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ABSTRACT
The application of autologous platelets that have been sequestered, concentrated, and mixed with thrombin to create growth factor–concentrated, autologous platelet–rich plasma for application to soft tissue wounds and for osseous healing has been a subject of great interest for much of the past 2 decades. Autologous platelet–rich plasma, which consists of both quantitative and qualitative components, has the greatest potency or ability to produce the desired effect. Manufacturers prepare autologous platelet–rich plasma with the ultimate goal of maximizing its benefits while minimizing potential risks. Unfortunately, the manufacturing processes for autologous platelet–rich plasma are highly variable, and the types of proprietary systems available on the market for soft tissue and osseous applications are numerous. The authors provide here an in-depth review of commercially available systems for delivery of autologous platelet–rich plasma that emphasizes the subtle yet important differences among systems. In addition, a detailed review of the literature regarding the use of autologous platelet–rich plasma in soft tissue and
osseous healing is provided. Although findings are not yet conclusive, autologous platelet–rich plasma has been shown to be safe, reproducible, and effective in mimicking the natural processes of soft tissue wound and osseous healing.

Keywords: autologous platelet–rich plasma; autologous platelet–poor plasma; bone graft; wound coverage; ulceration

INTRODUCTION

Autologous platelet–rich plasma, as the name implies, is a plasma concentrate harvested from the patient’s whole blood that comprises predominantly platelets (ie, thrombocytes). Platelets are produced by megakaryocytes that develop from stem cells in bone marrow and do not contain a nucleus. Platelets contain α granules, which store multiple growth factors. After tissue injury occurs, circulating platelets come in contact with exposed molecules of the damaged endothelium (eg, collagen, von Willebrand factor, fibronectin); they adhere to the damaged endothelial surfaces, releasing adenosine diphosphate (ADP), which stimulates further aggregation of platelets to the site. This process facilitates the production of thrombin from prothrombin, which initiates the conversion of fibrinogen to fibrin, resulting in polymerization into an insoluble form that links adjacent platelets together to form an irreversible platelet aggregate or “clot.” The application of thrombin onto platelets causes them to empty their α granules, which releases the stored growth factors that act as mitogens and chemoattractants to direct cellular growth and migration. Numerous growth factors are present within platelet α granules; some of the most important of these include platelet-derived growth factor (PDGF), transforming growth factor–beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF). PDGF initiates the chemotaxis of fibroblasts, macrophages, smooth muscle cells, and neutrophils, along with the stimulation of fibroblast and smooth muscle cell mitogenesis. Studies by Pierce et al suggest that PDGF-β exaggerates the inflammatory phase of wound healing, thereby leading to earlier matrix deposition caused by acceleration of the cascade of tissue repair. TGF-β is a potent inhibitor of immune reactivity that attracts macrophages, which then stimulate the secretion of additional cytokines. TGF-β also enhances fibroblast and smooth muscle cell chemotaxis, induces the deposition of bone matrix, and stimulates the synthesis of type I collagen. VEGF stimulates endothelial growth and promotes angiogenesis, while enhancing capillary permeability and leakage of tissue plasma into the tissue space. EGF is a cytokine that has been linked with angiogenesis and collagen deposition at wound sites; it has been shown to stimulate wound repair in fibroblasts and epithelial cells. IGF is a protein that exists in 2 forms: IGF-1 and IGF-2. IGF-1 has been associated with enhanced bone development. PDGF, TGF-β, VEGF, and EGF have been shown to be increased 3 to 7 times in autologous platelet–rich plasma as compared with baseline levels, but the concentration of IGF-1 has not been shown to increase.

In general, the production of autologous platelet–rich plasma involves extraction of a specific volume of the patient’s whole blood, which is then placed in an automated centrifuge to separate the layers of whole blood into 3 separate layers: (1) platelet-poor
plasma, (2) red blood cells, and (3) the “buffy coat” (ie, platelets and white blood cells). The amount of whole blood necessary to obtain autologous platelet–rich plasma is usually considered nonphysiologically significant and is directly dependent on the specific device that is used, although most systems require between 5 and 450 mL of whole blood for completion of the entire process. It is important to note that whole blood should be drawn before any intravenous fluid is administered, to avoid dilution and resultant lower platelet yield. Once the proper volume of whole blood has been obtained, it is mixed with an anticoagulant (ie, ascorbic acid or 10% calcium citrate) to prevent premature platelet rupture throughout the remaining steps. The mixture of whole blood and anticoagulant is then transferred to a disposable collection chamber or bucket and is placed in the automated centrifuge. Once the mixture has been properly spun down within the centrifuge, the platelet-rich plasma is drawn off and placed within a sterile delivery system. At the desired time of activity, the autologous platelet–rich plasma is usually mixed with 5000 IU topical bovine thrombin and 10% calcium citrate; this mixture activates platelets and releases concentrated growth factors to the target sites. (Note: Some companies have their own proprietary activation products, which can be used instead of those mentioned here.) The entire process of preparing the autologous platelet–rich plasma usually takes between 15 and 30 minutes.

Because the preparation of autologous platelet–rich plasma is dependent on the actual system employed, a review of each of the currently available systems is presented here in alphabetical order followed by a review of the literature on the role of autologous platelet–rich plasma in wound and bone healing.

