Autologous Platelet Gel — Clinical Function and Usage in Plastic Surgery

William J. Welsh, MD

Artificial methods to improve and enhance wound healing have been approached by many modalities. In recent years pharmacologic methods beyond antisepsis and antibacterial preparations have been investigated, and have reached the stage of clinical usage in acute and chronic wounds.

Among these pharmacologic modalities currently in clinical use are wound sealants including cyanoacrylates and both homologous and autologous fibrin preparations.

The method the author describes in this article provides an autologous preparation, including both wound sealant and a higher than native concentration of platelets, that can be utilized in both acute and chronic wounds and delivered at a lower cost and without the risk of blood bank error or the potential for homologous interactions of other preparations.

The use of the autologous platelet gel as a wound sealant in 108 acute cases and as a wound sealant and wound healing enhancement in five chronic cases is described. The search for an effective wound sealant involved the use of alloplastic substances, as well as homologous and autologous blood products. Recently, commercial products have become available in the United States.

Currently Utilized Wound Sealants

Cyanoacrylates

The acrylic plastic cyanoacrylates have been employed in the closure of tension-free wounds, but use is clinically limited. Although biodegradable and bacteriostatic, application of the short chain methyl- and ethylcyanoacrylates results in severe histotoxic responses - especially neural toxicity. Application of longer chain butyl- and isobutylcyanoacrylates to wound interfaces increases inflammatory responses and foreign body giant cell reactions. Mechanical blockage of neovascularization also precludes normal wound healing.1

Octylcyanoacrylate has recently been used topically and reported in clinical studies.2 This material is limited in its applications. These preparations are for external use only.

Dr. Welsh is a practicing plastic surgeon at Augusta Cosmetic Surgery and Day Spa in Augusta, GA.

Fibrin Glue

Improved fibrinogen concentration techniques allowed practical application of fibrin glue in severed nerve repair by Matras3 in Vienna in 1976. Fibrin glue utilization involves thrombin activation of concentrated fibrinogen in the presence of factor XIII, the plasma proteins: fibronectin and cold insoluble globulin, calcium chloride and aprotinin. The technique has been employed by numerous clinical investigators and found to be advantageous in reducing postoperative complications, including a study by Brück4 on ameliorating complications of facelifts.

Commercial preparation of fibrin glue involves cryoprecipitation of fibrinogen from pooled or single donor homologous human blood. Cofactors and stabilizers are added. The addition of thrombin and calcium at the time of usage eventuates a fibrin clot. The product is available under the brand names Tisseel™, Tissucol™ and Fibrin Sealant™ (Immuno A.G., Vienna.) The FDA ruled against its use in this country in 1978, as a possible viral vector of hepatitis.5 However, a fibrin sealant, Hemaseal®, has recently been licensed in the United States. These preparations cost from $200 to $500 per 2 cc usage.

Autologous cryoprecipitated fibrinogen preparations have been employed in the U.S. However, the technique is inconvenient (requiring a 3-day preparation, it cannot be prepared on an emergency basis) and yields limited amounts of usable end product. Autologous use in plastic surgery has been investigated in nonemergent clinical situations.6 Preparation of this product costs between $250 and $450. Homologous use has resulted in one case of HIV-1 transmission.7

A Sequestered Autologous Wound Sealant and Cytokine Delivery System

Blood Sequestration

Sequestration of blood in cardiac procedures has been exploited to avoid intraoperative depletion of blood components and subsequent deleterious effects on postoperative gas transport and coagulation. The technique has been instrumental in reducing the need for postsurgical homologous transfusion requirements and in cost, effectively shortening recovery time.

Preoperative separation of platelets and clotting factors from whole blood to circumvent damage to the
platelets in the heart-lung machine decreases the need for expensive postoperative platelet donor infusions. The undamaged, sequestered platelets can then be used as an autologous infusion after normal circulation is restored. The post-bypass hypocoagulable state is thus skirted with reduced risk and expense.

The ability to obtain platelet rich plasma in relatively high volumes has engendered investigation of its use as an autologous wound sealant without the theoretical and reported risks of fibrin glues. Oz was the first investigator to use sequestered platelets as a wound sealant. That study involved the use of sequestered platelets to seal experimentally induced incisions in rabbit liver and spleen. The preparation was deemed to be equivalent to cryoprecipitate-prepared fibrin glue in its hemostatic effect.

