Histologic Effects of Autologous Platelet Gel in Skin Flap Healing

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Objective: To supply animal model data in the area of autologous platelet gel (APG) in the application of plastic and reconstructive surgical procedures in which clinical observation purports augmented hemostasis and optimal wound healing.

Methods: Paired skin flaps were dissected on each side of the backs of 12 New Zealand white rabbits. Prior to suture closure, APG was placed in the wound bed on 1 side (hereinafter, APG wounds), and the wound bed on the other side served as a control. Punch biopsy specimens from each wound were obtained at 1-, 2-, and 3-week intervals and examined by a blinded pathologist.

Results: Histologic analysis revealed increased overall inflammation in the APG wounds, which was significant at week 3 ($P=.05$). The APG wounds also demonstrated significant increases in subdermal eosinophilia across the study period ($P=.01$). Neutrophilic and monocytic inflammation both increased over the time interval studied, but neither variable exhibited differences between control and APG wounds. No significant differences were observed in the degree of fibrosis or collagen deposition between the types of wounds.

Conclusions: The APG wounds demonstrated increased inflammation and tissue eosinophilia compared with the control wounds. These findings underscore the observation that concentrated platelets, although autologous, have a definite effect on postsurgical inflammation in a rabbit model.

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Recent trends in aesthetic and reconstructive surgery have included the application of autologous platelet-rich plasma, usually prepared in gel form, to the wound bed following procedures such as laser skin resurfacing, face-lift, and bony reconstruction. Purported advantages include more rapid reepithelialization; reduction in postoperative swelling, bruising, and pain; and reduced need for drains or compressive dressings. The biologic effect of platelets in thrombosis has been well described. Platelets aggregate to form a plug in the setting of exposed collagen, von Willebrand factor, and fibrin. Fibrin is generated by the action of thrombin during the intrinsic and extrinsic clotting cascades, both of which are calcium-dependent pathways. Thus, the role of an autologous platelet gel (APG) containing concentrated platelets plus thrombin and calcium in augmenting postsurgical hemostasis is clear. Clinically, this translates to the rapid sealing (within 3 minutes of application) of wound bed capillaries that has been observed. Platelets are also noted to contain autocrine and paracrine functional mediators that may influence wound healing. These include multiple growth factors, angiogenic factors, and mediators of inflammation. The concentration of platelets in platelet-rich plasma is variable depending on the method of preparation. Eppler et al demonstrated a platelet concentration that was 8 times that of whole blood. They also demonstrated that growth factor levels increased with platelet count. The biologic effect of supraphysiologic levels of platelets and their mediators has been incompletely elucidated to date, and unfortunately, techniques of APG preparation and application have yet to be standardized. Furthermore, most data regarding APG application in facial soft tissue surgery are based mainly on observations of patients. The goal of the present study was to examine histologic differences in wounds closed with vs without the application of APG in a rabbit skin flap model.

METHODS
see Health Science Center, Memphis. Twelve 3-kg New Zealand white rabbits were used. Following administration of conscious sedation, 8-mL blood samples were drawn from each rabbit through the saphenous vein. The blood was mixed with 1 mL of acid-citrate-dextrose anticoagulant (a weak anticoagulant routinely used to prevent in vitro clotting of banked blood products). The total volume of 9 mL was centrifuged for 15 minutes at 3500 rpm, resulting in a 3-phase separation with red blood cells sedimented at the bottom of the tube and platelet-poor plasma at the top. The middle layer, or "buffy coat layer," contained approximately 1.5 mL of platelet-rich plasma, which was aspirated with a pipette. The concentrated platelets were activated by combining them with 0.5 mL of a 10% calcium chloride solution containing 1000 U of bovine thrombin per milliliter. The final yield resulted in 2 mL of APG, all of which was used for application under the experimental skin wounds (hereinafter, APG wounds).