AUTOLOGOUS PLATELET–RICH PLASMA SYSTEMS: INDIVIDUAL TECHNIQUES

AutoloGel System

The AutoloGel™ System (Cytomedix, Little Rock, Ark) is a small-volume blood draw method for the production of autologous platelet–rich plasma that is specifically intended for application onto chronic wounds following proper débridement. The process begins when 5 to 40 mL of a patient’s whole blood is drawn into 5-mL vacuum containers, which are then centrifuged for approximately 5 minutes. Once the centrifuge process has been completed, the autologous platelet–rich plasma is drawn from each of the vacuum containers into a 20-cc syringe. The next sequence of steps occurs through a 3-way mixing chamber, wherein each syringe is connected to an individually marked, specific port. Depending on the volume of autologous platelet–rich plasma that is produced (2–8 cc), between 0.25 and 1 mL of ascorbic acid (500 mg/mL) is added through the mixing chamber and into the 20-cc syringe that contains the autologous platelet–rich plasma. A separate mixture of 5000 IU of topical bovine thrombin (5000 IU/mL) and 5 mL of 10% calcium chloride solution (100 mg/mL) is then created within a 5-cc syringe. The specific volume of calcium chloride varies at between 0.25 and 1 mL according to the volume of autologous platelet–rich plasma that is produced (2–8 mL), but the volume of topical bovine thrombin remains constant at 1 mL. The topical bovine thrombin and calcium chloride solution mixture within the 5-cc syringe is then attached and passed through the mixing chamber into the 20-cc syringe containing the autologous platelet–rich plasma and ascorbic acid mixture. The resultant autologous platelet–rich plasma

T. S. Roukis et al
Autologous Platelet–Rich Plasma for Wound and Osseous Healing: A Review
mixture is then gently blended by turning the 20-cc syringe over several times until it begins to thicken. Once the mixture has thickened into a gel consistency, it is applied to the wound bed as fully activated autologous platelet–rich plasma.

**Autologous Growth Factor**

Autologous Growth Factor™ (AGF™) (Ultraconcentrator U100; Interpore Cross International, Irvine, Calif) is a large-volume blood draw method for obtaining autologous platelet–rich plasma that is specifically intended for mixture with bone graft material and has been used primarily in spinal surgery. The procedure begins when approximately 1 unit of whole blood (450 mL) is drawn from the patient immediately before surgery is begun. The blood is transferred to a cell saver device for pheresis, and a 60-mL volume of “buffy coat” is obtained. Because the volume of whole blood that is drawn from the patient is 4 to 8 times greater than that used with other platelet concentrate systems and is considered physiologically significant, the “unused” layers (ie, platelet-poor plasma, red blood cells) are usually reinfused back into the patient to offset the volume of whole blood drawn. The buffy coat layer then takes multiple passes through the proprietary ultrafiltration, where excess water and low-molecular-weight molecules are removed and the remaining cellular elements, fibrinogen, and platelets are “ultraconcentrated.” In this manner, approximately 20 mL of ultraconcentrated autologous platelet–rich plasma is procured. The resultant autologous platelet–rich plasma is then combined with 1 mL of topical bovine thrombin (5000 IU/mL), which activates the platelets and creates the autologous platelet–rich plasma gel that is mixed with the bone graft material according to surgeon preference. This system has been shown to increase the platelet count by 575%, the PDGF by 546%, and the TGF-β by 380% over baseline measurements from the initial whole blood drawn (Table).

<table>
<thead>
<tr>
<th>Plasma System</th>
<th>AGF7</th>
<th>GPS II6</th>
<th>SmartPREP 2 APC8</th>
<th>Symphony II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet increase vs baseline</td>
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<td>8x</td>
<td>7x</td>
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<td>TGF-β (increase from baseline), %</td>
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<tr>
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<tr>
<td>EGF (increase from baseline), %</td>
<td>N/A</td>
<td>390</td>
<td>550</td>
<td>510</td>
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</table>

AGF, Autologous Growth Factor™; GPS II, Gravitational Platelet Separation™ II System; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; N/A, not available.
Gravitational Platelet Separation II System

The Gravitational Platelet Separation™ II System (GPS™ II System) (Cell Factor Technologies/Biomet Orthopedics, Warsaw, Ind) is a small-volume blood draw method that is used in the production of autologous platelet–rich plasma. This company offers 3 separate systems: (1) mini (27 mL blood draw); (2) single (55 mL blood draw); and (3) double (110 mL blood draw). The mini system process begins with the drawing of 27 mL of the patient’s whole blood, which is loaded into a specially designed, disposable 30-mL separation tube with a finely tuned “buoy” mechanism; it is then mixed with 3 mL of calcium citrate, which acts as an anticoagulant. The single-system process begins with the drawing of 55 mL of the patient’s whole blood, which is loaded into a specially designed, disposable 60-mL separation tube with a finely tuned “buoy” mechanism; it is then mixed with 5 mL of calcium citrate, which acts as an anticoagulant. The double system consists of 2 single systems combined and involves the same process as the single system performed twice; however, material from only 1 kit is used. Regardless of which system is selected, the separation tube that contains the whole blood and calcium citrate mixture is placed into the centrifuge with an equivalent counterbalance and is spun for 15 minutes at 3200 rpm. Under centrifuge forces, the buoy system “floats” within the separation tube to the optimal interface point of the red blood cell and buffy coat layers, regardless of the patient’s hematocrit. Once the centrifuge process has been completed, the platelet-poor plasma is contained within the topmost layer, the platelet-rich buffy coat is located just above the buoy, and the red blood cells are found at the bottom of the separation tube. If necessary, the platelet-poor plasma can then be removed by inverting the bottle and drawing off this layer through a separate, yellow extraction port on the separation tube.

To obtain the autologous platelet–rich plasma, platelets are initially suspended by shaking of the separation tube for between 15 and 30 seconds; this step is followed by removal through a separate, red extraction port into a 10-cc syringe. The mini system produces a total volume of 3 mL of autologous platelet–rich plasma; the single system produces 6 mL, and the double system, 12 mL. The resultant autologous platelet–rich plasma is then combined with 1 mL of topical bovine thrombin (5000 IU/mL) and 5 mL of 10% calcium chloride solution (100 mg/mL) and is applied to the area of interest, where platelets are activated and autologous platelet–rich plasma gel created. This system has been found to produce a platelet recovery rate of 85% and to increase platelet concentration 8 times over baseline whole blood values; it also increases PDGF-ββ by 510%, TGF-β by 360%, VEGF by 620%, and EGF by 390% over baseline whole blood values, with no increase noted in platelet surface receptor (ie, P-selectin; a direct measure of premature platelet activation) (Table).6

