The clinical efficacy of using autologous platelet rich plasma in hip arthroplasty: A retrospective comparative study

Atif Safdar[1], Hamid Shaaban, Restituto Tibayan, Richard Miller[1], Richard Boairdo[2], Gunwant Guron

Department of Hematology and Oncology, [1]Pulmonary and Critical Care, [2]Orthopedic Surgery, St Michael's Medical Center, 111 Central Avenue, Newark, NJ 07102, New Jersey, USA

Address for correspondence:
Dr. Hamid Shaaban, Department of Hematology and Oncology, St Michael's Medical Center, 111 Central Avenue, Newark, NJ 07102, New Jersey , USA
E-mail: hamidshaaban@gmail.com

Abstract

Background: Platelet rich plasma (PRP) is a blood derivative concentrate of platelets, fibrin and growth factors obtained through withdrawal and centrifugation of autologous blood and use for its inherent hemostatic and adhesive properties to promote wound healing. Hip arthroplasty is often associated with significant perioperative complications including blood loss necessitating blood transfusions, which can lead to multiple adverse reactions, infection transmission, and longer hospital stay. **Materials and Methods:** We conducted this retrospective comparative study to determine whether the use of PRP can reduce the bleeding complications in hip replacement surgeries and therefore decrease analgesic requirements and shorten the hospital stay. **Results:** Sixty patients had consecutive hip replacement surgeries. The study group (n=23) received PRP applications while the control group (n=37) were operated without PRP applications. Postoperative drop of hemoglobin, number of red blood cell (RBC) transfusions, analgesic requirements, and duration of hospital stay were recorded. There was no significant difference in the drop of hemoglobin preoperatively and postoperatively comparing study and control groups (P=0.75). There was no difference in transfusion requirements between the two groups (P=0.16) but there was trend toward less transfusion in the PRP-treated group. There were also no statistical differences in analgesic use (P=0.83) and lengths of hospitalization (P=0.68) between the two groups. **Conclusion:** We concluded that there is no clinical efficacy in using PRP in hip replacement surgeries. We recommend a larger prospective study be conducted to determine its clinical utility as an optimization strategy to improve outcome after hip arthroplasty

Key words: Autologous, hip arthroplasty, platelet rich plasma

INTRODUCTION

Significant perioperative blood loss is one of the major problems in elective orthopedic surgeries including hip arthroplasty. These procedures often require transfusions of allogeneic blood products averaging 2–4 red cell units per procedure.[1] Blood transfusion is associated with various adverse events including febrile reactions and transmission of infectious diseases.[1-3] It has an immunomodulatory effect resulting in increased infectious complications and wound healing disturbances.[2] Perioperative bleeding causes wound complications including hematomas and seroma formation, infection and adhesions leading to compromised clinical outcome such as poor wound healing, dehiscence, and impaired mobility. These were thought to cause prolongation of hospital stay even in elective surgeries.[2]

Multiple strategies had been employed to minimize perioperative blood loss and therefore improve outcome in orthopedic surgeries. These include use of platelet rich plasma (PRP) concentrate delivered directly to the surgical site.[1] Despite lack of hard evidence through clinical trials,

the use of PRP in humans has increased significantly. [1,2] PRP has found multiple uses in different surgical disciplines including orthopedics, maxillofacial surgery, neurosurgery, ophthalmology, urology, and cardiothoracic surgery for its hemostatic and adhesive properties.[4] Special interests had been explored on its use in sports and rehabilitative medicine in which patients desire and require a rapid recovery after sports-related injuries.[2-4] It stimulates physiologic wound healing and soft tissue reparative processes including bone regeneration.[3] Use of PRP, a bioactive component of whole blood containing concentrated platelets and platelet growth factors including platelet-derived growth factors (PDGF) and transforming growth factors β (TGF-β), in total knee arthroplasty (TKA) resulted to improved range of motion, decreased length of hospital stay, reduced incidence of arthrofibrosis, and decreased requirements for narcotics postoperatively.[4] It is derived from autologous blood and therefore there is low chance of rejection. It can be prepared at the time of the surgery in a simple and relatively inexpensive manner. It is also inherently safe and therefore free from concerns over transmissible diseases.[5]

Although PRP has been in use for more than two decades now, there has not been a published report in its application for hip replacement surgeries. This, to our knowledge and extensive literature search, will be the first clinical research to determine the impact of use of PRP application in hip arthroplasty in reducing bleeding complications, analgesic requirements, and length of hospital stay.

MATERIALS AND METHODS

This is a retrospective comparative study involving 60 patients who underwent consecutive hip arthroplasties in three hospitals performed by the same surgeon. All patients had primary diagnosis of osteoarthritis. Twenty-three patients were treated with PRP and 37 patients served as untreated control.

Patients were eligible for study if they are aged >18 years, with preoperative platelet count $\geq 150 \times 10^9$/L, hemoglobin level ≥ 10 g/dL and no preexisting coagulation defects. Preoperatively, patients were counseled on the risks and benefits of the PRP application. The study was approved by the Institutional Review Board (IRB).

PRP was prepared immediately prior to surgery by phlebotomizing 52 ml of whole blood from the patients in the PRP group and adding 8 ml of anticoagulant. The whole blood underwent centrifugation and separated into platelet-rich and platelet-poor plasma and the buffy coat using Accelerate Platelet Concentrating System (Exactech®

Biologics, Gainesville, Florida). Once separated, the deep layer of the platelet-rich plasma was aspirated and applied to the surgical field immediately before closure and after the surgical fields were rinsed with saline solution to remove all debris. PRP was prepared and applied by a person trained for this purpose toward the end of the surgery.