**Platelet Gel**

Use of autologous plasma as a tissue sealant by Oz et al has been succeeded by the use of the platelet rich plasma product of whole blood sequestration in combination with thrombin to produce a wound sealant that can be obtained on an emergency basis in large quantities with the added advantage of delivering young, large, healthy platelets to wound surfaces.

Enhanced harvesting of these undamaged platelets required some modification of the standard collection procedure for sequestration. First, the sequestration process is begun prior to operative heparinization using citrate-phosphate-dextrose preparation for anticoagulation. High dose heparin not only deactivates platelets, but inhibits later platelet reactivation. Citrate is reversible with calcium.

"Human life would remain a formless, helpless and ischemic mush in the absence of platelet derived growth factors."

—James H. Jandl

Secondly, a dual speed modification of the centrifugation with 5600 RPM and 2400 RPM speeds allows for separation of a platelet rich "buffy coat" with a specific gravity (sp.gr.) of approximately 1.06. This technique positions the platelets between the plasma layer (sp.gr. 1.03) and the red blood cells (sp.gr. 1.09). Separate harvesting of theuffy coat allows plasma and red cell return to the patient, averting hypovolemic consequences. The platelets can be stored at room temperature for up to 5 days and still maintain normal morphology and function.

**Platelets**

In his treatise, Blood, A Textbook of Hematology, James Jandl observes: “Human life would remain a formless, helpless and ischemic mush in the absence of platelet derived growth factors.”

Platelets are 1–7 µm anuclear fragments of megakaryocyte cytoplasm with a discoid shape and a normal blood level of 140–400,000 per cubic mm. The cytoplasm contains an open canalicular system that serves to increase the effective surface area of the fragment for intake of stimulating agonists and discharge of effector secretions. Mitochondria provide ATP in the inactivated state. The membrane is trilaminar with a glycoprotein receptor surface overlying and sometimes interspersed with and penetrating a polarized bilipid layer of phospholipids and cholesterol.

Transmembrane receptors mediate platelet function and secretion through activation of tyrosine kinase and the arachidonic and phosphoinositide cascades. Receptor deficiencies, intermediary alterations (e.g. aspirin interference with cyclooxygenase), storage pool deficiencies, genetic abnormalities and secretion defects can all lead to platelet dysfunction.

The submembrane region contains microfilaments of actin and myosin that mediate morphologic alterations. Contractions occur as an end result of the phosphoinositide cascade initiated by the agonists: Platelet Ac-
activating Factor (PAF), ADP, collagen, epinephrine, and thromboxane. ATP needed to energize the contractions is abundant in the platelet cytosol and can be further obtained by glycolysis.

With activation, the platelets assume a spheroidal shape with prickly extensions, but subsequent changes include extension and retraction of pseudopods, clot retraction and granule expulsion. Platelets can also be activated by air or foreign surfaces, especially silicone (glass) and silastic.

Thrombin binds to platelet surface receptors and activates serum factor VIII which complexes with factor IX on the platelet surfaces to activate factor X and initiate the coagulation cascade. The result is a platelet enmeshed fibrin web with a high tensile strength. A molecular flip-flop on the platelet surface membrane exposes thromboplastin, which binds factor VII and augments fibrin production.

Cytokines and other effector secretions of platelets, synthesized in the parent megakaryocyte, are concentrated and isolated from each other in the alpha, delta (dense) and lambda (lysosomal) granules of the anuclear cytosol. Isolation is important because many of the secretions are antagonistic. Regulation of secretions is responsive to differing concentrations and combinations of both intracellular and extracellular agonists.

One of the alpha granule secretions is factor V, which binds to activated factor X and calcium to catalyze prothrombin to thrombin, thereby enhancing clot formation. Alpha granules also contain supplemental factor VIII.

The actin myosin fibers are responsible for the eventual contraction of the fibrin-platelet plug. The releasate rich in platelet secretions is squeezed out like a sponge. Various studies have quantified and timed the levels of various released growth factors. The clot is limited in its propagation by PGII which is released as another of the end products of the arachidonic cascade and by nitric oxide (NO). PGII is normally released from endothelial cells to prevent clotting and NO is released from vasoconstricting smooth muscle cells for the same reason. These substances become temporarily overwhelmed on platelet activation. Vessel wall Thromboxanodulin in conjunction with Protein C and Protein S inactivates the clotting factors V and VIII. Plasminogen conversion to plasmin eventuates in clot degradation.

