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# Clinical impact and biomaterial evaluation of autologous platelet gel in cardiac surgery

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**We compared the clinical efficacy of autologous platelet gel (APG) and gelatine (CONT), including biomaterial evaluation. In a prospective, randomized, controlled trial, 64 patients undergoing complex coronary artery bypass graft (CABG) surgery and/or aortic surgery, in whom the surgeon was able to identify a bleeding site for which conventional means to stop bleeding were impractical or proved unsuccessful, were enrolled. Aortic punch biopsy from each patient was harvested in explant cell (EC) culture media. Hemostasis success for the "oozing" category was 89% in APG and 60% in CONT ($p< 0.05$). For the "heavy bleeding" category, the success rates were 92% in APG and 45% in CONT ($p<0.01$). Contact of gelatine inhibited EC proliferation and APG increased cell cycling and EC quantity. Phagocytic capacity (PC) was significantly higher in the APG group ($p<0.001$). APG was significantly better than CONT with respect to hemostatic success rate, effects on wound healing and increased resistance to infection (PC).** *Perfusion* (2008) **23,** 179–186.

**Key words:** extracorporeal circulation; platelet activation; platelet gel; platelet rich plasma; surgical hemostasis

## Introduction

Peri-operative bleeding leads to increased operating room time, blood product transfusions, pulmonary hypertension, and, potentially, to mortality. The coagulopathy induced by cardiopulmonary bypass and the multiple high pressure anastomoses created during cardiac surgery often result in bleeding, which is more effectively controlled with autologous platelet gel (APG) and topical hemostatic agents than with sutures or electrocoagulation. Several topical hemostatic agents are available which either provide clotting components (e.g. fibrin sealants and thrombin glues) or a surface for clotting to be stimulated (e.g. microfibrillar collagen, gelatin sponge, oxidized cellulose). The main advantage of a topical sealant is its tensile strength which helps in cessation of hemorrhage. Platelet-derived glues are somehow complementary, since they have a poor tensile strength, but promote tissue regeneration due to their content of growth factors. Also, sealants have varying degrees of efficacy, some carrying a potential risk of infection or allergic reaction, adhesion formation, prolonged preparation time and considerable expense.

APG was developed in the early 1990s as a by-product of platelet-rich plasma (sequestration during cardiac surgery). Although APG has been approved for post-operative healing, there have been a few studies that evaluate the hemostatic effects and biomaterial aspects of APG on open-heart surgery.[1,2]

We conducted a randomized, comparative, clinical trial to document the efficacy and safety of two standard clinical regimens of APG and gelatin (CONT). We have also collected an aortic punch biopsy from each patient enrolled in the study, harvested endothelial cell (EC) culture and evaluated agents on biomaterial aspects with respect to cytotoxicity and foreign body reaction, effects on wound healing, inflammatory reaction-resistance to infection and resorption-clearance time from the tissue.

## Patients and methods

### *Clinical evaluation*

*Patients*

During the period from May 2006 until November 2007, in a prospective, randomized, controlled trial in three centers, 195 patients undergoing complex coronary artery bypass grafting (CABG) and/or aortic surgery were screened and, of these, 64 patients were enrolled after standard surgical means to control bleeding had failed. The clinical trial involving

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human subjects was conducted in accordance with the Helsinki Declaration, as amended by the 41st World Medical Assembly in Hong Kong in 1989, and US Code of Federal Regulations. The Institutional Review Board approved the study before patient enrollment. Before the operation, each patient signed an Institutional Review Board–approved consent form to participate in the study. Patients were enrolled in the study intra-operatively if they did not have an active infection at the operative site, if there was bleeding that required the use of a topical hemostat, and if the use of a topical hemostatic agent was not contraindicated. A baseline blood sample was obtained within 24 hours before the operation to measure complete blood count, blood cell differentials, activated partial thromboplastin time, prothrombin time, and metabolic, hepatic, and renal panels. Peri-operative outcome was also documented. Patients were excluded if they had a known sensitivity to any components of bovine thrombin preparations or to any material of bovine origin, known coagulopathy, endocarditis and inability to obtain informed consent. Enrollment was limited to those patients in whom the surgeon was able to identify a bleeding site for which conventional means to stop bleeding (including direct pressure, suture, and electrocoagulation) were impractical or proved unsuccessful. After identification of a bleeding lesion requiring a topical hemostatic agent, the patients were randomized by closed envelope allocation to:

