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SPECIAL FOCUS

Use of Platelet-Rich Plasma and Bone Marrow–Derived Mesenchymal Stem Cells in Foot and Ankle Surgery

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ABSTRACT

Bone grafting is an important tool for filling osseous defects in foot and ankle procedures. Many alternatives exist for use by the surgeon, each with their own risks and benefits. Two adjunctive materials available for use to enhance the effects of these grafts are platelet-rich plasma and mesenchymal stem cells. We have developed an algorithm for use of each based on a cell proliferation triangle. Both materials have shown positive results in basic science and clinical studies and show promise as safe and cost-effective alternatives to augment bone healing.

Keywords: platelet concentrate, platelet-rich plasma, autologous mesenchymal stem cells

HISTORICAL PERSPECTIVE

Bone grafting is a common necessity in many foot and ankle procedures. Choices for the surgeon include autograft, allograft, or bone graft substitutes. No matter which is chosen, it is important to remember that cells are the only tissue capable of forming bone.[1] Autologous cancellous graft remains the criterion standard for bone regeneration because it possesses the 3 qualities necessary for bone healing: a scaffold providing osteoconductivity, signal proteins for osteoinductivity, and stem cells to become osteogenic. These are the components of the cell proliferation triangle (Fig. 1). For bone to be produced, all 3 parts of the triangle must be present. However, detriments in using autograft include the small amounts available and donor site morbidity.[2] No other single material used can provide the surgeon with all 3 necessary elements. Thus, when autograft is not a viable option, the surgeon must resort to combinations of various products including the purely osteoconductive ceramics, purely osteoinductive bone morphogenic proteins, and demineralized bone matrix (DBM) with some osteoconductivity and some osteoinductivity.[3] Platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs) may be effective in promoting bone growth in situations when these products are being considered.

Platelet-rich plasma, obtained from autologous blood, can be used to deliver high concentrations of osteoinductive growth factors to bony and soft tissue sites, initiating and/or augmenting the healing response of the body.[4] Platelet-derived growth factor, insulinlike growth factors, transforming growth factor β, and fibroblast growth factor have been shown to be present in platelets in vitro.[5] These same molecules are released by platelet degranulation during the initial inflammatory phase of tissue healing.[5] Cellular signals for chemotaxis, differentiation, and osteoblastic proliferation are controlled by these factors.[6,7]

Osteogenic precursors can be harvested from bone marrow in the form of MSCs using minimally invasive technique. Mesenchymal stem cells have been shown to be multipotential, meaning they can differentiate into various tissues, including bone.[8] Once the bone marrow is retrieved, the MSCs can be concentrated to enhance their effect, using several commercially available systems. The hematopoietic stem cell portion of the bone marrow, which has no role in bone formation, can be discarded.

Using PRP and MSCs with the addition of an osteoconductive carrier, such as porous hydroxyapatite or DBM, satisfies all 3 elements required for bone

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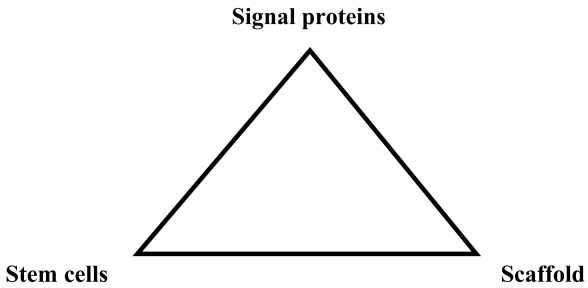

FIGURE 1. The cell proliferation triangle.

formation. Thus, surgeon's concerns over autograft donor-site complications and quantities of graft available may be lessened.