Each rabbit's back was trimmed with a handheld clipper and cleansed with chlorhexidine solution. On each side of the midline on the rabbit's back, 2 squares were marked, each measuring 4 x 4 cm. A local anesthetic (1 mL of 1% lidocaine with 1:100,000 epinephrine) was injected into a subdermal plane for each wound. Each wound margin was incised with a No. 15 blade, and 2 medially based wounds (1 on each side of the midline) were raised in the subdermal plane via sharp dissection with tenotomy scissors. No cautery was used. Each wound bed to the right of the midline was layered with 2 mL of APG, and all the wounds were closed with interrupted 4-0 Prolene sutures (Ethicon Inc, Somerville, New Jersey) (Figure 1). The wound to the left of the midline was closed without gel application (hereinafter, control wounds).

Punch biopsy specimens 4 mm in diameter were obtained from the rabbits after the administration of conscious sedation and local anesthesia (0.3 mL of 1% lidocaine with 1:100,000 epinephrine per punch) from each wound site at 1-, 2-, and 3-week intervals. Each biopsy site was separated from the incision lines and the adjacent biopsy sites by approximately 1 cm. Biopsy sites were matched on the contralateral side. The punch biopsy specimens were examined histopathologically to determine the degree of the inflammatory response, graded as 0 to 3 by a pathologist who was blinded to the nature of the specimen. For each specimen, 50 high-power fields (original magnification x400) were examined for the presence of an inflammatory infiltrate, defined as more than 20 inflammatory cells within the high-power field. The inflammatory score was graded semiquantitatively as follows: a score of 0 indicated that there were no inflammatory infiltrates; 1, infiltrate involving 10 or fewer high-power fields; 2, infiltrate involving more than 10 high-power fields; and 3, diffuse infiltrate throughout the dermis obvious even at low power.

Data were also collected regarding the character of the inflammatory infiltrate (neutrophils, eosinophils, and monocytes) and the degree of subepithelial fibrosis. Histologic data were analyzed statistically using the Wilcoxon, Kruskal-Wallis, and Mantel-Haenszel $\chi^2$ tests; $P \leq .05$ was considered significant.

RESULTS

Results of histologic analysis of inflammation are summarized in Figure 2 and the Table. There was greater overall inflammation in the APG vs the control wounds, which was statistically significant at week 3 ($P = .05$). Both types of wounds exhibited less overall inflammation at week 1, and this was most notable in the control wounds (Figure 3). Overall inflammation increased significantly from week 1 to week 3 in both APG and control wounds ($P = .03$).

When we examined the differences between types of wounds regarding the nature of the inflammatory infiltrate, the APG wounds exhibited a greater prevalence of tissue eosinophilia across the entire study interval (Figure 4; $P = .01$). However, the prevalence of eosinophilia did not significantly change from week to week during the 3-week period analyzed. The prevalence of eosinophilic infiltration did seem to peak at week 2, but this observation did not achieve significance.

Neutrophilic and monocytic infiltrates did not exhibit any significant differences between APG and control wounds. However, neutrophilic ($P = .04$) and monocytic ($P = .01$) inflammation did significantly increase after week 1 across both types of wounds. No differences were observed regarding subepithelial fibrosis between types of wounds or between time points during the study period analyzed. Gross observation demonstrated no difference between sides regarding wound edema at any time point, except for 1 animal that developed a seroma under the APG wound. Control wounds exhibited more...
Platelets are a key component in modulating the body’s response to injury. In addition to playing a hemostatic role, platelets actively influence tissue repair during the inflammatory and proliferative phases of wound healing and during remodeling. These effects are exercised through mediators such as histamine, arachidonic acid metabolites, serotonin, and epinephrine. These molecules have been implicated in a variety of physiologic processes, including inflammatory cell chemotaxis, regulation of vascular tone, and neurotransmission. Platelets also liberate a variety of growth and angiogenic factors, including platelet-derived growth factor, fibroblast growth factor, epidermal growth factor, transformation growth factors α and β, insulin-like growth factor, vascular endothelial growth factor, and platelet-derived angiogenesis factor.