Arachidonic acid, a polyunsaturated fatty acid, is released on activation from the inner platelet cell membrane and is oxidized to the eicosanoids including thromboxane A2 - the body's most potent vasoconstrictor and platelet aggregating agent. Phospholipase A2, responsible for releasing arachidonic acid from the cell membrane, is inhibited by steroids.

The released eicosanoids are extremely ephemeral - TXA2 has a half life of only 30 seconds, but its vasocostrictive effect lasts about 1 hour. This supplements 10-30 second post traumatic neurogenic vasospasm that initially occurs.

Granule exocytosis (ATP dependent degranulation) occurs as the contracting platelet positions the plasma membrane in contact with the granules. The lambda granules release enzymes.

In addition to factor V, alpha granules contain von Willebrand's Factor, platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), Platelet factor 4 (PF4), Interleukin-1 (IL-1), Epidermal Growth Factor (EGF) Platelet Endothelial Growth Factor, Insulin-like Growth Factor (IGF) and Fibronectin.

Platelet secretions can possibly be exploited to minimize or placate unwanted intermediary activities initiated by surgical trauma.

Two-thirds of the platelet adenine nucleotide pool (ADP) is concentrated in the dense granules. It is released to support coagulation reactions. This compartmentalized ADP is unavailable for intracytoplasmic generation of ATP used to support the phosphoinositol and arachidonic cascades. These reactions rely on cytoplasmic glycolysis.

Platelet secretions have multiple roles in wound healing and maturation and can possibly be exploited to minimize or placate unwanted intermediary activities initiated by surgical trauma. Control of bleeding and edema will facilitate the healing process. The exact combination of factors to optimize healing and minimize scarring has yet to be elucidated. Numerous studies have demonstrated both beneficial and detrimental effects of the various cytokines at different points in the chronology of wound healing. A proper matrix environment including optimal concentrations of hyaluronan, fibronectin and other integrins and various leukocytes ensures proper action of the cytokines in healing. Publication of these studies increases yearly.

Some of the growth factors released from platelets have been shown to be absent in chronic nonhealing wounds. PDGF, EGF, and TGF-β have all been shown to enhance healing in chronic animal wounds. Knighton used an autologous platelet releasate and Atrin used a homologous platelet releasate to study the effects on chronic wound healing with positive results. Studies of platelet releasate since Knighton and Atrin's studies have produced varied and even contradictory results. Fibronectin has been shown to enhance wound repair. Future investigations might demonstrate the need for timed application of growth factor, lymphokine or cytokine blocking agents, fibronectin or hyaluronic acid to optimize wound healing.

Polymorphonuclear and monocytic inflammatory cells are recruited from the circulation by endothelial selectins
Platelet Gel Function and Use

Fig. 2: Forehead lift, upper and lower blepharoplasties, and face and neck lift using platelet gel at 48 hours post operative.

and diapedese into the wound environment. The macrophage assumes the role of the main elucidator of growth factors replacing the platelet after the second day post trauma. These cells both augment cytokines in the wound environment and serve to free the environment of unwanted debris.

A major source of debris in the sterile wound is red blood cells. If the red blood cells can be diminished or even eliminated, by closure of dead space and potent hemostasis, the oxygen demand, lactate build up, and pH lowering that accompanies the metabolic processes of phagocytosis might be attenuated. The ephemeral vasoconstrictive elements from the platelets and the fibrin clot help achieve such an environment.

Perhaps each organism has its own independent recipe of optimal factors, amplifying the need for an autologous source of these factors and altering them only in the organism susceptible to wound healing abnormalities.

Studies of platelet clot release rich in growth factors and used in chronic wounds have had varying — and sometimes contradictory — results in terms of wound healing enhancement. Use of platelet gel at this time is described for the purpose of hemostasis, wound sealing, and support of particulate bone grafts. The expectation is to provide an autologous technique that is more readily available in greater quantities and at a reduced cost.