INDICATIONS/ CONTRAINDICATIONS/ PREOPERATIVE PLANNING

There are many clinical scenarios in which PRP and MSCs may be used. We consider the use of these 2 agents whenever concern for the "biology" of the bone is present, meaning the capacity for bone healing may be compromised because of a deficiency in the triangle. Nonunions, bone defects, difficult primary fractures with known poor healing rates, difficult primary arthrodeses with history of poor healing, large bone cysts, osteonecrosis, previous radiation therapy, sites of previous infection, and other disasters where amputation is the next option are good examples where sufficient osteoprogenitor cells and signal proteins may be absent.[9]

When deciding which orthobiologic will work best for a particular clinical scenario, we have found it helpful to use an algorithm based on the cell proliferation triangle (Fig. 2). We try to determine which component of the triangle is missing and use an agent that will satisfy that need. For small bone defects with good biology, DBM, autograft, or a ceramic may be used. If the biology is poor and allograft or ceramic is to be used, we supplement with PRP. In the case of medium-sized voids with good biology and in which autograft is not an option, we use PRP-augmented allograft or ceramic. These are supplemented with MSCs for poor biology. We treat very large defects the same as medium ones, if the biology is good. When there is a large defect with poor biology, we supplement our allograft and DBM with both PRP and MSCs.

Remember, PRP and MSCs are not substitutes for rigid internal fixation or good technique. Neither are they meant to be a stand-alone graft material. An