This company also offers 2 separate dedicated systems (CFT™ Graft Preparation System and VacPac™ Bone Grafting System) that can be used to impregnate demineralized allogenic bone graft material with autologous platelet–rich plasma, as described earlier. The CFT™ Graft Preparation System consists of demineralized allogenic bone matrix combined with a collagen-derived carrier from the same host (Boost™ DBM). With this system, demineralized allogenic bone matrix (5 or 10 mL) is placed into a 12-mL graft preparation chamber syringe, which is then gently compacted with the syringe plunger. Through a separate, closed valve system port on the side of the graft preparation chamber, a 30-mL vacuum syringe is placed; the vacuum syringe plunger is then fully withdrawn, and once the plunger has engaged...
the locking mechanism, the vacuum syringe is removed. This process compresses the demineralized allogenic bone graft material and creates a negative pressure vacuum inside the graft preparation chamber. The autologous platelet–rich plasma is prepared as described earlier and is attached to the closed valve system side port on the graft preparation chamber. Because of the previously created negative pressure environment within the graft preparation chamber, the appropriate volume of autologous platelet–rich plasma will automatically be withdrawn into the graft preparation chamber, uniformly impregnating the demineralized allogenic bone graft material. After the mixture has been allowed to hydrate for 5 minutes, the end-cap on the graft preparation chamber can be removed. The demineralized allogenic bone graft mixture that has been impregnated with autologous platelet–rich plasma is then expressed ready for implantation within the surgical site (Data on file; Cell Factor Technologies/Biomet Orthopedics, Warsaw, Ind).

The VacPac™ Bone Grafting System involves a specialized, vacuum-sealed, dual-compartment pouch; 1 compartment contains 10 cc of cancellous allogenic bone chips, and the other can be infused with up to 6 mL of autologous platelet–rich plasma. A rubber “zip strip” separates the 2 compartments. The vacuum effect that results deeply hydrates the cancellous allogenic bone graft chips with the autologous platelet–rich plasma, creating a resilient bone graft mixture that is then ready for implantation within the surgical site (Data on file; Cell Factor Technologies/Biomet Orthopedics, Warsaw, Ind).

**Magellan Autologous Platelet Separator**

The Magellan™ Autologous Platelet Separator (Medtronic Biologic Therapeutics and Diagnostics, Minneapolis, Minn), a small-volume blood draw method for the production of autologous platelet–rich plasma, uses a closed system that is purported to reduce operator error and decrease the potential for cross-contamination. The process begins when 30 to 60 mL of the patient’s whole blood is drawn and loaded into a specially designed disposable separation chamber; it is mixed with 5 mL of 10% calcium citrate, which acts as an anticoagulant. The 60-mL separation chamber that contains the whole blood and calcium citrate mixture is placed inside the closed system, which informs the operator of the phase of operation, the time remaining, and the volume of autologous platelet–rich plasma that has been selected (2–10 mL). The separation chamber and the tubing connected to the syringes can be reused up to 3 times in the same patient. Two custom-designed syringes are placed in the Magellan System adjacent to the centrifuge separator. Sterile tubing connects the centrifuge separator and the syringes through a closed system and delivers the autologous platelet–rich plasma through an automated pump. This ensures accurate delivery with minimum platelet activation. The resultant autologous platelet–rich plasma is then combined with 1 mL of topical bovine thrombin (5000 IU/mL) and 5 mL of 10% calcium chloride solution (100 mg/mL) and is applied to the area of interest, where platelets are activated and autologous platelet–rich plasma gel created.

**Secquire Cell Separator**

The Secquire® Cell Separator (PPAI Medical, Fort Myers, Fla) is a small-volume blood draw method used in the production of autologous platelet–rich plasma. The process begins when 50 mL of the patient’s whole blood is loaded into a 60-mL cell
separator, mixed with 5 mL of calcium citrate (which acts as an anticoagulant), and placed into a centrifuge with an equivalent counterbalance; it is then spun for 9 minutes at 3500 rpm. Once the centrifuge process has been completed, the packed red blood cells are removed from a specially labeled blood port, and the 60-mL cell separator tube with the remaining plasma and platelet concentrate is then placed back into the centrifuge with an equivalent counterbalance and spun for an additional 6 minutes at 3500 rpm. The 60-mL separation tube is removed, at which point the platelet-poor plasma is extracted from a specially labeled plasma port and is discarded or saved for later use. The process creates between 5 and 10 mL of autologous platelet-rich plasma. So that autologous platelet-rich plasma can be obtained, the platelets are initially suspended by shaking of the separation tube for between 15 and 30 seconds; this step is followed by removal through an extraction port into a 10-cc syringe. The resultant autologous platelet-rich plasma is then combined with 1 mL of topical bovine thrombin (5000 IU/mL) and 5 mL of 10% calcium chloride solution (100 mg/mL) and is applied to the area of interest, where platelets are activated and autologous platelet-rich plasma gel created. This system has been found to produce a platelet recovery rate of 77% and to increase platelet concentration by 10.2±2.4 times over that of baseline whole blood (Data on file; PPAI Medical, Fort Myers, Fla).

**SmartPReP®2 APC+ System**

The SmartPReP®2 APC+™ System (Harvest Technologies, Plymouth, Mass) is a small volume blood draw method used to produce autologous platelet-rich plasma. The process begins when 50 or 100 mL of the patient’s whole blood is drawn; this produces 3 to 9 mL or 10 to 20 mL of autologous platelet-rich plasma, respectively. The whole blood is separated equally into 60-mL syringes if a 100-mL whole blood sample is used, and into one 60-cc syringe if a 50-mL sample is used. After the whole blood has been drawn, 5 mL of 10% calcium citrate–based anticoagulant (100 mg/mL) is added to each syringe, and the resultant mixture is emptied into a chamber within a disposable container that has 2 side-by-side chambers. These chambers are connected at their top ends and use a specialized filter to separate plasma from red blood cells. Both side-by-side chambers are placed in a vertical position and rotate into a horizontal position when the centrifuge is started; this allows the plasma to escape the filled chamber and flow into the adjacent empty chamber. At the completion of the centrifuge process (ie, after approximately 14 min), when the plasma extraction is complete, a “pellet-like” precipitate of plasma concentrate is created at the bottom of the plasma chamber, and the remainder is filled with platelet-poor plasma. The platelet-poor plasma is then removed and the platelet concentrate resuspended through the centrifuge several times so that very concentrated autologous platelet-rich plasma is obtained. The resultant autologous platelet-rich plasma is then (1) removed from the plasma vial, (2) combined with 0.2 mL of topical bovine thrombin (5000 IU/mL) and 1 mL of 10% calcium chloride solution (100 mg/mL) through 2 side-by-side syringes (ie, 1-cc syringe containing the thrombin–calcium citrate activator and a 10-cc syringe of autologous platelet-rich plasma) with 1 common plunger and 1 common applicator tip, and (3) applied to the area of interest, where platelets are activated and autologous platelet-rich plasma gel created. This system has been found to increase PDGF-β by 600%, TGF-β by 727%, VEGF by 428%, and EGF by 550% over baseline whole blood values (Table).
Symphony II Platelet Concentrate System