Group 1: Autologous platelet gel (APG) (N=31) (Harvest Smart Prep 2, Harvest Technologies, Plymouth MA, USA)
Group 2: Gelatine (CONT) (N=33): (Microval-G.R.F., Saint-Just-Malmont, France)

Gelatine consists of an aqueous mixture of gelatine and resorcine. The polymerization occurs by the addition of a hardener made of formaldehyde and glutaraldehyde. CPB was instituted on a roller pump (System 1, TCM Heat exchanger, Terumo) via a conventional uncoated open system (Capiox, Terumo, USA).

Anticoagulation or antiplatelet therapy was discontinued 7 days before the operation in all patients.

*Operative technique*

Anesthesia was induced by fentanyl (35 µg /kg) and muscle relaxation was established with pancronium (0.1 mg/kg). The patients were intubated endotracheally and ventilated with 100% oxygen. A Swan-Ganz catheter was placed via the internal jugular vein. Patients were administered full dose 300 IU/kg heparin (Liquemine, Roche, Istanbul, Turkey) with a target activated clotting time (ACT) over 480 s. ACT was measured by the Hemochron 801 (International Technodyne Corporation, Edison, NJ). The ascending aorta was cannulated for arterial inflow, and the right atrium for venous return. Patients were cooled down to 28°C. Blood flow was maintained at 2.0-2.4 L/min/$m^2$ during CPB. After cross-clamping of the aorta, the heart was arrested with 4:1 blood cardioplegia, 10-15 mL/kg, and maintained at 20-minute intervals. Warm blood cardioplegia was administered before the aortic cross-clamp was released. Bilateral internal mammary artery or radial artery grafts were used for coronary artery lesions, if not utilized in the first operation, and saphenous vein grafts were used as an alternative in other instances. Rewarming was initiated during last grafting. At 36.5°C, CPB was discontinued and heparin was reversed by protamine sulphate (Protamine, Roche), 3.1 mg/kg. The adequacy of protamine reversal was checked by ACT and corrected when necessary. Prime for CPB was identical for all patient groups; 60 ml of mannitol 20% + 1000 ml of hydroxyethyl starch (Voluven 130.4, Fresenius, Turkey) and 300 ml of crystalloid (Plasmalyte A, Eczacibasi, Turkey) with a total of 1360 cc. Cell washing was not performed. No anticoagulants were used. The bleeding severity at each site was characterized as "oozing" (slow leak out through anastomotic sites) or "heavy bleeding" (flowing or spurting). After application of the hemostatic product, the occurrence of continued bleeding was recorded at 1, 3, 6, and 10 minutes. Re-application of the assigned product was allowed, and the primary endpoint was cessation of bleeding of the first treated site within 10 minutes. Secondary endpoints included the outcome of additional treated bleeding sites. Safety and adverse effects were assessed at 12 to 36 hours and 6 to 8 weeks post-operatively. Adverse events were categorized as mild, moderate or severe and were assessed by the surgeons as unrelated, possibly related, or probably related to the products used. Hematologic and blood chemistry assays were performed as before at 12 to 36 hours and 6 to 8 weeks post-operatively.