Method for Intraoperative Use of Platelet Gel

Whole blood is harvested from the patient preoperatively, in the elective situation. Optimum platelet concentrations should be achievable in the preoperative nonedated period, as sedatives can increase platelet marginalization lowering midstream concentrations. In the emergent situation, intraoperative harvesting may be necessary.

There are 450 gms of whole blood harvested from a peripheral vein or arterial line and collected in a blood collection bag containing 63 cc of citrate anticoagulant. (If a very small amount of platelets is needed, harvest only 300 cc of blood. The excess citrate will not alter the coagulability of the mixture.) Direct draw situations can have the citrate added to the line feeding the sequestering bowl. A 125 cc bowl is used for the differential centrifugation necessary to separate the platelet rich plasma. The unused portion of blood is returned to the patient, resulting in minimal volume loss.

The harvested platelet rich plasma is hung from IV pole and attached to an intravenous infusion line, which is attached to a sterile extension line in the operative field. (Usually two extension cords are necessary for useful motion in the operative field.)

The distal end of the extension tubing is attached to the side port of a three-way stopcock. A 5 cc syringe is attached to the stopcock along with an 18 g plastic angiocath, and 1 cc of air is aspirated into the syringe. The stopcock valve is turned and 3.5 cc of platelet rich plasma is drawn into the syringe.

The valve is again turned and .5 cc of bovine thrombin (5000 units) in 10% CaCl2 (5 cc) is added to the syringe, which is agitated gently until coagulation begins. The gel is then expressed over the surgical interfaces to be approximated and allowed to cascade over the entire field. The interfaces are approximated, the skin surface is swept with the hand to remove excess gel from under the flap, and light pressure is applied for twenty seconds. The wound is then closed.

Drains are not placed in the wound, as they will only remove the gel and its releasant, defeating the purpose of the gel application.
Platelets mechanically activated during the sequestering process will resume a resting discoid state if left to quiesce in the collection reservoir. It is not recommended to keep the gel longer than 6 hours at room temperature, even though the platelets may still be activated for several days.

The preparation will contain between 500,000 and 1.5 million platelets and a fibrinogen concentration equal to that of the circulation.

Alternatively, an autologous source of thrombin can be obtained by adding one third of a cc of CaCl₂ to a red top phlebotomy tube. Blood is then drawn into the tube and the tube placed on its side for 4-8 minutes. When a clot is formed, it is removed from the tube and squeezed to eluciate 6-8 cc of thrombin-rich solution. The solution is added to the platelet rich plasma in the same ratio of 7:1 as in the bovine thrombin technique. This autologous thrombin should be used within 10 minutes of its procurement and will necessitate correct timing of preparation. There have been some objections to the use of bovine thrombin in the literature in relation to immune reactions and to the concentrations of factors V and XII in the commercial preparation. Recent reports of prion mediated diseases also cause concern.

RESULTS

To date, the preparation has been used in 85 facial plasties, three blepharoplasties without sutures and five with sutures, eight facial laser resurfacings, one seroma evacuation, five split thickness skin grafts, three sacral decubitus flaps, seven breast reductions and one rhinoplasty with iliac bone graft for a total of 113 cases.

Facial plasty patients have had their sutures all removed by postoperative day 4, unless a weekend situation necessitated removal on day 5. Most sutures were removed on postoperative day 2. There have been no postoperative or intraoperative hematomas in these patients. One patient had an intraoperative hemorrhage from a facial vein laceration that required hospitalization. None of these patients has been turned from the second operated side and found to have blood collections on the first operated side necessitating longer operating time. There has been one partially dehisced postauricular incision.

Only two patients were smokers at the time of surgery. One smoker had a small (2 sq cm) necrotic area postauricularly. One non-smoker developed a 1.5 sq cm preauricular necrotic ulcer (probably a too superficially dissected flap).

There have been three hypertrophic intraauricular scars, one in a smoker. There were also 12 patients with palpable ridges under the cheek flap where the skin was thought to be thinly elevated. Too much gel under a thin flap can be detrimental, and too thin facial skin flaps should be avoided. Frequent ridge formation in the dependent portion of the anterior cervical flap was noted. Attempts at allowing the gel to clot in a sterile basin and then inserting releasate alone in the anterior neck had a similar result. Therefore, use of the gel is not recommended in the anterior neck. The resulting ridges can take many weeks to resolve. Bruising is noticeably greater in the anterior neck postoperatively when the gel is not applied to that area.

