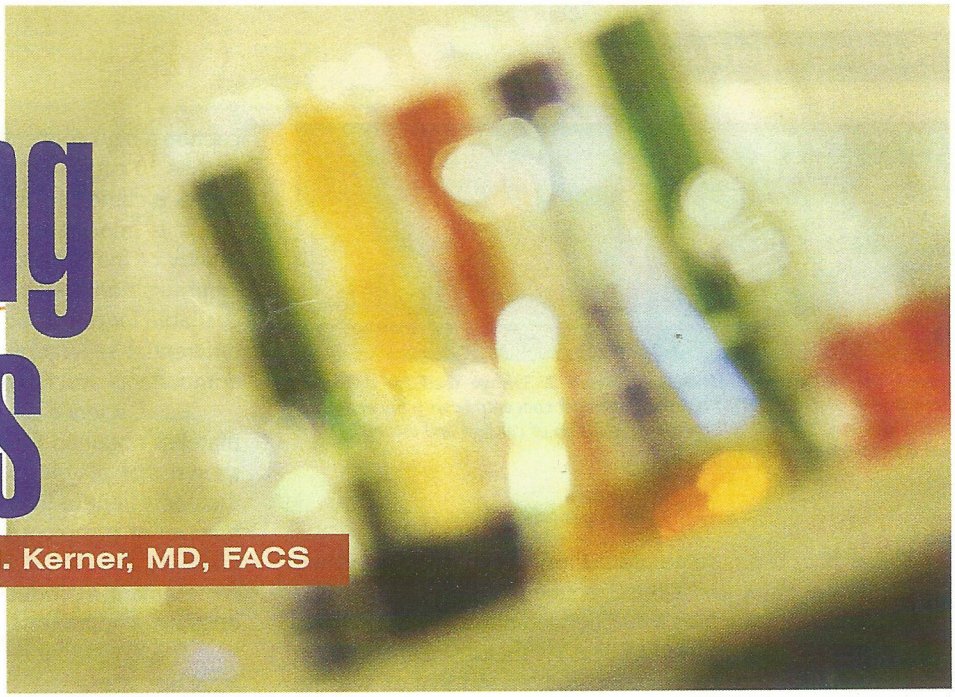
Healing Agents



by Marc M. Kerner, MD, FACS

APGs are proving to be a useful adjunct in facial plastic surgery by improving hemostasis and accelerating wound healing

As we begin to understand the molecular biology of wound healing and the relationship of growth factors (proteins) and cellular mechanisms in this complex process, the methodologies to augment healing by manipulating these various factors can be applied. For years, physicians have sought to identify substances that can enhance hemostasis and promote or accelerate tissue regeneration. Autologous platelet gels (APG) are concentrations of plasma and platelets that can be directly delivered to any surgical wound.

The basic mechanisms responsible for wound healing have been well described.[1,2] Wound healing can be divided into three basic phases: 1) inflammatory, 2) a proliferative phase, and 3) tissue remodeling. The inflammatory process is primarily mediated by platelets, but includes the release of cellular factors responsible for hemostasis and the initiation of cellular influx into the wound. The initial phase includes the activation of fibrin from fibrinogen mediated by thrombin and calcium, and the development of an organized clot consisting of platelets and cross-linked fibrin strands.

Platelet growth factors have been extensively studied. Platelets release a number of cytokines, growth factors, and proangiogenic proteins that direct the wound healing process and initiate the hemostatic cascade. The cytokines attract neutrophils and fibroblasts to the wound to direct the proliferative or second phase of wound healing, and subsequently to attract fibroblasts which are crucial to the remodeling or third phase.

The inflammatory phase begins immediately after wounding, and typically lasts for 1 week. A number of platelet-derived growth factors have been identified and include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-b (TGF-b), insulin-like growth factors, and fibroblast growth factor. These factors are also integral in the angiogenic phase in which new blood vessel formation is stimulated within the wound milieu. As the wound matures, a matrix forms between the crosslinked strands of fibrin and platelets. As additional cells such as macrophages, monocytes, and neutrophils are attracted to the wound, additional growth factors are released.