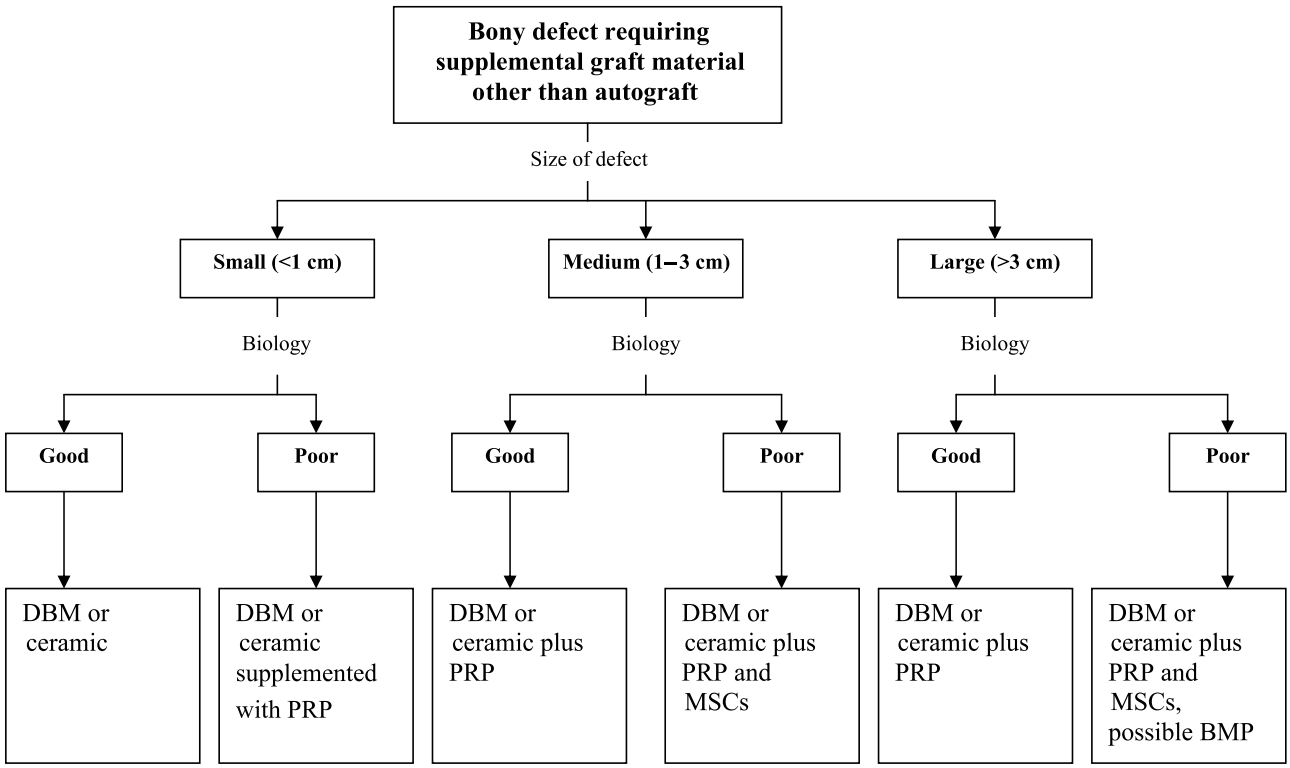

FIGURE 2. Our algorithm for use of PRP and MSCs when autograft solely is not an option. BMP indicates bone morphogenic protein.

osteoconductive scaffold is necessary for PRP or MSCs to have a beneficial effect.[10–12] As an example, DBM makes an attractive option because its osteogenic potential lies primarily in its osteoconductive properties, although it does have some osteoinductive properties.[13]

Reported contraindications for using PRP include platelet dysfunction syndrome, critical thrombocytopenia, hypofibrinogenemia, hemodynamic instability, sensitivity to bovine thrombin,[14] septicemia, and use of aspirin or other medications altering platelet function a few days before surgery. To our knowledge, there have been no contraindications in the orthopaedic literature to using MSCs from the iliac crest.

It is also important to make sure trained staff who are familiar with the processing and handling of these agents and all necessary equipment will be available in the operating room on the day of surgery. Many hospitals may not keep these systems at hand, and company representatives should be notified to assist if needed.

TECHNIQUE

A number of commercially available systems for the preparation of PRP and retrieval of stem cells are currently in use, with some variation of technique. The Symphony Platelet Concentrate System and the Cellect Selective Retention stem cell concentration system (DePuy, a Johnson & Johnson company, Warsaw, Ind) are the devices used primarily at our institution.

Aseptic technique is used throughout. Platelet-rich plasma preparation begins with drawing 6 mL of anticoagulant citrate dextrose into a 60-mL syringe. Next, 54 mL of the patient's blood is drawn into the same syringe. Blood is drawn just after induction and before administration of fluids and incision. This is vital to prevent dilution of the blood and avoid bacterial contamination, thus lowering the available platelets and risking infection of the operative site, respectively. The mixture is then inverted several times to promote mixing of the blood and anticoagulant. The solution is injected in to the blood chamber side of a disposable processing device and placed in a centrifuge. A counterbalance weight is inserted opposite the device, and the lid is closed. The centrifuge is run for a cycle of approximately 14 minutes. Plasma separates from the cellular component of blood, ending up in a separate plasma chamber. Using a syringe with a spacer attached to avoid the platelet pellet at the bottom, a volume (usually 10 mL) of platelet-poor plasma (PPP) is removed from the plasma chamber. This is preserved to resuspend the platelets later. The remaining PPP is then removed and discarded, leaving a pellet of platelets in the chamber. This can now be resuspended with the saved 10-mL of PPP, creating PRP. The PRP is now withdrawn in a 10-mL syringe and placed on to one side of an applicator assembly. A 1-mL syringe is filled with a thrombin mixture (made from 5,000 U of thrombin powder and 5 mL of 10% calcium chloride) and is attached to the other side. When the applicator is depressed, a 10:1 ratio of fluid is expressed on to the bony surfaces, allograft, or wound, and a platelet gel is created. This gel is a fibrin clot with a highly concentrated amount of platelets and their growth factors.[15] With this system, a 5-fold concentration of platelets can be obtained, resulting in a 225% increase in platelet-derived growth factor and other growth factors at the site of application (data on file at DePuy).

