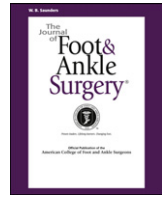




Contents lists available at ScienceDirect

# The Journal of Foot & Ankle Surgery

journal homepage: [www.jfas.org](http://www.jfas.org)

## Original Research

### The Use of Autologous Platelet Gel in Toenail Surgery: A Within-Patient Clinical Trial

Antonio Córdoba-Fernández, DP, PhD<sup>1</sup>, Rafael Rayo-Rosado, DP, PhD<sup>2</sup>, José María Juárez-Jiménez, DP, PhD<sup>2</sup>

<sup>1</sup>Professor of Podiatric Surgery, School of Health Sciences, Department of Podiatry, Seville University, Seville, Spain

<sup>2</sup>Assistant Professor, Department of Podiatry, Seville University, Seville, Spain

#### ARTICLE INFO

Level of Clinical Evidence: 1

Keywords:

inflammation

pain

platelet rich plasma

recovery

surgery

#### ABSTRACT

A number of studies have stressed the importance of platelets in acute and chronic wound healing, although their clinical utility remains controversial. To analyze the use of autologous platelet gel in the surgical treatment of ingrown toenails, a within-patient clinical trial was conducted. Thirty-five healthy volunteers (70 feet) underwent surgical treatment for bilateral ingrown hallux nails. Recovery time (days), postoperative pain (analog chromatic scale), and inflammation (digital circumference) at 48 hours postoperative were the outcomes of interest. Recovery time and postoperative pain were less in the experimental group, although the differences of means were not statistically significant. Based on these results, we suggest that local application of APG in surgical ingrown toenail wounds may produce a slight increase in acute inflammatory phase dermal wound healing, but it does not cause a statistically significant reduction in recovery times or postoperative pain.

© 2010 by the American College of Foot and Ankle Surgeons. All rights reserved.

Numerous clinical trials have been carried out to evaluate the effectiveness of platelet-derived growth factors in the treatment of chronic and acute wounds. Most of the studies stressed the important role of platelets in the cicatrization process, especially in hemostasis, acute inflammation, and proliferative phases (1). Platelets are believed to have concentrated levels of naturally occurring growth factors and other substances that have the potential to accelerate healing. The platelets contain substantial reservoirs of cytokines, notably platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF- $\beta$ ). These substances exert their effect on the cells, acting in all stages of cicatrization, especially hemostasis and early fibroplasia (2, 3). Currently autologous platelet gel (APG) is being used clinically in several surgical specialities in an attempt to improve tissue healing and innovative devices for processing autologous blood to concentrate platelet-rich plasma into APG have recently become available. The use of APG to reduce ecchymosis, bleeding, and edema has been evaluated in several trials and its efficacy has been demonstrated (4–7). Most studies agree that the APG accelerated vascular growth, increased fibroblast proliferation and collagen production, but did not appear to accelerate the epithelialization (8–12). In some of these studies, the researchers mention that the patients reported a significant decrease in wound pain following

application of the hemoderivative (13); some authors have suggested that platelet gels may reduce postoperative pain as a result of APG increasing all phases of the dermal wound-healing process, but few studies have analyzed this variable in advance using objective scales of pain (13–16). The capacity to modulate the inflammatory response, and recruit and activate leukocytes at the site of infection has been attributed to various platelet-derived growth factors. TGF- $\beta$ 1, the most-abundant platelet factor, plays a crucial role as modulator of the inflammatory response, whether infection is present or not (17–19). Some studies have demonstrated that APG applied to wounds increases inflammation and tissue eosinophilia compared with control wounds. These findings underscore the observation that concentrated platelets, although autologous, have a definite effect on postsurgical inflammation (20); however, the use of APG has received mixed reviews in clinical reports, and its clinical use remains controversial (14–16, 21, 22). APG is also associated with safety concerns; however, this gel is a platelet concentrate that contains no allogeneic or animal blood products (human or bovine thrombin), thereby avoiding anaphylactic reactions, autoimmune coagulopathies, or potential for disease transmission (23, 24).