The Symphony™ II Platelet Concentrate System (PCS) (DePuy ACE, Warsaw, Ind) is a small-volume whole blood draw method (55–110 mL) used for the production of autologous platelet–rich plasma; it is nearly identical to the SmartPReP®2 APC+™ System (Harvest Technologies) described earlier. The process begins when 55 mL of the patient’s whole blood is drawn, mixed with 5 mL of 10% calcium citrate–based anticoagulant (100 mg/mL), and placed into a specially designed, disposable 60-mL container with a finely tuned “floating shelf” mechanism very similar to the Gravitational Platelet Separation™ II System (Cell Factor Technologies/Biomet Orthopedics) described earlier. The 60-mL tube that contains the whole blood and calcium citrate mixture is placed into the centrifuge with an equivalent counterbalance and spun for approximately 12 minutes. Under centrifuge forces, the shelf system will “float” within the tube to the interface point of the red blood cell and buffy coat layers, regardless of the patient’s hematocrit. Once the centrifuge process has been completed, the platelet-poor plasma is contained within the topmost layer, the platelet-rich buffy coat is located just above the shelf, and the red blood cells are found at the bottom of the separation tube. The resultant autologous platelet–rich plasma is then combined with 1 mL of topical bovine thrombin (5000 IU/mL) and 5 mL of 10% calcium chloride solution (100 mg/mL) and is applied to the area of interest, where platelets are activated and autologous platelet–rich plasma gel created. This system has been shown to increase the concentration of autologous platelet–rich plasma growth factors approximately 3 to 6 times over baseline whole blood values and allows for a mean of 80.2% of available platelets to be recovered. This product has been found to increase PDGF by 310%, TGF-β by 620%, EGF by 510%, and VEGF by 290% over baseline whole blood values (Data on file; DePuy ACE, Warsaw, Ind) (Table).

This company also offers a dedicated system (Symphony™ Graft Delivery System) by which β-tricalciumphosphate synthetic bone graft material (Conduit™ TCP Granules) combined with autologous platelet–rich plasma can be impregnated, as described earlier. This particular synthetic bone graft is a porous, osteoconductive ceramic that is similar (ie, 70%) to the mineral constituents of natural bone; it consists of irregularly shaped granules and has interconnected varying pore sizes (1–600 µm) that have been shown to be important for fibrovascular tissue ingrowth (Data on file; DePuy ACE, Warsaw, Ind).9,10 With this product, β-tricalciumphosphate synthetic bone graft material is placed into a 15-mL graft preparation chamber syringe that has been attached to a robust manifold stand. The previously collected autologous platelet–rich plasma and topical bovine thrombin–10% calcium chloride solution mixture are placed into their own syringes, which are attached to the manifold stand through their own separate ports. The autologous platelet–rich plasma and topical bovine thrombin–10% calcium chloride solution mixture syringes are then compressed simultaneously, thus uniformly impregnating the β-tricalciumphosphate synthetic bone graft material. After the mixture is allowed to hydrate, the graft preparation syringe is removed from the manifold stand, and the β-tricalciumphosphate synthetic bone graft mixture that has been impregnated with autologous platelet–rich plasma is ready for implantation within the surgical site as a bioactive bone graft substitute (Data on file; DePuy ACE, Warsaw, Ind).
Accelerate Platelet-Rich Plasma Gel

Accelerate™ Platelet-Rich Plasma (PRP) Gel (Exactech Inc., Gainesville, Fla) is a small-volume, sterile blood draw method for the production of autologous platelet–rich plasma that is similar to the Secquire® Cell Separator (PPAI Medical). The process begins with the drawing of 50 mL of the patient’s whole blood directly from the surgical field (ie, greater saphenous vein) following sterile preparation and draping of the involved extremity. The withdrawn whole blood is then loaded into a specially designed, disposable 60-mL cell separator tube; it is mixed with 5 mL of calcium citrate, which acts as an anticoagulant, and is then placed within a sterile centrifuge capsule adapter located inside of the second centrifuge capsule adapter. It is transferred from the surgical site and is placed into the centrifuge with an equivalent counterbalance, where it is spun for 9 minutes at 3500 rpm. Once the centrifuge process has been completed, the centrifuge capsule adapter is removed from the centrifuge and opened, the original centrifuge capsule adapter is removed in sterile fashion and is returned to the operative field, where it is opened and the packed red blood cells removed from a specially labeled blood port on the sterile 60-mL cell separator tube. The sterile centrifuge capsule adapter that contains the 60-mL cell separator tube with the remaining plasma and platelet concentrate is then placed back inside the second sterile centrifuge capsule adapter, which is transferred from the surgical site and placed into the centrifuge with an equivalent counterbalance and spun for 6 minutes at 3500 rpm. The sterile centrifuge capsule adapter is then opened and the 60-mL cell separation tube removed, at which point the platelet-poor plasma is extracted from a specially labeled plasma port and is discarded or saved for later use. This process creates 5 to 10 mL of completely sterile, ultraconcentrated autologous platelet–rich plasma. To obtain autologous platelet–rich plasma, the platelets are initially suspended by shaking of the separation tube for between 15 and 30 seconds; this step is followed by removal through an extraction port into a 10-cc syringe. The resultant autologous platelet–rich plasma is then combined with 1 mL of topical bovine thrombin (5000 IU/mL) and 5 mL of 10% calcium chloride solution (100 mg/mL) and is applied to the area of interest, where platelets are activated and autologous platelet–rich plasma gel created.