As the wound continues to mature, epithelialization begins by attracting stem cells from the edges of the wound into the wound bed. These keratinocyte and fibroblast precursors migrate in a complex process that involves cytokines, metalloproteinases, and connective tissue substances such as fibronectin and collagen. This phase is clinically recognized as scar formation.

The biology of scar healing and maturation occurs in the remodeling phase. In this phase, collagen matures and is remodeled through a complex interaction of collagenases and other metalloproteinases lasting approximately 2 years. The process of collagen turnover is also regulated by the myriad of growth factors released by platelets and macrophages.

The platelets contain high concentrations of these growth factors in the alpha-granules and release them in the initial phase of wound healing. This release initiates a cascade that results in chemoattraction of stem cells, and the differentiation of stem cells called proliferation, to develop into mature tissues.

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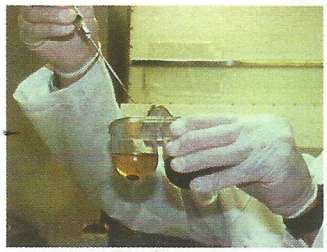

Figure 1. The left canister contains the concentrated platelet clot within the plasma solution. The red cell component is in the right canister.

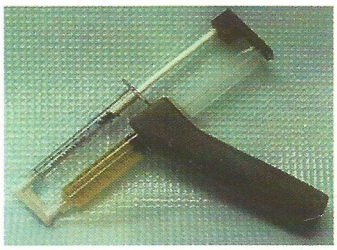

Figure 2. Syringe used for delivering platelet concentrate with the activating solution of calcium and thrombin.

Terminology of Wound Healing Agents

There has been some confusion as to the difference between tissue glues and wound accelerants. Tissue glues can be either synthetic, such as those derived from cyanoacrylates, or biologic, such as fibrin glue. The synthetic sealants form a strong bond in a closely approximated wound that allows the physcian to avoid using sutures or staples, but does not contain the biologic factors that would promote wound healing via chemoattraction.

Fibrin glue on the other hand, is a biologic product derived from a pool of plasma donors. It contains a high concentration of fibrinogen, which exceeds 40 times the levels found in nascent plasma. To complete the preparation, the fibrinogen is added to factor XIII and pooled plasma proteins. Exogenous calcium, thrombin, and fibrinolytic inhibitors are added to the product, which results in the formation of a stable clot matrix. The greatest disadvantage of fibrin glue is that it must be developed from pooled donors to achieve this high concentration and has the inherent risk of blood borne disease transmission. The processing for single donor or autologous fibrin glue is cumbersome, requiring days to complete; however, the final product has excellent hemostatic properties.

The current methodology for acquiring autologous tissue glues involves collecting whole blood from the patient and centrifugation of this blood into the plasma and cellular components. The plasma is collected and mixed with exogenous calcium and thrombin. This combination results in the activation of the fibrin matrix. Since this autologous fibrin glue product contains only the plasma concentration of fibrinogen, it differs clinically from the commercially prepared fibrin glues that contain a 40 times increase in fibrinogen concentration. Moreover, there have been reports that the fibrin matrices resulting from commercially prepared fibrin glue are so dense that they may actually inhibit wound healing.[3] In addition, the clot matrix derived from fibrin glue does not contain growth or cellular proteins capable of performing the functions of recruiting stem cells, and stimulating the proliferative and remodeling phases of wound healing.[2]

Characterization of Platelet Concentrate

Platelet gels were first identified through the use of plasmapheresis. Large volumes (up to a liter) of blood were initially required to collect a significant concentration of platelets. The components resulting from the differential centrifugation of whole blood include the red cell component, the platelet-rich plasma concentrate, and the platelet-poor plasma component. Clinical systems are now available to capture a high concentration of platelets from a small volume (as little as 20 cc) of whole blood in approximately 15 minutes. This makes it clinically applicable to any surgical procedure.

It is important to understand the distinctions among the quality of the platelet concentrates obtained with a particular centrifugation system. There are at least seven clinical systems available for preparing autologous platelet gels.[1] All involve the differential separation of whole blood into the three primary components described above.