Bilateral ingrown hallux nail surgery is one of the most common surgical procedures in foot surgery and patients are an ideal model in which to study the effectiveness of the product. Studying the effectiveness of APG in patients with bilateral ingrown hallux nail surgery allows for a paired research design, reduced between-subject variability, and a smaller sample size (25). To analyze the effectiveness of APG application in ingrown hallux nail surgery, a within-patient clinical trial was designed. This type of design has been previously used in similar trials with APG (14). Our primary objective was to

**Financial Disclosure:** None reported.

**Conflict of Interest:** None reported.

Address correspondence to: Antonio Córdoba Fernández, DP, PhD, <sup>1</sup>School of Health Sciences, Department of Podiatry, Seville University, C/ Avicena, s/n, Seville, Seville 41009, Spain.

E-mail address: [acordoba@us.es](mailto:acordoba@us.es) (A. Córdoba-Fernández).

assess whether the topical application of APG during bilateral ingrown hallux nail surgery would reduce the recovery time compared with standard care. Secondary objectives were to assess whether the application of APG would result in less postoperative pain and, on the other hand, analyze the influence of APG in postoperative inflammatory response.

### Patients and Methods

#### Study Population

The samples were obtained from patients with ingrown toenails in the hallux of both feet requiring treatment in the Podiatry Clinic of Seville University (Spain). The patients had previously received conservative treatment without definitive results. The inclusion criteria were patients with an ingrown toenail in stage I and IIa according to the Mozena classification system (26) (fold depth less than 3 mm). The patients with erythema, drainage, and acute pain were given conservative treatment before surgery (removal of the ingrown nail spicule, and local treatment with antiseptic) until the clinical signs and symptoms disappeared. Patients older than 60 years, smokers, and those with circulatory problems, infectious disease history, coagulation disorders, cicatrix formation, noncontrolled diabetes mellitus, and abnormal platelet count in peripheral blood (platelet count was measured in every patient before treatment) were excluded. Individuals who had been taking an anticoagulant or a steroid medication over the previous month or had an allergy to local anesthetic, nitrofurazone, cephalosporins, acetaminophen, or povidone iodine were also excluded. The study was approved by the Ethics and Experimental Committee of Seville University (opinion No. NN-310-196/1/06 from May 11, 2006) and was carried out between February 2007 and September 2009.

#### Outcome Measures

To assess the effectiveness of APG, we designed a within-patient, randomized, single-blind controlled trial. The patients were blinded about the side of treatment (the surgeon knew which treatment was being applied in each foot). The hallux control group received standard treatment care with administration of a water-soluble nitrofurazone ointment, Furaclín (Seid SA, Barcelona, Spain), and the hallux experimental group received APG treatment. Healing was monitored for spontaneous wound closure by clinical assessments and by digital photographs over 1 month. Both treatments were repeated after 48 hours, and from the fifth day the treatment administered to both the control and experimental groups consisted of applying an antiseptic solution of povidone iodine 10%, Betadine (Meda Pharma, SAU, Madrid, Spain). All participants returned for regulated, standardized dressing changes. Participants were seen 48 hours after the surgical procedure by the same surgeon and the initial treatment was repeated in both groups. From the fifth day patients were seen approximately every 48 hours until the recovery time period was complete. To limit subjectivity in the assessment of recovery time, clinical indicators of recovery time were considered when there was absence of drainage (no exudate evident), when granulation tissue was covered by a scab (no evidence of hypergranulation tissue), when there were no signs of erythematous tissue without evidence of infection, and the patient was able to use normal footwear (Fig. 1). All criteria had to be met before the recovery time was reached. From the fifth day, when participants presented for re-dressing, 2 blinded experienced clinicians (R.R.-R., J.M.J.-J.) in wound care assessed the wound independently. Recovery time was considered when both clinicians had independently agreed that all the criteria had been met. The recovery time was the interval between the application of the first dressing (at the time of surgery) and the clinical indicators were completely achieved. To measure the postoperative pain, an analog chromatic visual scale for self-evaluation of pain (scale from 0 = absence of pain to 10 = unbearable pain) was used. The analogue chromatic continuous scale has proven to be more sensitive than the traditional visual analogue scale and is very easy to use (27). The intensity of pain was recorded subjectively for both groups, according to colors from white to dark red, corresponding to the numbers from 0 to 10, during the 3 days following surgery. Postoperative pain was treated with 500 mg of acetaminophen, Termalgin (Novartis Farmacéutica SA, Barcelona, Spain), orally every 6 to 8 hours (no more than 4 g/d) when the pain measured with the chromatic scale was less than 5 and 1000 mg when it was more than 5. Each patient was instructed to report the pain level, and how to correlate the intake of the analgesic drug to it; the analgesic drug was always taken after such report. All patients completed a questionnaire where pain intensity was scored according to the scale in each of their feet for the 3 days following surgery. For the analysis of postoperative inflammation, the digital circumference (in millimeters) was measured using a flexible millimeter rule (Devon Industries 1-800, Inc., Devon, PA) at the level of the proximal fold of the nail (Fig. 2). A measurement of the digital circumference was made before the procedure and at 48 hours (acute inflammatory phase of the cicatrization) in both groups (both sides of each patient); a single researcher (A.C.-F.) performed all measurements. Patients were blinded as to which foot received the treatment. All parties involved in postoperative care, with the exception of the surgeon (A.C.-F.) were blinded. This included the nurses who collected the pain questionnaires.