This company also offers a system that can be used to impregnate demineralized allogenic bone graft material (Opteform™ RT Allograft Paste Room Temperature) with autologous platelet–rich plasma, as described earlier. Each lot of this particular allogenic bone graft is tested with the athymic nude rat muscle implantation model for osteoinductive activity. This product combines a demineralized bone matrix for osteoinductivity, precisely shaped and sized corticocancellous bone chips for osteoconductivity, and a thermoplastic insoluble inert biological carrier matrix that affords irrigation-resistant structural integrity (Data on file; Exactech Inc., Gainesville, Fla). Through this approach, the autologous platelet–rich plasma is delivered directly into the sterile mixing jar that contains the demineralized allogenic bone graft powder. With a specialized spatula, the autologous platelet–rich plasma is hand-mixed into the demineralized allogenic bone graft powder for approximately 1 minute until the desired handling characteristics (ie, formability) of the bone graft mixture are achieved. The resultant demineralized allogenic bone graft mixture that has been impregnated with autologous platelet–rich plasma can then be molded into the desired shape (eg, disc, cylinder, strip) and implanted within the surgical site (Data on file; Exactech Inc., Gainesville, Fla).
ROLE OF AUTOLOGOUS PLATELET–RICH PLASMA IN WOUND HEALING

Wound healing represents an intricate process that involves a series of elaborate and well-orchestrated steps and a complex interplay of numerous humoral factors and cellular constituents. This process consists of 3 overlapping phases: inflammatory, proliferative, and remodeling. During the inflammatory phase, platelets aggregate and release a number of cytokines, growth factors, and hemostatic factors. During the proliferative phase, macrophages remove debris and bacteria, fibroblasts synthesize ground substance, and migrating endothelial cells, while responding to the influence of chemostatic factors, create angiogenesis. Epithelialization commences from the edges of the wound and continues through the remodeling phase, which can last up to 2 years. It appears that the increased concentration of growth factors contained within autologous platelet–rich plasma would be ideally suited for wound healing.

Veterinary medicine has utilized autologous platelet–rich plasma for wound healing of the lower equine limb. In a well-constructed equine model study, 2 full-thickness skin wounds, each measuring 2.5 cm², were created on a generally healthy 16-year-old male horse. These wounds were specifically placed 3 cm apart on a single lower limb. One wound was treated with autologous platelet–rich plasma and the other was left untreated. Both wounds were covered with sterile nonadherent gauze and a circumferential bandage. The wounds were retreated every 4 days according to the technique described previously. After day 28, dressings were applied every 8 days. An 8-mm surgical biopsy specimen was taken for histologic examination at 7, 36, and 79 days after wounding. This examination showed that the area treated with autologous platelet–rich plasma produced more dense and better organized collagen bundles compared with untreated areas.

The plastic surgery literature reveals that platelet-rich and platelet-poor plasma concentrates have been used successfully in many soft tissue procedures. Man et al, in a prospective cohort study involving 20 patients between 25 and 76 years of age who were undergoing cosmetic facial flap surgery, showed that the use of autologous platelet–rich plasma accelerated primary flap healing and markedly reduced peri-incision erythema. Welsh followed patients with 108 acute and 5 chronic wounds (ie, 85 facial plasties, 3 blepharoplasties, 8 facial laser resurfacings, 1 seroma evacuation, 5 split-thickness skin graft application sites, 3 sacral decubitus flaps, 7 breast reductions, and 1 rhinoplasty) who had been treated with autologous platelet–rich plasma and showed that this treatment acts as an effective wound sealant, and that it enhances wound healing. The authors concluded that the use of autologous platelet–rich plasma resulted in improved primary healing, limited incision site dehiscence, formation of hematoma, and bruising in the group of patients who underwent facial plasty or split-thickness skin graft application. Marx compared application of autologous platelet–rich plasma with standard treatment (ie, control site) for 2 side-by-side, thin (0.016 inch), split-thickness skin graft harvest sites from a single patient’s thigh. The split-thickness skin graft harvest sites were evaluated at 6 days and 6 months postoperatively. At 6 days, the control site revealed ≤5% epithelialization with increased peripheral erythema and an abundance of granulation tissue. In contrast, the harvest site to which autologous platelet–rich plasma was applied showed no peripheral edema and a thin epithelial covering over...
95% of the area. Histologically, the control site showed no epithelial budding, and granulation tissue consisted of only immature fibroblasts and macrophages; however, the site to which autologous platelet–rich plasma was applied showed epithelial budding and a mature dermis. At 6 months, the control site showed an abundance of scarring and a greater loss of pigmented cells compared with the site treated with autologous platelet–rich plasma. The authors concluded that the application of autologous platelet–rich plasma to split-thickness skin graft harvest sites results in more expeditious and complete healing, as well as decreased scarring and greater melanocyte survival.17