*APG preparation*

An intravenous infusion line was inserted into the medial cubital vein using a 17-g needle. For each donor, whole blood donation was distributed as 2 aliquots. First, a 60-ml syringe was pre-filled with 6ml of citrate dextrose A (ACD-A) anticoagulant solution and 53ml of whole blood was slowly drawn via an intravenous catheter. The syringe was inverted five times to ensure proper mixing with the anticoagulant before platelet-rich plasma (PRP) was

prepared using the Harvest Smart Prep 2 system. Two milliliters of ACD-A were drawn into a 3-mL syringe and transferred into the plasma chamber of a process disposable (PD). The total volume collected was also transferred into a blood chamber of PD. The system was loaded with PD and started.

Following PRP preparation, the solution was treated with autologous platelet concentrate activator (typical ratio of thrombin to 10% calcium chloride is 1000 units/mL). A dual syringe technique was used with the platelet concentrate drawn into a 10-mL syringe while the bovine thrombin/$CaCl_2$ solution was drawn into a 1-mL syringe. The APG was created by mixing the platelet concentrate with the reconstituted thrombin in a ratio of 10 parts concentrate to 1 part thrombin/$CaCl_2$

The quality and standardization of APG were verified by platelet count, levels of beta-thromboglobulin and platelet-derived growth factor (PDGF-AB). APG was prepared before surgery for all patients and used for wound care in cases where there was not any significant bleeding.

During the entire procedure, platelet activation was determined by measuring plasma levels of beta-thromboglobulin using Asserachrome beta-thromboglobulin kits (Stago, Asnieres, France). Platelet growth factor concentration in whole blood, PRP, concentrated PRP, and APG supernatants were determined by commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis MN, USA) that had been validated for measuring PDGF-AB (Quantikine PDGF-AB) according to the manufacturer's instructions.

*Peri-operative follow-up*

For each patient, the following factors were evaluated before discharge and documented: hemodynamic parameters, perfusion and cross-clamp duration, intubation period, post-operative hemorrhage, the use of blood (units/patient) and blood products (fresh frozen plasma or plasma), incidence of arrhythmia, use of inotropic support, complications and infection, the duration of ICU stay and hospital stay, peri-operative mortality, New York Heart Association Classification (NYHA), and Doppler echocardiography. Comparison between groups was performed retrospectively.

### *Biomaterial evaluation cell culture*

Aortic punch biopsy from each patient was harvested in primary explant cell culture media. Cells were treated by Dulbecco's Modified Eagle's Medium (Sigma Chemical Co., St.Louis, MO) containing 3% penicillin and streptomycin. Incubation lasted 10 days. Cell migration was followed under microscopy. Proliferated cells were passaged when 80% were confluent in order to increase cell quantity. For verification of EC, samples were centrifuged by cytospin (ThermoShandon, Inc., Pittsburgh, PA). Papanicolaou staining demonstrated cell quantity sufficiency. The avidine biotine peroxidase method was employed for immune-histochemistry (Shandon Sequenza, Thermo Fisher Scientific, Inc., Waltham, MA). Cells were treated by three different monoclonal antibodies: CD31/PECAM-1 (Lab Vision, MS-1873-R7, Thermo Fisher Scientific, Inc.), CD34 (Lab Vision, MS363-R7) and FVIIIRA/Von Willebrand Factor Ab-2 (Clone F8/86, Lab Vision, MS1237-R7). Results were expressed as cells/ml according to diffuse cytoplasmic staining (Figure 1). After obtaining pure endothelial cultures, cells were subjected to topical agents for consecutive testing.

*Cytotoxicity and effects on wound healing*

Cytotoxic effects of gelatine and APG were studied by MTS proliferation bioassay, documenting cell viability after EC were co-cultured with agents for 48 h. Cultured cell quantity was measured after 48 h until the end of one week after being in contact with topical agents. The increase or decrease in cell number was documented.