Fig. 1. Clinical photograph (12-days postoperative) of a representative nail wound site with absence of drainage and scab-covered granulation tissue.



Fig. 2. Preoperative measurement of digital circumference at the level of the proximal nail fold using a flexible millimeter rule.

### Surgical Technique

Local anesthesia was achieved with 3 to 4 mL of mepivacaine 2% (Scandinibsa, Laboratorios Inibsa SA, Barcelona, Spain) using hallux block technique. The surgical procedure consisted of a partial removal of the nail plate, followed by separation of the nail bed and matrix with a scalpel and in one piece, following the Suppan I technique (28). The procedure was carried out at both nail borders of the hallux. Treatments were selected intraoperatively and randomly (by tossing a coin; heads = right foot, treatment with APG, and left foot, treatment with nitrofurazone ointment; tails = right foot, treatment with nitrofurazone ointment, and left foot, treatment with APG). All the patients received antibiotic prophylaxis with cephalexin 2 g, Kefloridina forte (Lilly, Madrid, Spain) orally in 1 single dose 60 minutes before the procedure. A single surgeon (A.C.-F.) performed all the interventions.

To obtain APG, eight 4.5-mL aliquots of anticoagulated blood (3.8% anticoagulant trisodium citrate solution) were obtained from each subject by venipuncture (Vacutainer, Belliver Industrial Estate, Plymouth, UK). Each aliquot was processed with PRGF equipment (PRGF System Biotechnology Institute, Vitoria, Spain). This method, in a single centrifugation, yields 1 mL platelet-rich plasma (3 to 5 times more than the subject's baseline platelet count) from each aliquot, thereby a total of 8 mL of platelet-rich plasma from each subject was obtained in a single centrifugation step and with outpatient suitability. When activated with added 10% calcium chloride solution (Braun, Barcelona, Spain), the gel was easy to manipulate and could be packed into the operated nail grooves (Fig. 3). The protocol follows that of an autologous graft, using the patient's own blood exclusively (thrombin is not added), thereby avoiding immunological and allergic rejection responses (23, 24). The platelet counts in peripheral blood were measured in a hematology analyzer (Advil 120, Bayer, Germany).