Basic science studies have characterized the quality of the platelets obtained from differential centrifugation.[4] It has been shown that platelets obtained from differential centrifugation are equivalent to transfusable platelets when studied against a number of parameters including pH, platelet aggregation percentages, hypotonic stress, and P-selectin values. Additionally, the resultant reproducible recovery yield of platelets in a concentrate is approximately 68%.[4]

This consistent centrifugation results in a uniform four-fold increase in platelet concentration as compared to the initial platelet count in the patient's whole blood. Growth factors have also been measured and compared to their initial plasma concentrations. The mean percentage increase over baseline of proteins, such as PDGF, TGF-b, and EGF, is approximately 450%. Additionally, the concentration of growth factors rises in a linear fashion when plotted against the platelet concentration, and the viability of these growth factors obtained from platelet concentrates has been shown to last approximately 7 days.[1,4]

Obtaining Platelet Concentrate

Surgical incisions activate the clotting cascade and can result in premature activation of platelets and release of proteins, so blood is drawn from the patient at the time of surgery before any incisions are made. The volume of blood drawn required to obtain 10 cc of platelet concentrate is approximately 50 cc of whole blood. This is mixed with 5 cc of citrate phosphate dextrose anticoagulant. This prevents the activation of the clotting cascade. The blood is placed into a chamber of a disposable apparatus and placed into the centrifuge. The resulting product contains red cells in one chamber and plasma and a platelet concentrate in the other chamber (Figure 1).

The platelet concentrate is drawn into a 10-cc syringe. The activator consisting of 5,000 units of bovine thrombin and 5 mL of 10% calcium chloride is drawn into a 1-cc syringe. The syringes are attached to a clip and connected to various applicator tips for either spray or stream application (Figure 2). The combined platelet mixture forms a gel that can be placed directly into the wound in a thin coating, or mixed with autogenous bone graft materials and then placed into the wound bed. When using it in soft tissue surgery such as a facelift or other large skin flap procedure, the gel can be sprayed or the wound surface can be coated with a thin layer of the mixture. Any type of graft material can be mixed with APG. Mixing the platelet-poor mixture with the calcium/thrombin activator results in an

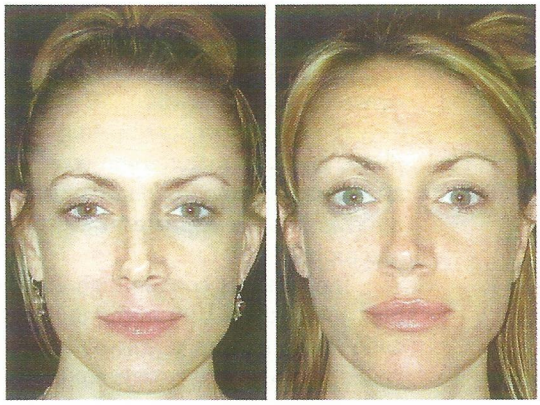

Figure 3: Pre- and postoperative photos of patient 8 days after endoscopic brow lift, revision rhinoplasty, lip augmentation, and lipotransfer procedure.

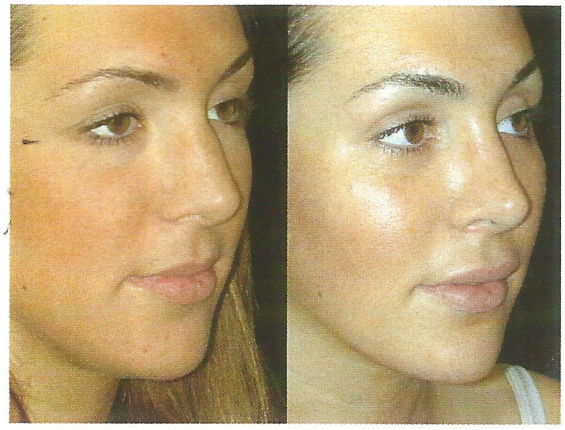

Figure 4: Pre- and 1 year postoperative photo illustrating lipotransfer to cheeks and lips using APG. The patient was back to work within 7 days with virtually no bruising or swelling.