*Resistance to infection*

Phagocytic capacity (PC) of cultivated EC was compared before and after contact with topical agents. Collected cells were investigated using monodisperse poly glycidil methacrylate (Poly GMA) microspheres. Baseline blood sample of each agent was used as control. Microspheres were mixed with cultured cells with an amount of 200,000 cells/$mm^3$ incubated at 37°C. Cellular phagocytosis rate of cells and the total internalized particle amount was reported. Samples were examined with an optical microscope to determine the number of cells

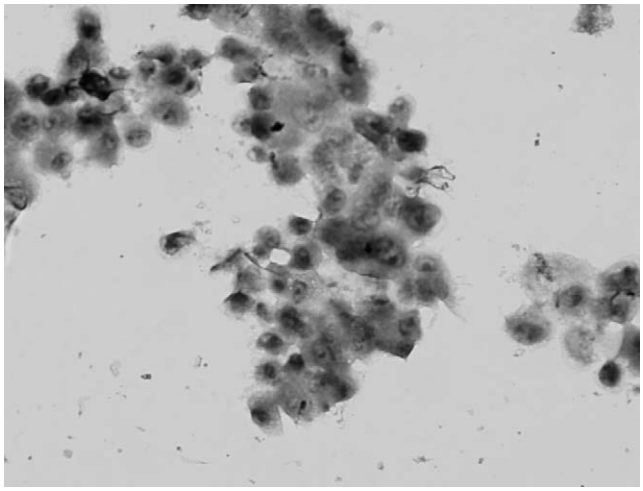

**Figure 1** F VIII staining of EC.

phagocyting and the number of microspheres ingested per cell.

$$PC = \frac{Number\ of\ cells\ with\ microsphere\ inside}{Total\ number\ of\ cells}$$

*Resorption*

The cell cultures were observed by iodine testing until two weeks after being in contact with topical agents, checking for the clearance of agents from the cell clusters.

### *Statistical analysis*

Data are expressed as the mean ± the standard error of the mean. The Mann Whitney-U test was used to compare demographic and non-parametric data. Two-way analysis of variance, with factor group and repeated factor time, was used to analyze differences over time in each group and for differences between groups. The post hoc test (Bonferroni correction) was applied whenever a significant difference was detected. Effectiveness results were statistically analyzed in an "intent-to-treat" fashion using the Cochran-Mantel-Haenszel test stratified by site. A *p*-value less than 0.05 was considered significant. Data were analyzed using an SPSS program.

## Results

### *Clinical*

Demographic and pre-operative surgical data are presented in Table 1. The patients in the APG group had a total of 57 and the CONT group 61 bleeding sites that were treated.

Platelet concentration, platelet activation and growth factor concentration of APG were verified by platelet count, beta-thromboglobulin and PDGF-AB levels (Table 2).

**Table 1** Pre-operative evaluation of patient cohorts

| Demographics | Group 1 (APG) | Group 2 (CONT) |
|---|---|---|
| Age (y) | 68 ± 2.6 | 68.9 ± 2.38 |
| Male sex | 14 | 16 |
| BSA ($m^2$) | 1.74 ± 0.05 | 1.76 ± 0.02 |
| NYHA class | 3.1 ± 0.13 | 3.26 ± 0.11 |
| Ejection fraction | 0.45 ± 0.01 | 0.43 ± 0.01 |
| LVEDP (mm Hg) | 15.2 ± 0.8 | 14.6 ± 0.95 |
| Type of surgery | | |
| CABG + LV Aneurysm Repair | 4 | 3 |
| Redo CABG | 12 | 14 |
| AVR + CABG | 10 | 9 |
| Redo AVR | 4 | 4 |
| Ao Aneurysm Repair | 0 | 2 |
| Ao Dissection | 1 | 1 |

BSA, Body surface area; NYHA, New York Heart Association Class; LVEDP, Left ventricular end-diastolic pressure; CABG, Coronary artery bypass grafting; AVR, Aortic valve replacement.

**Table 2** Quality and standardization of APG

| | PLT Count ($\mu L^{-1}$) | β-TG ($IU.mL^{-1}$) | PDGF-ab ($pg.mL^{-1}$) |
|---|---|---|---|
| Baseline | 197.250 ± 75 | 1564 ± 560 | 4250 ± 735 |
| APG | 1.396.000 ± 245 | 13450 ± 2550 | 32500 ± 1250 |
| Increase above baseline | ×7 | ×8.5 | ×7.6 |

APG, Autologous platelet gel; β-TG, Beta thromboglobulin; PDGF-ab, Platelet-derived growth factor-ab.