Increasing the concentration of these mediators and factors during the early phases of wound healing is thought to augment optimal healing after various forms of injury. In addition to having an application in facial soft tissue surgery, autologous platelets have been applied in multiple clinical scenarios, including osseous reconstructive surgery, orthopedic surgery, oral-maxillofacial surgery, and periodontal medicine. Adhesion of platelets has been implicated in the consolidation of cancellous bone and the repair of comminuted fracture segments. In addition, autologous platelets are thought to promote ossification in sites of tooth extraction, augment ossification around titanium implants, promote

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**COMMENT**

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**Table. Prevalence of Inflammatory Cells by Time Point***

<table>
<thead>
<tr>
<th>Week</th>
<th>Control Wound</th>
<th>APG Wound</th>
<th>Control Wound</th>
<th>APG Wound</th>
<th>Control Wound</th>
<th>APG Wound</th>
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<tr>
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<td>41.7</td>
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<td>41.7</td>
<td>50.0</td>
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<tr>
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<td>33.3</td>
<td>41.7</td>
<td>41.7</td>
<td>83.3</td>
<td>91.7</td>
</tr>
</tbody>
</table>

Abbreviation: APG, autologous platelet gel.

*Data are presented as the percentage of specimens demonstrating each type of inflammatory infiltrate. Wounds treated with APG (APG wounds) exhibited more eosinophilia (Mantel-Haenszel χ²) across the entire study, but the degree of eosinophilia was stable from week to week during the study. Neutrophilia and monocytic infiltrate were lowest at week 1 (Mantel-Haenszel χ²) and increased thereafter, but no differences were observed between APG wounds and control wounds.

*P* = .001.

*P* = .04.

*P* = .01.

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**Figure 3.** Punch biopsy specimen taken at week 1 demonstrating grade 0 inflammation (hematoxylin-eosin, original magnification ×100).

**Figure 4.** Punch biopsy specimen taken at week 2. A, Grade 3 inflammation (hematoxylin-eosin, original magnification ×100) throughout the dermis. B, A mixed infiltrate that includes eosinophils (hematoxylin-eosin, original magnification ×600).
the healing of ulcers and burns, accelerate muscle or tendon repair, and improve skin graft survival.\textsuperscript{1,3,9} The use of autologous platelet preparations as drug delivery systems has also been suggested.\textsuperscript{10}

The model used herein is most applicable to the use of APG after face-lifting. Surgeons who have applied APG in a large number of face-lift procedures have observed significant advantages, such as augmented hemostasis as well as diminished postoperative edema, bruising, and pain.\textsuperscript{9} The observations of the current study underscore the finding that although concentrated platelets may be derived from an autologous source, the use of APG is certainly not inert with respect to postsurgical inflammatory changes. Specifically, the present data suggest that APG is associated with increased overall inflammation and tissue eosinophilia during the first 3 weeks after surgery. One hypothesis for the recruitment of eosinophils into the wound involves chemotaxis from platelet-derived histamine. Activated platelets are also known to release products of arachidonic acid metabolism, including leukotrienes, which are generated via the lipoxygenase pathway. These molecules also have a chemotactic effect for eosinophils. The increase in neutrophilic and monocytic inflammation during the study period seems consistent with postsurgical inflammation and healing because no differences were observed between APG and control wounds. Fibrosis did not differ between control and APG wounds or among time points, suggesting that the 3-week study interval may have been insufficient to detect changes in this variable.

Although wound complications are not a primary outcome measure of this study, we did observe a greater tendency for bleeding at the time of biopsy in the control wounds. In contrast, the only wound complication (seroma) occurred in an APG wound. Thus, surgeons must be cautioned against thinking of APG as a panacea. However, when considering clinical observations as well as cost factors (APG costs only approximately $300 per case), the use of APG may be appropriately characterized as a “value-added service” and may improve surgical outcomes for patients with potential healing problems, such as smokers or those with diabetes mellitus.\textsuperscript{1}

In conclusion, the results of the present investigation underscore the finding that APG does influence the biologic effects of soft tissue healing beyond the role of the platelet in hemostasis. The effect of the observed tissue eosinophilia in the ultimate healing process remains an area of active study. The exact microenvironment in wounds treated with APG also awaits characterization with regard to the influence of various platelet-derived mediators and growth factors. In addition, further study is necessary to determine the effect of supranormal concentrations of platelets (and their mediators) on long-term healing and wound remodeling. The present study lays the groundwork for future investigation into these issues.

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REFERENCES