No short- or long-term differences were clinically discernible between the blepharoplasties closed with platelet gel and Steristrip® application without sutures versus platelet gel with suturing.

The CO₂ laser resurfacing areas that were treated with the gel did not show any advantage in healing over absorbant dressings, and the technique was discontinued.

The seroma was a post traumatic right thigh seroma in a 9-year-old who was struck by a bus. The 3-week-old seroma was evacuated of 100 cc of fluid and 4 cc of platelet gel was injected into the pocket. No drains were placed and the seroma did not recur.

All the treated (small series) split thickness skin grafts survived uneventfully except a great toe wound in a diabetic with a neuropathy, which took initially, but was rubbed off again by the patient who resumed wearing his shoe against medical advice. Gel was placed over the donor site prior to application of a transparent film dressing. Efficacy was not assessed.

The sacral decubitus ulcer repairs were performed by excising bursae over the sacri of two paraplegics. Re-
Platelet Gel Function and Use

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Product</th>
<th>Adhesion Potential</th>
<th>Hemostasis</th>
<th>Contamination</th>
<th>Ease of Procurement</th>
<th>Available Product</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyanoacrylates</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hemaseal®</td>
<td>+++</td>
<td></td>
<td></td>
<td>(+)</td>
<td>(+)</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Homologous</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>(+)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>cryoprecipitate</td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autologous</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>(+)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>cryoprecipitate</td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autologous</td>
<td>++</td>
<td>+++</td>
<td></td>
<td>(+)</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Platelet Gel</td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pair was accomplished with locally transferred flaps measuring 200-300 sq cm. No drains were inserted under the flaps. The patients were wheelchair ambulating in 1 week. The ulcers have not recurred after 18 months and 6 months, respectively. The third patient had an ischial ulcer. This repair is short-term.

No advantage was seen in subflap application of the gel in the breast reduction patients; therefore, the technique was abandoned.

The rhinoplasty was a non-Caucasian woman desiring more nasal projection. A previous calvarial graft had been unsuccessful. Bone was harvested from the right ilium, particulated and impregnated with gel in the technique described by Whitman et al.24

A successful graft take eventuated and the patient was satisfied. No patients experienced intraoperative or recovery room fevers, rashers, or pruritis. No evidence of delayed hypersensitivity reactions was experienced.

Autologous platelet gel has now been used in a multiplicity of operative situations in multiple specialty areas. Of interest to plastic surgeons are the applications in bone healing, dural rent repair,25 radical neck dissection, seromas, orocutaneous fistulas, vascular grafts, skin grafting, flap survival, and chronic wound healing. Many of these uses are in papers currently in preparation by other investigators.

Discussion

The enhancement of wound healing necessitates a clean environment with the presence of adequate chemical and cellular mediators to ensure the optimum sequence of events leading to an uncomplicated wound. Adequate hemostasis and closure of dead spaces help to ensure that environment.

Chemical mediators like fibrin glue, whether homologous or autologous, of such an environment can be helpful. An autologous derivation of such mediators is preferential to avoid the complications of procurement and cross reactivity. If the cost of an autologous source of wound nutrients/enhancers is less than the cost of other sources and is available in large quantities, its usage is further justified (Table 1).

The platelet is a readily accessed storehouse of mediators that can be collected and administered directly to the wound environment in high concentrations. Autologous platelet gel is readily accessible and useful in controlling some of the effects of surgical intervention and adds another modality to the armamentarium of surgeons seeking to reduce such effects.

The process will be aided in the future by advances in the technology to reduce the size of machines, reduce the cost of the necessary disposables, and to reduce the number of steps required to administer the product. New machines may incorporate the procurement of an autologous thrombin simultaneous to the procurement of the platelet rich plasma. Appliances to facilitate mixture and application are also being developed.

Disclosure of Interest: Medtronic Corporation donated use of a Sequestra 5000 centrifugation machine for the purposes of this study. All the disposables were paid for by the author. The author presented his findings at several advanced autotransfusion symposia sponsored by Medtronic and was given an honorarium for doing so.

References