Comparison of blood assays between baseline, 12 to 36 hours, and 6 to 8 weeks post-operatively demonstrated no statistically significant differences that were judged to be clinically significant. No allergic reactions occurred in any groups. Hemostasis success for the "oozing" category in 10 min. was significantly better than CONT at first at all bleeding sites ($p < 0.05$) (Figure 2A). For the "heavy bleeding" category, the success rates were significantly different in APG vs CONT ($p<0.01$) (Figure 2B). Peri-operative data are summarized in Table 3.

### *Biomaterial*

The MTS bioassay showed that contact of gelatine did not contribute to EC proliferation. Cells died within five days. The suppressed cell cycling was significantly less in CONT than APG ($p<0.0001$).

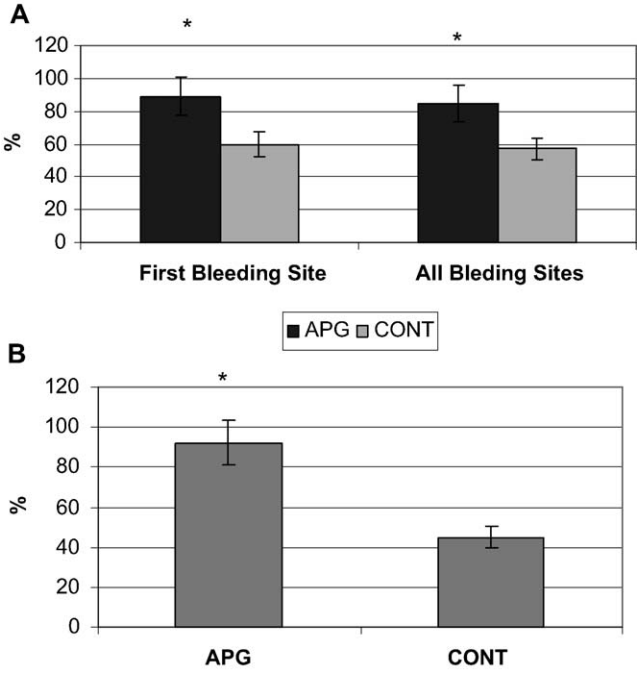


**Figure 2** (A) % Hemostatic success of oozing category in first and all bleeding sites. $*P < 0.05$ vs. control. (B) % Hemostatic success of heavy bleeding category $*P < 0.01$ vs. control.

**Table 3** Peri-operative evaluation of patients

| | APG | CONT | *P* |
|---|---|---|---|
| Duration of CPB (min) | 105 ± 4.2 | 109 ± 3.7 | NS |
| Duration of x-clamp (min) | 80.3 ± 3 | 85.2 ± 3.2 | NS |
| t-intub (h) | 15 ± 0.6 | 17 ± 1.5 | NS |
| Post-operative hemorrhage (mL) | 790 ± 23 | 857 ± 30 | NS |
| Arrhythmia (n) | AF:7 | AF:10 | NS |
| Blood transfusion (Unit/patient) | 2.0 ± 0.21 | 2.78 ± 0.26 | $P = 0.04$ |
| Blood products (Unit/patient) | 2.4 ± 0.35 | 2.9 ± 0.3 | NS |
| Inotropic support (n) | 12 | 15 | NS |
| Post-op NYHA Class | 3.1 ± 0.1 | 2.9 ± 0.1 | NS |
| IABP (n) | 3 | 4 | NS |
| ICU stay (day) | 3.9 ± 0.24 | 4.6 ± 0.27 | NS |
| Post-operative EF (%) | 44 ± 1.5 | 39.4 ± 1.4 | NS |
| Hospital stay (day) | 10.7 ± 0.5 | 12 ± 1.1 | NS |
| Mortality (N) | 4 | 5 | NS |

CPB, Cardiopulmonary bypass; X-clamp, Aortic cross-clamp; t-intub, respiratory support time; ICU, Intensive care unit; NYHA, New York Heart Association Class; EF, Ejection fraction.