### Data Management and Statistical Plan

The calculation of sample size for the study was conducted using the program nQuery Advisor, Release 6.01 (Statistical Solutions 2005, Los Angeles, CA). This determined that to detect a clinically relevant difference of 1 day between the means in the experimental and control groups with respect to recovery time, taking into account the bilateral nature of the study (paired samples), an  $\alpha$  error of 5% and 80% of the power (1–0.2), the minimum number of individuals necessary in each group was 10 patients (pairs). For the statistical analysis, we used the statistical package SPSS, version 15.0 (SPSS, Inc., Chicago, IL). The first step in analyzing the studied variables between the experimental and control groups was to determine whether the sample of the differences between the 2 groups of each variable had a normal distribution. Because the sample was composed of fewer than 50 elements, the Shapiro-Wilk test was used to test normality. The test was normal for the sampling of the differences of inflammation and recovery time and for the sampling of the differences of pain between the first and third days, but not for the sampling of the differences of pain in the 3 days postoperative. To compare equality of means between the 2 groups for the variables recovery time and inflammation, the independent-samples Student *t* test was used. To compare the equality of means of pain between the first and third day in the 2 groups, the 1-tailed Student *t* test for paired samples was used. To compare the equality of means between the 2 groups for the variable of pain in the 3 days postoperative, the 1-tailed Wilcoxon rank sum test was used. Statistical significance was defined at the 5% ( $P \leq .05$ ) level.



Fig. 3. Autologous platelet gel packed into the operated nail grooves.

Table 1

Recovery time (days) in the treatment groups (N = 70 feet in 35 patients)

Days	Control (n = 35)	APG (n = 35)	P value*
Mean $\pm$ Standard deviation	12.3 $\pm$ 1.99	11.79 $\pm$ 2.07	.864
Median (minimum, maximum)	12 (8,16)	12 (7,14)	

Abbreviation: APG, autologous platelet gel.

\* Independent samples Student *t* test.

### Results

The final study sample consisted of 35 patients (70 feet), of whom 19 were males (54.29%) and 16 (45.71%) were females, with a mean age of  $33.8 \pm 23.36$  years, and, by virtue of the study design, mean age was not statistically different between the treatment groups. The average difference in recovery time (in days) between groups was not statistically significant (Table 1). The average difference, in relative values, of digital circumference (inflammatory process) was slightly greater in the experimental group than in the control group, but this difference was not statistically significant (Table 2). The evolution of measurements of pain observed on the scale during the first 3 days postoperative was similar in both groups (Fig. 4). The result of the Wilcoxon test showed that the average differences in pain in the 3 days postoperative did not differ significantly between the groups (control group, median: 3.45 and range: 13.15; APG median: 3.75 and range: 17.29,  $P = .103$ ). The 1-tailed Student *t* test revealed statistically significant changes in regard to the evolution of pain measured between the first and third postoperative days for both groups (Table 3). The evolution of pain measurements at 3 moments in time (each of the 3 days postoperative) and each treatment was analyzed using the nonparametric Friedman test. This was significant for both groups ( $P < .001$ ), showing that the mean values of pain differ at the 3 moments of the study and for both treatments. To discover the causes of this significance, the 1-tailed Wilcoxon test was applied (2 at 2 moments during the 3 days postoperative) to each treatment group; it was always significant ( $P < .0005$ ). Therefore, it can be stated that there is always a significant decrease on the pain scale measured from the first to third day for each of the treatments (Table 4).

### Discussion

The results of previous studies with APG about postoperative benefits in different surgical procedures have reported contradictory results (2–16). We have observed that APG does not improve outcome after bilateral ingrown toenail surgery. Application of APG after surgery did not reduce postoperative pain or improve wound healing. In our study we conducted a checking of wounds every 48 hours from the fifth day with clinical indicators to reach what has been considered as recovery time. We noted that the combined use of antibiotic prophylaxis and povidone-iodine antiseptic solution had eliminated the experimental mortality of risk of infection in both groups, even taking into account that nail surgery is considered dirty surgery (29). Currently the use of antiseptics on wound healing remains controversial; however, there has been no conclusive evidence to support the use of other medicated dressing or forms of amorphous hydrogels

Table 2

Comparison of outcomes between the treatment groups in inflammation (increase of digital circumference in relative values) in first 48 hours (N = 35).

