biologic activity. There are hematopoietic stem cells that give rise to other blood cells. These are found in bone marrow and to a lesser degree in peripheral blood. Mesenchymal stem cells (MSC) can differentiate into bone, cartilage, and fat. Mesenchymal stem cells can be obtained from bone marrow, adipose tissue, synovial tissue, and periosteum.

Mesenchymal stem cells are found in bone marrow and are readily obtained. Mesenchymal stem cells make up 0.01 percent of cells in bone marrow. This bone marrow aspiration is usually centrifuged to concentrate the cell numbers. Even with centrifuge, it still has fairly low numbers available. Stem cells can be cultured in vitro but this limits their clinical application and has a significant cost associated.

Mesenchymal stem cells can be obtained in adipose tissue. An aspiration of adipose tissue produces lipo-aspirate cells of which approximately two percent are stem cells. This is more than 500 times the level in a bone marrow aspirate per gram.[29] Adipose tissue mesenchymal cells are a more recent method of obtaining mesenchymal stem cells.

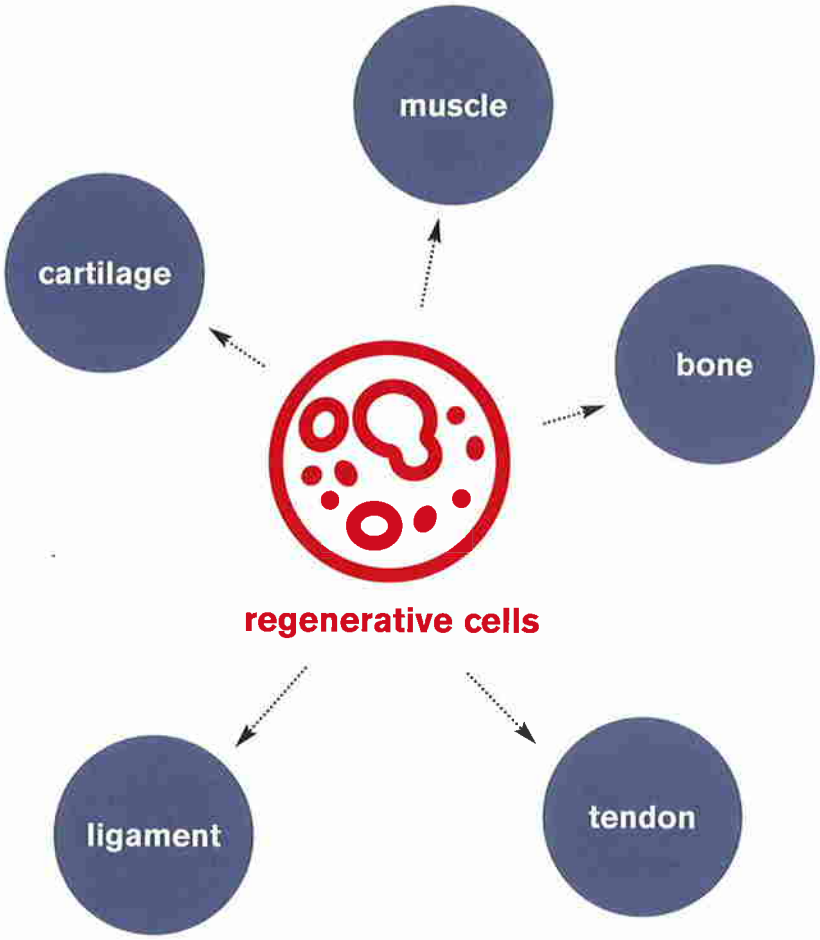
Many animal studies have showed mesenchymal cells in the animal model that enhance meniscal repair and better healing of cartilage defects with mesenchymal stem cell injections. There has also been noted improvement in microfracture results as well, with better histology, with microfracture plus bone marrow aspirate injections.