The contact of APG increased EC proliferation incrementally within five days. The cell cycling was significantly higher than baseline (p<0.001) (Figure 3 A). PC was significantly higher in APG vs CONT (p<0.001). These data demonstrated the increased resistance to infection (Figure 3B). Resorption of agents from cultured media was significantly shorter for APG than CONT (p<0.001) (Figure 3C).

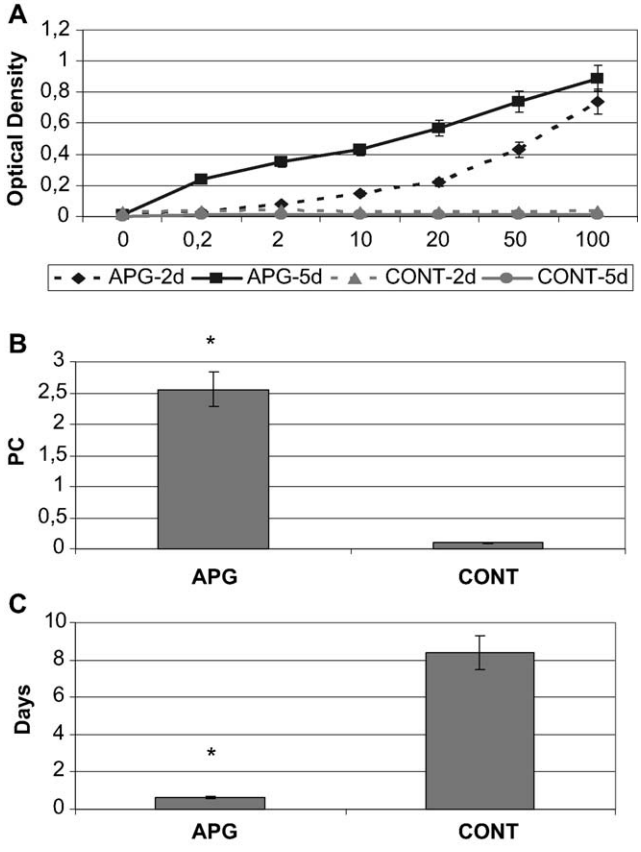


**Figure 3** (A) MTS bioassay of EC (2-day and 5-day) co-cultured with APG and CONT. *$P < 0.01$ vs. control. (B) Phagocytic capacity of groups *$P < 0.001$. (C) Resorption of APG and gelatine from cultured media *$P < 0.001$.

## Discussion

Platelets play a central role in hemostasis and wound healing. The latter is mediated by the release of secretor proteins on platelet activation, which directly or indirectly influences virtually all aspects of the wound-healing cascade. Studies in basic science have shown a dose-response relationship between the platelet concentration and levels of secretory proteins, as well as between platelet concentration and certain proliferative events of significance to the healing wound.[1] Technologies to provide autologous platelet-rich plasma to the repair site are now being used in a wide variety of clinical applications, with the majority of such studies suggesting a role in the surgeon's armamentarium. Little standardization in the field exists, which has made it difficult to fully evaluate the literature on the subject and unequivocally establish applications for which the technology truly has merit.[3]

APG made directly from sequestered buffy coat has been marginally promoted for a number of years by the leading autotransfusion companies. First proposed as an autologous fibrin sealant able to be fabricated in the operating room, it gave the surgeon an alternative to allogeneic cryoprecipitate from the blood bank. Like other fibrin glues, it has been most extensively used in soft tissue applications (including sinus lifts, dural tears, and burns).