	APG (mean $\pm$ SD)	Control (mean $\pm$ SD)	P value*	CI (95%)
Inflammation, %	7.47 $\pm$ 3.54	6.47 $\pm$ 3.91	0.102	–2.20 to 0.21

Abbreviations: APG, autologous platelet gel; CI, confidence interval.

\* Independent samples Student *t* test.

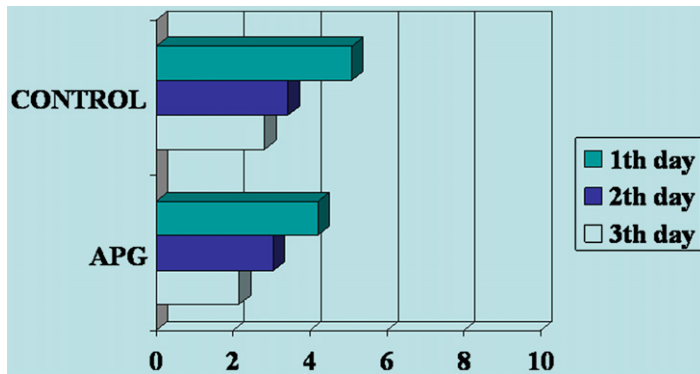


Fig. 4. The evolution of the mean of pain measured in 3 postoperative days for both treatments.

in nail surgery (30). We believe that povidone iodine may contribute to scab formation; however, we do not believe that it inhibits healing after granulation tissue has formed. In our study we have analyzed and studied the pain manifested by our patients, using a self-evaluation chromatic scale in the 3 days following surgery. As the study had a within-patient design, we avoided skew from one patient to another when quantifying the subjective perception of pain stimulus. As in previous studies, the mean pain observed on the scale during the first 3 postoperative days was slightly less in the experimental group, although the reduction was not statistically significant (14–16). Although in our study the sample size was estimated based on the variable recovery time, we feel that it is unlikely we would have achieved significant differences by adapting the sample size to the other variables. As in previous studies, we have found that inflammatory process, in relative values, was slightly greater in the experimental group than in the control group without statistically significant differences (20). From the clinical standpoint, this could indicate that the inflammatory process induced and/or modulated by the APG does not substantially modify the inflammatory phase of healing, although it is true that it increases it slightly. The growth factors present in APG are mainly PDGF-AB (proinflammatory cytokine) and TGF- $\beta$ 1 (antagonist of proinflammatory cytokines), whose concentrations are closely related with the number of platelets. Anitua et al (31) argue that for any individual, a concentrate of platelets will always contain between 2.5- and 3.0-fold more TGF- $\beta$ -1 than PDGF-AB, the ratio remaining constant whatever the number of platelets. These circumstances make the inflammatory response modulate perfectly, independently of the number of platelets contained in the platelet-rich plasma.

Several limitations in our study should be taken into account. The APG application and digital circumference measurements were performed in a single-blinded fashion. Another limitation in our study is that the platelet count in peripheral blood varies from one subject to another and the platelet counts in platelet-rich plasma should have been performed. The technique of the PRGF system used in this study provides a concentration of platelets between 3 and 5 times more

**Table 3**  
Comparison of mean differences in postoperative pain between first and third days (N=70 feet in 35 patients)

Pain	Control group	APG
Mean of difference	2.27	2.03
Standard deviation	2.03	1.97
Typical mean error	0.35	0.034
95% CI (lower limit, upper limit)	1.57, 2.98	1.34, 2.74

Abbreviations: APG, autologous platelet gel; CI, confidence interval.

**Table 4**

Pain between first and third postoperative days, by treatment group (N = 70 feet in 35 patients)

	Postoperative day	Control (n = 35)	APG (n = 35)	P value
Pain	1	5.0 $\pm$ 2.24	4.2 $\pm$ 2.22	
	2	3.4 $\pm$ 2.22	3.0 $\pm$ 1.79	<.005*
	3	2.8 $\pm$ 2.16	2.1 $\pm$ 1.55	<.001†

Abbreviation: APG, autologous platelet gel.