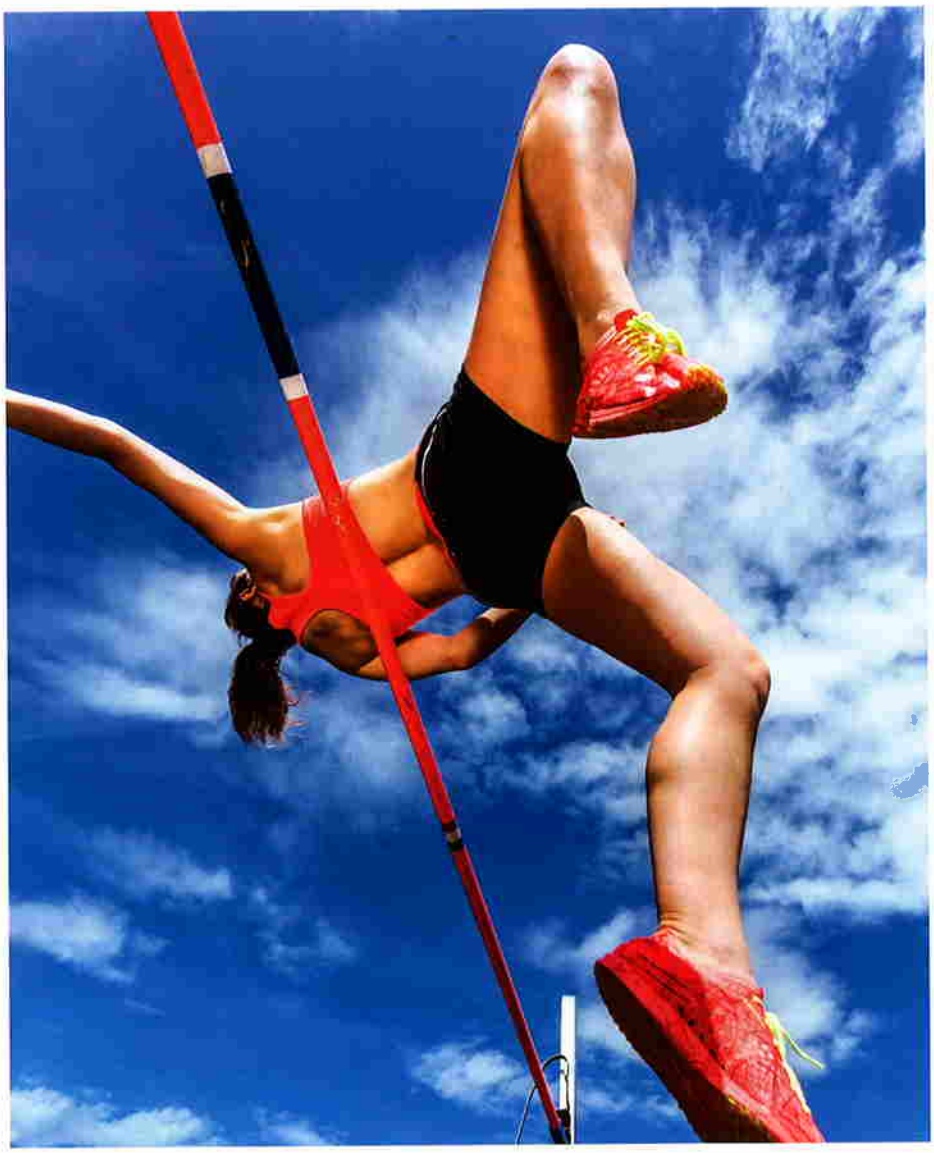
Adipose drawn stem cells for osteoarthritis in animal model has been studied. An article by Ter Huurne showed that adipose derived stem cells inhibited synovial thickening and cartilage destruction in mice with early stage arthritis.[30]

Mesenchymal stem cells in human studies have shown some promising, yet mixed results. An article by Nejadjnik in the *American Journal of Sports Medicine* showed no difference in clinical outcomes between an ACI group, versus bone marrow mesenchymal stem cells for cartilage defects when periosteal patch was used.[31] Saw et al. in *Arthroscopy* 2011 did a study with the stem cells and microfracture and showed that the histology in this human study of repaired cartilage improved when this was coupled with mesenchymal stem cell injections and the microfracture.[32] Another study by Saw et al. in *Arthroscopy* 2013 compared microfracture with hyaluronic acid with microfracture with hyaluronic acid and peripheral blood stem cells having a better repair with the stem cells, but had similar clinical outcome scores.[33]

Mesenchymal stem cells in the United States are available with autologous mesenchymal stem cells from whole blood, bone marrow aspirate (BMA), and bone marrow aspirate centrifuge (BMAC) as well as adipose derived mesenchymal stem cells. Allogeneic are available with allograft bone matrix as well as placental derived tissue.

Autologous mesenchymal stem cells from whole blood have very low numbers of mesenchymal stem cells. Bone marrow has .001 percent of mononuclear cells. Because of this low number this bone marrow aspirate is usually centrifuged to concentrate





the numbers of stem cells. Usually the bone marrow aspirate is centrifuged and applied to the site within 15 minutes.

The stem cells can be increased via culture process. This takes several weeks and significantly increases the cost. There are concerns of this culture growth causing immunogenicity problems as well as the possibility of genetic instability which leads to the possibility of tumors developing. With the product being taken out of the initial patient sterile environment, transported, and then reapplied, increased infection risks are always a concern. Currently there are no approved therapies for these cultured stem cells outside of ACI usage. There are also ways to increase mesenchymal stem cells with cell surface markers that are either fluorescently activated cell sorting or magnetically activated cell sorting, but this can be costly and also raises the question of antibody exposure.

Adipose derived mesenchymal stem cells are produced from a lipoaspirate that is agitated and microfractured in a closed system. It is concentrated for injection and it is approved for homologous use.

Placental derived allograft products also are available. Currently it is mainly used in wound healing applications. There are some animal studies, but there is a concern that with the non-homologous use of the product. This seems to be a gray area for the FDA and may limit its use.

The Future and Beyond

The future of biologics in the treatment of sports medicine and musculoskeletal disease is exciting. Sports medicine physicians are in the early stage of understanding its clinical uses and application. As of right now, orthopaedic and sports medicine applications for PRP show some good data available, but with so many products available, with varying concentration levels and variability in the application, this makes clinical interpretation difficult. With the heterogeneity in tendons and tendonopathies defining protocols is important. There is some good data, but the results are not completely clear. Protocols need to be standardized. Identifying what works in the human environment is also needed. Future research must define the correct platelet leukocyte count and the balance ratio between the two, as well as what plasma proteins are helpful in a specific clinical setting. The future may have second generation PRP that will neutralize the negative or unwanted growth factors and enhance the positive growth factors that would be beneficial for a prescribed clinical setting. Maybe there will be a combination of PRP or other blood products added to a stem cell or some scaffold that will produce a more improved and predictable clinical result.

Future methods to improve clinical efficacy include improving the formula for the specific indication. As we learn more, we can try to exclude unwanted growth factors and possibly concentrate the positive growth factors, and maybe use blood sources other than platelets.

Mesenchymal stem cells have promise, and some recent data show immense promise. This indeed is an exciting frontier of possible future treatments to the athlete for the sports medicine physician.

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