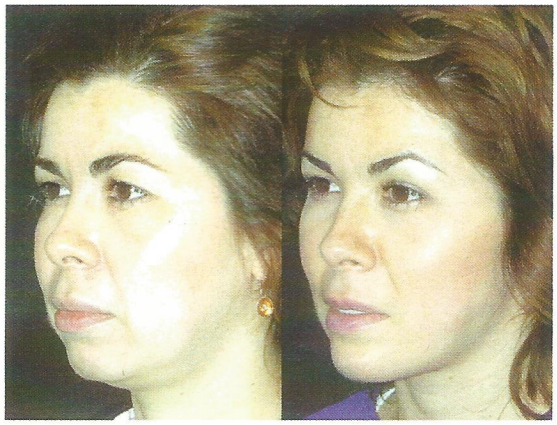

Figure 5: Pre- and 7 days postoperative facial recontouring procedure including lipotransfer to cheeks and chin and submental resculpting. The patient has maintained this result at 1 year.

autologous tissue glue. This can also be applied directly to the wound for additional hemostasis.

Clinical Applications

The clinical applications of platelet gels are limited only by the physician's imagination. We presented the first data on the use of APG as a postsurgical dressing in endonasal sinus surgery.[5] Platelet gels have wide application in procedures involving bone or tissue grafting,[6] as well as craniomaxillofacial applications.[7] Marx and colleagues have published animal studies that show significant improvement in graft survival and decreased time for graft incorporation in an in vivo bone graft model.[6] Extrapolating from this data, we have incorporated APG in virtually all of our graft procedures as well as soft tissue surgical procedures.

The primary clinical effects seen with the use of APG include a marked decrease in postsurgical swelling and bruising, decreased surgical site pain, and what appears to be accelerated wound healing. We use APG in all cosmetic procedures including facelifts, lipotransfer procedures, endoscopic brow lifts, midface lifts, and laser resurfacing, in addition to any craniomaxillofacial trauma procedures and bone grafts (Table 1). After applying the platelet-rich solution, we typically use the activated platelet-poor solution as an additional hemostatic agent (Figures 3-5).

Since we introduced autologous platelet gels into our clinical practice more than 3 years ago, we have virtually eliminated the need for drains, pressure dressings, and endonasal packing. I believe the cost of the disposable materials required to obtain the platelet concentrate is far less than the cost of the intangible factors of swelling, bruising, patient discomfort, and time missed from work.

In contrast, commercially available fibrin glues provide excellent hemostasis, but cannot provide the protein load necessary to accelerate wound healing, and has the added disadvantage of not being derived from autologous tissues. Platelet gels, on the other hand, contain the hemostatic backbones of wound healing in addition to providing an increased protein load that can drive wound healing through all of its various phases (Table 2).

The clinical applications of our current knowledge of wound healing are still in their infancy. Future studies will improve our understanding of wound biology and allow us to adjust the precise tissue-engineered growth factors to any surgical or traumatic wound. The current state of the art holds that autologous derived platelet gels can provide these biologic materials at virtually no risk to the patient. ■

Table 1: Procedures used in conjunction with activated platelet gel

- Endoscopic sinus surgery
- Craniomaxillofacial trauma
- Brow lifts
- Laser resurfacing
- Split thickness skin grafting
- Parotidectomy
- Orthognathic surgery
- Cranioplasty with hydroxyapatite cement
- Nasal reconstruction and rhinoplasty
- Rhytidectomy
- Autologous fat transfers
- Bone grafting for sinus lift procedures
- Postradiation neck dissection
- Iliac crest donor site
- Thyroidectomy

Table 2: Comparison between fibrin glue and autologous platelet gel

Protein/growth factor	Fibrin glue	APG
Fibrinogen	present	present
Platelets	absent	present
Platelet-derived growth factor	absent	present
Transforming growth factor-b	absent	present
Epidermal growth factor	absent	present
Vascular endothelial growth factor	absent	present

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