\* Friedman's nonparametric test

† Wilcoxon rank sum test between first and third postoperative days by treatment group.

than the subject's baseline platelet count and some studies have shown that when the platelet count in APG was more than 5 times the subject's baseline platelet count, the beneficial effects of APG became more clinically apparent and consistent (11). However, excessively high platelet-gel concentrations may inhibit the angiogenic process (32). Finally, with regard to the pain variable, the study design did not allow us to analyze postoperative pain in terms of analgesic requirements in each of the groups, unlike other clinical trials (17, 18). Although some authors have suggested that APG may reduce postoperative pain by increasing all phases of the dermal wound-healing process, we think that this potential increase is not translated into clinically significant decreases of postoperative pain. In conclusion, we believe that the benefits of using APG in hallux nail surgery are limited. Our results do not support the use of APG as a method to decrease recovery time and postoperative pain after bilateral ingrown hallux nail. The evidence to support their use is lacking. Our findings suggest surgeons should critically examine the effectiveness of these products for soft tissue healing. More studies are needed to determine the effectiveness and to know if their use in other surgical procedures may improve morbidity and faster uptake activities in the daily lives of patients undergoing surgery.

## References

- Martínez-Zapata MJ, Martí-Carvajal A, Solà I, Bolibar I, Angel Expósito J, Rodríguez L, García J. Efficacy and safety of the use of autologous plasma rich in platelets for tissue regeneration: a systematic review. *Transfusion* 49:44–56, 2008.
- Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source for healing and tissue regeneration. *Thromb Haemostasis* 91:4–15, 2004.
- Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. *Wound Repair Regen* 16:585–601, 2008.
- Giuffrè G, Caputo G, Misso S, Peluso F. Platelet-rich plasma treatment and hemostasis in patients with hemorrhagic risk. *Minerva Stomatol* 55:599–609, 2006.
- Cillo JE Jr, Marx RE, Stevens MR. Evaluation of autologous platelet-poor plasma gel as a hemostatic adjunct after posterior iliac crest bone harvest. *J Oral Maxillofac Surg* 65:1734–1738, 2007.
- Englert SJ, Estep TH, Ellis-Stoll CC. Autologous platelet gel applications during cardiovascular surgery: effect on wound healing. *J Am Soc Extr Corp Tech* 37:148–152, 2005.
- Córdoba-Fernández A, Rayo-Rosado R, Juárez-Jiménez JM. Platelet gel for the surgical treatment of onychocryptosis. *J Am Podiatr Med Assoc* 98:296–301, 2008.
- Kakudo N, Minakata T, Mitsui T, Kushida S, Notodihardjo FZ, Kusumoto K. Proliferation-promoting effect of platelet-rich plasma on human adipose-derived stem cells and human dermal fibroblasts. *Plast Reconstr Surg* 122:1352–1360, 2008.
- Kazakos K, Lyras DN, Verettas D, Tilkeridis K, Tryfonidis M. The use of autologous PRP gel as an aid in the management of acute trauma wounds. *Injury* 40:801–805, 2009.
- Danielsen P, Jorgensen B, Karlsmark T, Jorgensen LN, Agren MS. Effect of topical autologous platelet-rich fibrin versus no intervention on epithelialization of donor sites and meshed split-thickness skin autografts: a randomized clinical trial. *Plast Reconstr Surg* 122:1431–1440, 2008.
- Hom DB, Linzie BM, Huang TC. The healing effects of autologous platelet gel on acute human skin wounds. *Arch Facial Plast Surg* 9:174–183, 2008.
- Henderson JL, Cupp CL, Ross EV, Shick PC, Keefe MA, Wester DC, Hannon T, McConnell D. The effects of autologous platelet gel on wound healing. *Ear Nose Throat J* 82:598–602, 2003.
- Zavadiil DP, Satterlee CC, Costigan JM, Holt DW, Shostrom VK. Autologous platelet gel and platelet-poor plasma reduce pain with total shoulder arthroplasty. *J Extra Corpor Technol* 39:177–182, 2007.