The critical differences in composition between APG and conventional sealants are the presence of a high concentration of platelets and a native concentration of fibrinogen in APG. We believe that APG offers significant advantages over previously

described fibrinogen-based wound sealants. The inclusion of a buffy coat of platelet- and leukocyte-enriched plasma appears to have several beneficial effects. With platelet counts of millions per ml, as demonstrated in our study, the various cytokines and mediators found in the platelet's alpha and dense granules can promote angiogenesis and collagen synthesis, thereby, enhancing soft-tissue wound healing. These mediators include platelet-derived growth factor (PDGF), platelet-derived epidermal growth factor, fibroblast growth factor, transforming growth factor beta, and platelet-derived angiogenesis factor. PDGF is known to be chemotactic for monocytes, macrophages, and fibroblasts; it is also an activator of collagenase within the later stages of wound healing, allowing for remodeling of collagen to promote wound strength. These function in attracting additional platelets to the developing clot, thus, enhancing the hemostatic response. Our clinical results also verified the hemostatic success of APG versus conventional topical sealant.[4]

The native concentration of fibrinogen in APG increases its working time, and once activated by calcified thrombin it imparts a gelatinous adhesive consistency to the gel, allowing for ease of injection into the surgical site. As we have prepared the APG before the procedure, it was ready to use upon request. Once the PRP is prepared, it is stable, in the anticoagulated state, for 8 hours or longer, permitting the blood to be drawn before surgery and used, as needed, during lengthy procedures.[5] Available APG products in the market can be prepared by both autologus thrombin and bovine thrombin. Not all countries have access to bovine thrombin and must use autologous thrombin. We believe bovine thrombin is easier for application, decreases the time taken for the APG to set and has a potential knock-on effect on hemostatic success rate. We did not document any side or adverse effect by bovine thrombin.

Application was as easy as conventional sealants. The processing time required for separation of the autologous blood into the components by this technique is less than 30 minutes and the option exists for the anesthesiologist to return the remaining erythrocytes and platelet-poor fractions to the patient before the induction of anesthesia.

If an autologous alternative to conventional sealants is desired, the collection of blood in the immediate pre-operative period avoids a time-consuming visit to the blood bank for the patient. Furthermore, use of APG eliminates the risk of clerical errors when the blood is pre-donated and moved to a site distant from the patient. More patients are eligible for this procedure which is performed in the peri-operative period, because the strict criteria for blood bank donation do not have to be met.[6]

Consistently, in vitro studies demonstrate the ability of platelet-derived products to enhance proliferation of cultured cells. The results of in vivo studies are often conflicting, highlighting the need for an in-depth analysis of the many variables of study designs and technical protocols of the pertinent in vitro and in vivo studies.[7]

PRP accelerated proliferation of fibroblasts, osteoblasts, and periodontal ligament cells while reducing the proliferation of epithelial cells. Two-dimensional and three-dimensional in vitro studies should increase our understanding of the mechanisms of action of platelet derivatives. Most in vitro studies using EC have been conducted on rigid two-dimensional tissue culture plastic substrata. Under these conditions, EC express many of the phenotypic characteristics of their in vivo counterparts, such as expression of von Willebrand factor, platelet/EC adhesion molecule-1 and VE-cadherin response to mitogens, such as vascular endothelial growth factor and basic fibroblast growth factor; and binding and internalization of acetylated low-density lipoprotein. A recent review outlined points for debate: in vitro observations may oversimplify the in vivo situation; platelet concentration and methods of preparing PRP are not (yet) standardized, which may influence success rates; more studies are needed to differentiate the roles of platelets and plasma proteins; and the cost-effectiveness of the procedures may influence the use of APG. Platelet gels release growth factors slowly over 7-10 days, prolonging their chemotactic and mitogenic activities.[8]

We have formed EC cultures of the patients constructed on a polymer layer, targeting a three-dimensional design in a tissue engineering perspective. These products also may be used to prime artificial scaffolds with bioactive molecules to prepare new more and more effective bio-constructs.