Mesenchymal stem cells are harvested using an anterior approach to the iliac crest through a small incision. The provided trocar is used to puncture the cortical bone. Once seated in cancellous bone, 2 to 4 mL of fluid are withdrawn via syringe connected to the trocar. Once this amount is collected, it is important to advance the needle at least 1 cm or reenter the bone by piercing another portion of the cortex. If the volume of fluid extracted exceeds this amount from any one site, the concentration of osteoblast progenitor cells decreases because of dilution with peripheral blood.[9] When sufficient marrow fluid has been harvested, it is passed through a concentration chamber (Cellect; DePuy). A matrix of tricalcium phosphate or DBM with specific surface properties to attract osteoprogenitor stem cells then binds the MSCs, allowing hematopoietic cells from the marrow to pass through.[16] Stem cells are then concentrated and capable of being used for their desired purpose, already mixed with a carrier.[17] This particular system is capable of capturing an average of 80% of the MSCs present (data on file at DePuy).

COMPLICATIONS

One of the main benefits of using these agents is the low morbidity associated with their techniques. They are autologous and readily available to the surgeon. Those complications associated with obtaining PRP are the same as with any percutaneous venipuncture. When done appropriately, harvesting of iliac crest stem cells is very safe, with minimal postoperative discomfort and complications.[18] This provides a more attractive option than harvesting iliac crest bone, which has been associated with 27% chance of chronic donor site pain at 24 months as well other complications.[19]

POSTOPERATIVE MANAGEMENT

Because these materials are used in a variety of locations and scenarios, the postoperative management of each patient will have to be individualized. The use of these 2 graft materials should not influence

the protocols already in place for a particular procedure. Clinical and radiographic assessments should be performed as usual. The site for the venipuncture and/or iliac crest incision should be examined at the first postoperative appointment. There are no other specific monitoring requirements regarding postoperative care with respect to the patient receiving either PRP or MSCs.

RESULTS

Animal studies have demonstrated benefits with the use of PRP. It has increased the amount and density of bone formed in bony defects, improved bone growth into hydroxyapatite, and enhanced skin, muscle, and tendon healing.[20–24]

Human studies have shown positive effects in areas outside orthopaedics, including improved hemostasis and decreased infection in cardiac surgery wounds,[25,26] reconstruction of periodontal soft and hard tissue in oral and maxillofacial surgery,[27] and multiple uses in cosmetic surgery flaps.[28]

In orthopaedic surgery, Lowery et al,[29] found no pseudoarthroses in 19 patients after augmenting autograft and coralline hydroxyapatite with PRP in posterior and anterior instrumented lumbar fusions. There was no control group, however. Weiner and Walker[30] performed a retrospective review of single-level lumbar fusions using experimental (autograft and PRP) and control (autograft-only) groups. The experimental group showed a 62% fusion rate versus 91% in the controls. They determined bone morphogenic protein within the autograft might have an antagonistic effect when used with PRP. These findings go against many of the basic science and animal studies and only stress the importance of more quality clinical trials.

As for foot and ankle surgery, the senior author (G.C.P.) recently reported results in 2 studies using PRP-augmented autograft for syndesmotic fusion in total ankle arthroplasty (TAR).[31,32] In both series, PRP was sprayed on the cut surfaces of the tibia and talus and the porous coating of the prosthesis and mixed with local autograft derived from resected bone. In the first study, 20 TARs were performed, and there were no delayed unions or nonunions of the syndesmosis. This was compared with a historical control group with a delayed/nonunion rate of 62%. No subsidence or change in implant position was noted on follow-up radiographs. In the second study, 66 TARs prepared as above with PRP were compared with a control group of 114. A statistically significant improvement in the rate of syndesmotic fusion was seen at 8 and 12 weeks, as well as less delayed unions and nonunions reported at 6 months.

Bibbo et al[33] reported results on their experience using PRP alone and in combination with autograft and allograft in high-risk foot and ankle fusions. They characterized high risk as those patients who actively smoked; were diabetic, immunocompromised, or nutritionally deficient; or had a history of poor bone healing, 2 previous operations at the site, or 1 operation for a high-energy trauma. There were no statistically significant differences noted in time to fusion, whether PRP was used alone or mixed with bone graft. Their overall fusion rates were 94% in this difficult population.