14. Anzarut A, Guenther CR, Edwards DC, Tsuyuki RT. Completely autologous platelet gel in breast reduction surgery: a blinded, randomized, controlled trial. *Plast Reconstr Surg* 119:1159–1166, 2007.
15. Peerbooms JC, de Wolf GS, Colaris JW, Bruijn DJ, Verhaar JA. No positive effect of autologous platelet gel after total knee arthroplasty. *Acta Orthop* 80:557–562, 2009.
16. Nin JR, Gasque GM, Azcárate AV, Beola JD, Gonzalez MH. Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? *Arthroscopy* 25:1206–1213, 2009.
17. McNicol A, Israels SJ. Beyond hemostasis: the role of platelets in inflammation, malignancy and infection. *Cardiovasc Hematol Disord Drug Targets* 8:99–117, 2008.
18. Zarbock A, Polanowska-Grabowska RK. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* 21:99–111, 2007.
19. Qian L, Wei SJ, Zhang D, Hu X, Xu Z, Wilson B, El-Benna J, Hong JS, Flood PM. Potent anti-inflammatory and neuroprotective effects of TGF-beta1 are mediated through the inhibition of ERK and p47phox-Ser345 phosphorylation and translocation in microglia. *J Immunol* 181:660–668, 2008.
20. Chandra RK, Handorf C, West M, Kruger EA, Jackson S. Histologic effects of autologous platelet gel in skin flap healing. *Arch Facial Plast Surg* 9(4):260–263, 2007.
21. Buchwald D, Kaltschmidt C, Haardt H, Laczkovics A, Reber D. Autologous platelet gel fails to show beneficial effects on wound healing after saphenectomy in CABG patients. *J Extra Corpor Technol* 40:196–202, 2008.
22. Vang SN, Brady CP, Christensen KA, Allen KR, Anderson JE, Isler JR, Holt DW, Smith LM. Autologous platelet gel in coronary artery bypass grafting: effects on surgical wound healing. *J Extra Corpor Technol* 39:31–38, 2007.
23. Beierlein W, Scheule AM, Antoniadis G, Braun C, Schosser R. An immediate, allergic skin reaction to aprotinin after reexposure to fibrin sealant. *Transfusion* 40:302–305, 2000.
24. Streiff MB, Ness PM. Acquired FV inhibitors: a needless iatrogenic complication of bovine thrombin exposure. *Transfusion* 42:18–26, 2002.
25. Argimon JM, Jimenez J. Estudios experimentales II: otros diseños. In: *Métodos de investigación clínica y epidemiológica*, Edited by JM Argimon Pallás, J Jiménez Villa, Elsevier España, S.A, Madrid, 2004.
26. Mozena JD. The Mozena classification system and treatment algorithm for ingrown hallux nails. *J Am Podiatr Med Assoc* 92:131–135, 2002.
27. Grossi E, Borghi C, Cerchiari EL, Della Puppa T, Francucci B. Analogue chromatic continuous scale (ACCS): a new method for pain assessment. *Clin Exp Rheumatol* 1:337–340, 1983.
28. Weisfeld M. Illustrated technique for complete removal of nail matrix and hyponychium without skin incisions (Suppan nail technique no.2). *J Am Podiatry Assoc* 65:481–489, 1975.
29. Wolf EW, Hodge W, Spielfogel WD. Periungual bacterial flora in the human foot. *J Foot Surg* 30:253–263, 1991.
30. Dovison R, Keenan AM. Wound healing and infection in nail matrix phenolization wounds. Does topical medication make a difference? *J Am Podiatr Med Assoc* 91:230–233, 2001.
31. Anitua E, Andia IPRGF. (Plasma rico en factores de crecimiento). *Dental Dialogue* 3:1–15, 2004.
32. Giusti I, Rughetti A, D'Ascenzo S, Millimaggi D, Pavan A, Dell'Orso L, Dolo V. Identification of an optimal concentration of platelet gel for promoting angiogenesis in human endothelial cells. *Transfusion* 49:771–778, 2009.