The specific application of autologous platelet–rich plasma to chronic wounds has been studied in detail, and a number of studies have demonstrated a significant increase in the limb salvage rate (ie, amputation prevention) among high-risk diabetic patients,18-21 as well as reduced total treatment expenses when combined with a comprehensive local wound care protocol. Knighton et al conducted a prospective study involving the application of autologous platelet–rich plasma in 41 patients with a total of 71 chronic wounds of various causes and locations.24 The mean time from initiation of autologous platelet–rich plasma application to 100% epithelialization was 7.47±6.53 weeks, regardless of patient age, chronicity of the ulceration, or anatomic location.24 Atri et al conducted a prospective study involving the application of homologous platelet–rich plasma in 23 patients with a diabetic or venous stasis ulceration that had not responded to a mean of 25 weeks of conservative therapy.25 During the 25 weeks of conservative therapy alone, 3 (11%) wounds achieved 100% epithelialization. In contrast, all of the remaining 24 wounds achieved 100% epithelialization following application of homologous platelet–rich plasma at a mean of 9.7 weeks.25 Knighton et al conducted a prospective, double-blind, randomized, placebo-controlled, crossover trial of 24 patients with 34 wounds, in whom 21 wounds were treated with autologous platelet–rich plasma and 13 were treated with a placebo.26 At the 8-week crossover point, 17 (81%) of the wounds treated with autologous platelet–rich plasma had achieved 100% epithelialization, which was attained by only 2 (15%) of the wounds treated with placebo. After treatment with autologous platelet–rich plasma was provided, the crossover placebo wounds each achieved 100% epithelialization. Mean times to 100% epithelialization were 8.6 weeks in the group treated with autologous platelet–rich plasma primarily and 15 weeks in the crossover group.26 Steed et al conducted a prospective, double-blind, placebo-controlled study involving 13 patients with diabetes who had a total of 13 wounds that remained unhealed after 8 weeks of conservative therapy.27 Six of the wounds were treated with placebo and 7 were treated with homologous platelet–rich plasma that had been diluted 1:100 in an aqueous buffer. At a mean of 15 weeks, 5 (71%) of the wounds treated with the homologous platelet–rich plasma were substantially healed, but only 1 wound (17%) in the placebo-treated group showed equivalent healing at a mean of 20 weeks.27

Holloway et al conducted a prospective, double-blinded, placebo-controlled, dose-ranging, multicenter study involving 70 patients with 70 wounds treated with placebo or various dilutions of homologous platelet–rich plasma (1:10, 1:30, or 1:100).28 Although 63% of the wounds treated with homologous platelet–rich plasma healed versus only 29% of the placebo-treated wounds, it was found that the 1:100 diluted homologous platelet–rich plasma group demonstrated the most efficacious healing (80%).28 Crovetti et al performed a study involving the application of autologous platelet–rich plasma or homologous hemocomponent platelet–rich plasma...
to 24 chronic cutaneous wounds from 24 patients with a mean age of 74 years. Of the 24 patients enrolled in the study, only 3 were able to undergo autologous whole blood withdrawal; the remainder used homologous hemocomponent platelet–rich plasma instead. At the completion of the study, 9 wounds were fully healed following a mean of 10 applications of platelet-rich plasma. Seven of the wounds had decreased in volume by 50% or more at the completion of the study; 4 patients had ceased treatment for various reasons, and 2 were ultimately covered with a split-thickness skin graft. Of a total of 323 platelet-rich plasma applications, no adverse reactions were noted, and only 2 localized infections developed; these were successfully resolved with oral antibiotic therapy. Each patient stated that pain at the ulceration site was markedly decreased during the treatment process. The authors observed a significant increase in the volume of healthy granulation tissue after the platelet-rich plasma was first applied. Unfortunately, the separate effect that autologous platelet–rich plasma versus homologous platelet–rich plasma has on healing chronic wounds was not discussed and remains a matter for conjecture.

ROLE OF AUTOLOGOUS PLATELET–RICH PLASMA IN BONE GRAFT HEALING

The discovery of bone morphogenetic protein in the 1960s has led to the identification and utilization of various growth factors for bone graft incorporation and osteogenesis. In this regard, some of the most widely studied and most frequently used growth factors have been shown to be present in greatly increased concentrations within autologous platelet–rich plasma.

Autologous platelet–rich plasma has been used in oral and maxillofacial surgery to stimulate bone growth and maturation. Marx et al conducted a study involving 88 elective cancellous cellular marrow bone grafts applied to mandibular defects ≥5 cm, some of which had been impregnated with autologous platelet–rich plasma during the bone milling phase of graft preparation (those not so impregnated were used for the control arm of this study). In each group, bone grafts were allowed to consolidate for a 6-month period. Standardized radiographs obtained at 2-, 4-, and 6-month intervals were evaluated by 2 blinded investigators for perceived maturity of the grafts. Bone grafts that had been impregnated with autologous platelet–rich plasma were consistently assessed at approximately twice their actual maturity level with ratios at each interval as compared with the control bone grafts. Bone grafts impregnated with autologous platelet–rich plasma showed a greater trabecular bone density compared with control bone grafts (74.0%±11.0% vs 55.1%±8.0%, respectively). The authors concluded that growth factor additions from the autologous platelet–rich plasma produced quantifiably enhanced bone density and maturation, as well as an improved ultimate result, when compared with control bone grafts.

The spine surgery literature has supported the use of autologous platelet–rich plasma in lumbar fusion. Lowery et al performed a retrospective review of 39 lumbar fusions in 21 patients between 30 and 72 years of age. Each patient was followed postoperatively for at least 6 months; 19 patients (91%) were available at a mean follow-up evaluation of 13 months (91%). Of these 19 patients, 9 had undergone previous back surgery at the area of interest, 8 were active tobacco users, and 6 were morbidly obese (body mass index ≥27). Three patients underwent hardware removal at 6 months,
and 2 others were treated by intradiscal fusion for disc degeneration at adjacent levels to the index fusion sites. In each of these patients, direct visualization of the fusion sites revealed solid, well-consolidated bone graft application sites. The remaining 14 patients were noted on serial radiographs to have solid union across fusion sites. In each patient, early bone maturation and solid fusion occurred with no major complications associated with use of autologous platelet-rich plasma. The authors concluded that when used as an adjunct to an autologous bone graft, autologous platelet-rich plasma concentrate offers theoretical advantages that warrant further study. 