Literature with direct implications on foot and ankle surgery with respect to stem cell usage is lacking. Several animal studies have shown promising results, especially in the areas of spinal fusion, nonunions, and bony defects. Wang et al[34] found that lumbar interbody fusions in rhesus monkeys showed greater biomechanical stiffness with a hybrid ceramic-SC graft than using ceramics alone. They also noted equal stiffness between the hybrid graft and autograft. Lindholm et al[35] documented significantly more rapid posterior thoracic fusions in a rabbit model with the use of DBM and DBM augmented with marrow over bone marrow alone. In another study in rats, Lindholm et al[36] concluded that using a diluted bone marrow mixed with DBM increased induction of host MSCs to form bone at extraskeletal sites. This outperformed DBM, bone marrow, or whole bone marrow mixed with DBM. They believed the dilution of the marrow actually made the osteoprogenitors within the marrow more readily available. Tiedeman et al,[37] showed a synergistic effect of percutaneously injected DBM along with unconcentrated bone marrow in a canine nonunion model. Healing of a 6-mm tibial defect using bone marrow and DBM was better than using each alone and was comparable to open autogenous grafting.

In human studies, Romih et al[38] showed a significantly larger number of osteoprogenitors available in the iliac crest versus the vertebral interbody space and advocated the use of bone marrow aspirate to increase fusion rates of interbody fusions. Hernigou et al[39] reported the results of percutaneously injected stem cells obtained through centrifugation of bone marrow aspirates into 60 atrophic nonunions of the tibia. They found significantly lower total numbers and concentration of osteoprogenitors in the graft used for the 7 nonunions that occurred. Also, positive correlations between volume of callus at 4 months and total number and concentration of cells were seen. They concluded that efficacy of stem cells from autogenous bone marrow was directly related to increased concentration, and poorer results may be expected if the aspirate is not concentrated. Connolly[40] reported excellent results using percutaneously injected concentrated bone marrow aspirates with or without DBM in the treatment of difficult grade

III tibial fractures as well as nonunions or delayed unions of scaphoid, humeral, femoral, and tibial fractures. It was emphasized again that DBM or some other carrier should be used to prevent diffusion from the operative site. Also, the importance of using rigid fixation techniques together with the graft was stressed. Using DBM and autogenous unconcentrated bone marrow injected into aneurysmal bone cysts, Docquier and Delloye[41] achieved healing in 11 of 13 patients with a minimally invasive technique.

CONCERNS AND FUTURE OF TECHNIQUE

There is a scarcity of research in the foot and ankle literature concerning these 2 alternatives for bone and soft tissue healing at this time. Prospective randomized studies comparing these with current techniques need to be pursued because these may represent a better option in certain populations of patients who require significant amounts of bone graft or who lack the biologic capability to heal bony or soft tissue defects on their own. Many more basic science studies will be needed as well to determine if the populations of cells being used are optimal, if there exists a better source of easily obtainable stem cells such as fat, muscle, or skin, and whether angiogenic or stem cell renewal factors are necessary.[42] These advances may make stem cells more easily obtainable and cause even less morbidity than bone marrow aspiration.

COST

At our institution, the price of creating PRP and harvesting MSCs is much lower than that of more expensive recombinant growth factors such as bone morphogenic proteins. There are currently no published studies that have directly compared the cost-benefit ratios of PRP and stem cells with these other products.

There is a need for options in foot and ankle surgery that provide an alternative to the high cost of recombinant products and the morbidity of autograft. We believe, based on positive results from the previously mentioned studies, many of the difficult cases faced by foot and ankle surgeons can be helped by using PRP and MSCs. Continued research is needed to better define their role.

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