We have studied specifically EC. The endothelium, which lines the vascular system, is endowed with a variety of capacities and functions. Not only do EC provide a non-thrombogenic surface to the entirety of the vascular tree, but they also regulate vessel tone through the production of vasoactive mediators, such as nitric oxide, and permit selective adhesion for binding and extravasation of inflammatory cells. In addition, EC from different parts of the vasculature display properties that provide tissue specializations, such as a high degree of fluid filtration in the kidney or maintenance of barrier function in the brain and retina. The ability to culture both large vessel and microvascular EC has dramatically increased our understanding of the endothelium.

Investigation of EC in this system will allow a better approximation of the endothelium that lines the adult vascular system. As endothelium from large vessels has both phenotypically and functionally different characteristics from microvascular endothelium, it is important to have an in vitro model that represents large vessel endothelium in addition to the numerous models of microvessel formation already established. Information from models of large vessel endothelium will be essential for assessing drug effects on EC and for tissue engineering efforts, such as the development of large vessel vascular grafts.[9]

Although currently speculative, the high concentration of leukocytes in the buffy coat should add an antimicrobial effect to APG as well as elaborate additional regulatory factors that enhance the wound-healing process. We have demonstrated the increase of PC in leukocytes within APG which contributed the resistance to infection.[10]

The advantages of APG over previously described biologic sealants include safety and convenience for the patient as well as improved support for tissue healing. Cytotoxicity tests were supportive for APG and cell proliferation capacity was far better than synthetic products. Resorption and clearance rate was shortest. There is a theoretical concern, but not definitive answer, to the possibility that in-situ delivered growth factors may induce pre-existing malignant cells to proliferate; we have not observed such proliferation within two weeks in the culture.[11]

It is difficult to design a clinical study model incorporated to three-dimensional biomaterial background for APG evaluation in cardiovascular surgery.

The majority of previous clinical studies showed a significantly improved effect on soft tissue healing and bone regeneration when APG was used. Strikingly, in most studies, data were obtained in oral and maxillofacial surgery, wound care, and cosmetic surgery, mainly because of the availability of histological specimens under these treatment conditions.[12] In our study, we have constructed a design of histological specimens obtained from the patients and followed outside the body.

Data from human and animal studies provide both direct and indirect evidence that APG plays a considerable role in tissue regenerative processes. Nevertheless, some uncertainty is present, and some clinicians remain skeptical of the clinical benefits of APG and are uncertain about the ideal biological setting (e.g., percentage of vital bone cells, volume of PRP) for the application of the APG. Poor clinical outcome might have derived from a series of circumstances: insufficient concentration of the APG products, loss of growth factors across the preparation of the concentrate, local conditions (e.g., infections, metabolic disturbance) that impaired or counteracted the proliferation and poor receptor availability in the target cells.[13] Some of these circumstances may be kept under the investigator's control whereas others cannot.

We have standardized the preparation process by following the quality of APG meticulously.

We have studied hemostatic efficacy of APG confirmed by a three-dimensional biomaterial back-up targeted to mechanisms of therapeutic implications. Clinical outcome summarized in Table 3 does not give a significant difference between groups. We believe patient population may be one of the reasons. The high standard of current topical hemostatic systems has made it increasingly difficult to test improvement in clinical studies involving relatively small patient groups. In most cases, the statistical power of such studies will not suffice to show a significant clinical benefit associated with different agents. Nevertheless, it is difficult to correlate the benefits of a novel product demonstrated in laboratory studies with significant clinical benefits in patients. One cannot ignore the moderate effect sizes obtained through data analyses; however, power needs to be increased to determine if a treatment effect with APG does affect patient healing and recovery post cardiopulmonary bypass surgery.

Future studies should include large samples and measures of product quality. If multiple teams report on sufficiently large samples, meaningful conclusions can be made concerning the use of APG. Our pilot work is a start to induce following clinical trials required to study the potential of the use of APG and to provide material for sound clinical decision-making in the near future.

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