Siebrecht et al conducted a study involving 17 athymic nude rats to assess osseous ingrowth following subperiosteal implantation of a bone conduction chamber at the level of the proximal tibia. Each rat served as its own control in that 1 tibia received a bone conduction chamber that contained hydroxyapatite synthetic bone graft material impregnated with autologous platelet–rich plasma, and the other contained the hydroxyapatite synthetic bone graft material alone (ie, control group). At 4 weeks after implantation, the bone conduction chambers were cut along their long axes, and the total areas of osseous tissue and soft tissue were measured with a computerized video system. The bone conduction chambers that contained the hydroxyapatite synthetic bone graft material impregnated with autologous platelet–rich plasma revealed an osseous ingrowth distance of 1.8 mm compared with an osseous ingrowth distance of 0.8 mm for those in the control group. Total soft tissue ingrowth distance was also increased from 2.9 mm in the control group to 4.1 mm in the bone conduction chambers that contained the hydroxyapatite synthetic bone graft material impregnated with autologous platelet–rich plasma. The authors concluded that the addition of autologous platelet–rich plasma enhances the induction of hydroxyapatite synthetic bone grafts for osseous replacement. 

Fennis et al evaluated 28 goats following resection of the bone marrow in a segment of the mandible, which left the outer cortices intact; the defect was filled with autogenous corticocancellous anterior iliac crest bone graft ground in a bone mill alone (ie, control group) or the same graft that had been impregnated with autogenous platelet–rich plasma. Two cortical bone “trays” were filled with the bone graft—1 with the active material and 1 with the control bone graft—and were placed into the osseous defect. The mandible was then secured by 2 plates, with 5-mm gaps separating the cortical trays from the remaining mandible. Burr holes were drilled into the outer cortex of the cortical bone graft trays to facilitate vascular ingrowth. Radiographic evaluation was performed at 3, 6, and 12 weeks by 2 independent physicians, who used a scoring system that included variables for bone gaps, perforations, formation of callus, and central and angular resorption. The cortical bone trays filled with the autogenous ground iliac crest graft impregnated with autogenous platelet–rich plasma showed statistically significant accelerated osseous ingrowth. 

Aghaloo et al conducted a randomized, blinded, prospective study to investigate the effects of autologous platelet–rich plasma alone, autologous bone graft impregnated with autologous platelet–rich plasma, autologous bone graft alone, and treatment that involved no grafting at all on the healing of four 8-mm-diameter cranial defects from 15 rabbits. Each rabbit was evaluated postoperatively by digital subtraction radiography at 1, 2, and 4 months. Although they were not statistically significant, results demonstrated a histomorphometric tendency toward enhanced bone healing, regardless of time frame, in the group given autologous bone graft impregnated with autologous platelet–rich plasma compared with those given...
autologous bone graft alone. However, what was clear was that defects filled with autologous bone graft alone or autologous bone graft impregnated with platelet-rich plasma showed a statistically significant increase in percentage of bone healing compared with those given the autologous platelet–rich plasma alone or the group given no treatment at all.

The use of autologous platelet–rich plasma during distraction osteogenesis has been evaluated in some preliminary clinical studies. Robiony et al conducted a study of 5 patients with atrophy of the mandible who were treated with distraction osteogenesis so that the mandible could attain appropriate height and density to accept dental implants. Autologous bone graft harvested from the iliac crest impregnated with autologous platelet–rich plasma was applied to the distraction gap at the time of the corticotomy. Following a 15-day latency period, distraction osteogenesis was initiated at a rate of 0.5 mm per day until the defect was fully corrected; this phase was followed by a 60-day consolidation period. Following consolidation, the external fixation distraction device was removed and the dental implants seated. In each patient, the necessary mandibular height was successfully restored and the mean distraction osteogenesis was 10.3 mm (range, 8.5–11.5 mm). Although the study population included no control group, the authors concluded that the use of autologous iliac crest bone graft impregnated with autologous platelet–rich plasma during distraction osteogenesis of the mandible affords more rapid osseous maturation than can be achieved with corticotomy and distraction alone.

In another distraction study, Kitoh et al used a combination of mesenchymal stem cells impregnated with autologous platelet–rich plasma during distraction osteogenesis of 3 femora and 2 tibiae in 2 patients with achondroplasia and 1 patient with congenital pseudoarthrosis of the tibia. At the time of corticotomy and external fixation application, mesenchymal stem cells were harvested from the iliac crest, cultured with osteogenic supplements, and differentiated into osteoblasts. Once distraction of callus was completed, harvested osteoblasts were mixed with autologous platelet–rich plasma and injected into the distracted tissue site at the callus. Results of this study were then compared with outcomes of 8 patients with the same types of disorders who underwent only distraction osteogenesis without augmented tissue application (ie, control group). Among these patients, mean age was documented as 16.7 years, and mean limb length achieved was 8.7 cm. Patients in the treatment group in whom harvested osteoblasts and autologous platelet–rich plasma were applied had a mean age of 13.9 years with mean limb length of 10 cm. The most significant difference between the 2 groups was the osseous regenerate healing index, which was found to be 37.8 days/cm in the control group and 22.0 days/cm in the group treated with harvested osteoblasts and autologous platelet–rich plasma. Authors concluded that the application of harvested osteoblasts and autologous platelet–rich plasma to osseous regenerate following distraction osteogenesis in this patient population is a safe alternative to autologous or allogenic bone grafting procedures, is minimally invasive, and has great potential for clinical applications.

Several animal model studies have shown that the addition of autologous platelet–rich plasma accelerates bone ingrowth into titanium implants in the tibiae of rats and the mandibles of both mini-pigs and mongrel dogs. Kim et al used 30 titanium mandibular implants in 10 mongrel dogs, which were separated into 3 groups: (1) dogs treated with demineralized bone protein impregnated with autologous platelet–rich plasma, (2) those given demineralized platelet–rich plasma alone, and...
(3) patients who received no treatment at all (ie, control group). Histomorphometric results at 6 and 12 weeks revealed a higher percentage of bone contact within the group treated with demineralized bone protein impregnated with autologous platelet-rich plasma than was seen in either of the other groups. At 6 weeks, mean direct implant-to-bone contact percentages were 10%±18.4% for the control group, 48%±10.5% for the demineralized bone protein only group, and 74%±13.3% for the group treated with demineralized bone protein impregnated with autologous platelet-rich plasma. At 12 weeks, mean direct implant-to-bone contact percentages were 17%±2.5% for the control group, 56%±15.9% for the demineralized bone protein only group, and 80%±15.2% for the group treated with demineralized bone protein impregnated with autologous platelet-rich plasma. On the basis of these results, the authors concluded that the addition of autologous platelet-rich plasma to demineralized bone protein accelerated and improved bone formation when compared with demineralized bone protein alone.

Although the vast majority of published literature on the use of bone graft impregnated with autologous platelet-rich plasma has shown remarkably positive results, a number of clinical studies have evaluated the use of autologous platelet-rich plasma for sinus floor augmentation to improve the height of the posterior maxilla and have identified no statistically significant benefit. Jakse et al and Butterfield et al performed animal studies in sheep and rabbits, respectively, that showed no statistical significance with the utilization of autologous platelet-rich plasma alone for sinus floor augmentation. Several other studies that evaluated the use of autologous platelet-rich plasma impregnated within an organic bone mineral for sinus floor elevation showed no benefit, but unfortunately, these studies included no control group and so the findings are questionable. Wiltfang et al performed 45 sinus floor elevations in 39 patients with 22 sites at which β-tricalciumphosphate impregnated with autologous platelet-rich plasma was applied and 23 sites where β-tricalciumphosphate alone was used (ie, control group). Findings from biopsy specimens of sinus floor elevations revealed that osseous regeneration reached an average level of 29% for the β-tricalciumphosphate control group compared with 38% in the group treated with β-tricalciumphosphate that had been impregnated with autologous platelet-rich plasma. The author concluded that the addition of autologous platelet-rich plasma accelerates de novo bone formation if target cells such as osteoblasts and osteocytes are present.

From an in-depth review of the available literature on sinus floor elevation, it appears that the regional anatomy of the sinus floor and the use of synthetic organic bone material as opposed to demineralized bone matrix or autogenous bone graft require the addition of osseous cellular components beyond those that can be delivered by autologous platelet-rich plasma alone. For example, the porous nature of demineralized allogenic bone graft would seem to allow full infiltration of autologous platelet-rich plasma, which would enhance the osteoinductive, proliferative, and chemoattractive signals already present in the bone graft material (ie, bone morphogenic protein-2, bone morphogenic protein-4, and bone morphogenic protein-7) and should signify greater healing potential over synthetic organic bone material such as β-tricalciumphosphate. Until additional study findings become available, it seems that the benefit of using bone grafts impregnated with autologous platelet-rich plasma during surgery that involves the sinus floor remains a matter for conjecture.
DISCUSSION

The application of autologous platelet–rich plasma to soft tissue and for osseous healing has been a subject of great interest for much of the past 2 decades. The growth factors discussed earlier, which are present in high concentrations within autologous platelet–rich plasma, have been shown to initiate and modulate soft tissue and osseous healing. Unfortunately, little consistency or agreement has been attained in the actual manufacturing process of autologous platelet–rich plasma, and a variety of proprietary systems may be used for various applications, as described in detail earlier. The ultimate goals are to maximize the benefits of autologous platelet–rich plasma and minimize the potential risks. The benefits of autologous platelet–rich plasma can be summarized as follows: (1) offers the highest potency, (2) has the ability to produce the desired effect, and (3) consists of both quantitative and qualitative components. Quantitative potency refers to the percentage of platelet yield, and qualitative potency represents actual platelet viability, survival, and function. A number of activities conducted throughout the manufacturing process of autologous platelet–rich plasma can directly affect both quantitative and qualitative potency (Table).

Although final growth factor content has not been shown to be strongly correlated with platelet count in whole blood, the greatest concentration of autologous platelet–rich plasma can be attained when whole blood is drawn from a peripheral vein before any intravenous fluid is administered; this prevents dilution of the whole blood and resultant lower platelet yield. Furthermore, although sex and age have not been shown to have a significant influence over the final platelet count or growth factor concentration within autologous platelet–rich plasma, advanced age does imply the use of various medications secondary to disease states associated with advanced age, as well as renal and liver disease, cardiopulmonary bypass, and primary bone marrow disorders. Each of these factors is known to adversely affect platelet aggregation and therefore the potential potency of autologous platelet–rich plasma. Although the autologous nature of the whole blood ensures the safety of the procedure for patients (except with Accelerate PRP Gel [Exactech Inc.]), the nonsterile sequence of steps required to fully prepare the autologous platelet–rich plasma, as well as the number of different medical personnel involved in the process, remain potential sources of contamination and disease transmission, respectively. Additionally, the requirement that the autologous platelet–rich plasma concentrate be used immediately (ie, no ability to store the final product for later use) and the need for specialized equipment that is specific to a single company can create problems.

Finally, the vast majority of studies performed on the role of autologous platelet–rich plasma in wound healing and osseous incorporation have involved relatively small numbers of subjects and have not been powered to show statistical significance; limited or no control groups were included, and imprecise means of determining the desired effect (ie, radiographs for determining osseous healing rather than direct visualization, biopsy, or more specific imaging modalities) were employed. Despite these significant weaknesses, it appears that autologous platelets that are sequestered, concentrated, and mixed with thrombin to yield autologous platelet–rich plasma represent safe, reproducible, and effective means of mimicking the natural processes of wound healing and osseous incorporation. Added trauma to the patient caused by the whole blood draw, potential for contamination of the implanted product, lack of shelf life, dependence on a single company’s equipment, and additional costs associated
with the various commercially available systems must be taken into account when one is deciding whether this technology should be used. It is the authors’ opinion that this technology should be reserved for the most appropriate clinical and surgical situations, as documented in the medical literature reviewed earlier (ie, chronic wounds that have failed to heal with appropriate conservative therapy, nonunion, or high-risk osseous defects).

CONCLUSIONS

The authors have provided an in-depth review of commercially available systems used to procure autologous platelet–rich plasma and have emphasized the subtle yet important differences between these systems. In addition, a detailed review of the literature regarding the use of autologous platelet–rich plasma in soft tissue and osseous healing has been provided. Although not yet conclusive, autologous platelets that are sequestered, concentrated, and mixed with thrombin to yield autologous platelet–rich plasma appear to be safe, reproducible, and effective in mimicking the natural processes of wound healing and osseous healing when applied in appropriate clinical and surgical situations.

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