All patients received standard postoperative pain protocol including intraoperative site instillation of methylprednisolone, morphine, and bupivacaine (cocktail). As needed, pain coverage included use of oxycodone/ acetaminophen (Percocet®), hydrocodone/acetaminophen (Vicodin®), hydromorphone (Dilaudid®), morphine, meperidine, and ketorolac. For comparison purposes, all analgesics used during hospital stay were converted to equivalent morphine dosages. All patients received thrombosis prophylaxis with low molecular weight heparin daily before the operations and until 6 weeks postoperatively.

Hemoglobin levels preoperatively and 3 days postoperatively and red cell units transfused were used as markers for blood loss. Length of hospital stay was recorded as the number of hospital days from admission to the discharge date. Analgesic requirements, as stated, were based on the amount of IV and oral pain medications used during hospital stay converted to equivalent morphine dosages.

Statistical analysis

Categorical variables were evaluated for statistical significance by Fisher's exact test; relative risk (RR) was used as the measure of clinical relevance. Continuous variables were examined for fit-to-normality by the D'Agostino–Pearson omnibus normality test. Group-wise comparisons were made using appropriate parametric (unpaired *t*-tests) or nonparametric (Mann–Whitney tests) based on the results of normality testing. Comparisons of pre- and posttreatment measures were made using two-way analysis of variance (ANOVA) methods with groups (PRP – treated/not treated) represented as independently – assorted data and time factor (pre/post) represented as repeated measures. For normally distributed variables, means ± 1 standard deviation (SD) are given; for nonnormally distributed data, medians and interquartile ranges (IQR) are presented.

All data were analyzed using Prism® software (GraphPad Corp., San Diego, CA) on a Windows-PC platform. In this study, α was set at 0.05; *P*<0.05 (two-sided) was required for statistical significance

RESULTS

Between January 2005 and February 2009, 60 patients

underwent consecutive hip replacements at three different hospitals under the same surgeon. Twenty-three patients were treated with PRP and 37 patients served as untreated control.

The mean ages of the PRP-treated patients and the control group was 64 and 65, respectively. There were 60% female among all patients in the study with higher percentage of female in both groups.

The average hemoglobin values preoperatively and postoperatively among PRP-treated group was 13.63 and 10.76 g/dl, respectively, with a mean difference of 2.87 g/dl. The average hemoglobin values preoperatively and postoperatively in the untreated control was 12.94 and 9.88 g/dl, respectively, with mean difference of 3.05 g/dl. In both groups there were significant differences in the hemoglobin levels before and after surgery ($P<0.001$). However, comparing PRP and non-PRP-treated groups, there was no significant difference in the drop of hemoglobin ($P=0.75$) [Figure 1].

The mean number of transfused red blood cells (RBCs) in PRP-treated group was 0.39 units while 1.05 were transfused in the untreated control. Although our sample size was not large enough, there appeared to be a trend toward less transfusion, however, this did not reach statistical significance ($P=0.16$). There was no significant difference ($P=0.78$) [RR=0.87, 95% CI 0.41–2.85] between subjects in the study and control groups. In the former, 30% required transfusion. The latter group had 35% of patients transfused. The largest number of RBC units transfused to a single patient was six units from the untreated control [Figure 2].

There was no significant difference in the length of hospital stay between the groups ($P=0.68$). The length of stay for PRP-treated and control groups were 3.56 and 3.51 days, respectively. Incidentally, patients who received blood transfusions stayed longer in the hospital with average of 4.5 days compared with those who were discharged without transfusion who had average of 3.05 days [Figure 3].

Finally, the use of analgesia did not yield any significant difference between the groups ($P=0.83$). A mean average of 25.85 and 36.12 mg of morphine equivalent analgesics were used by the treated and untreated groups, respectively.

DISCUSSION

Musculoskeletal diseases are prevalent and the most common causes of severe long-term pain and disability, and they affect hundreds of millions of people around the world costing billions in terms of health care expenditures and loss of earnings.[6] The bulk of disease burden

Figure 1: When the PRP and non-PRP-treated groups were compared, there was no significant difference in the drop of hemoglobin ($P=0.75$)

Figure 2: The mean number of transfused red blood cells (RBCs) in PRP-treated group was 0.39 units while 1.05 were transfused in the untreated control. There appeared to be a trend toward less transfusion, however, this did not reach statistical significance ($P=0.16$). There was no significant difference ($P=0.78$) [RR=0.87, 95% CI 0.41–2.85] between subjects in the study and control groups.

expenditures had been attributed to longer duration of hospitalization secondary to perioperative complications including bleeding, infections, wound dehiscence, and impaired mobility.[6]

For the past 20 years, there had been increasing interest with use of PRP as an optimization strategy to improve outcome by reducing postoperative bleeding and promoting safe and natural healing. An *in vitro* study using porcine partial-thickness skin wound model showed reduction of bleeding by 70% at 5 min using PRP application compared with placebo. The platelet poor plasma showed only 10% reduction of bleeding using the same model.[7]

Freshly drawn autologous blood can be fractionated by blood cell separator and centrifugation devices into platelet-poor plasma, platelet-rich plasma (PRP), and RBCs. The PRP fraction, a rich concentration of platelets, fibrin, cytokines, and growth factors, can be activated to create a viscous solution known as platelet-leukocyte gel (PLG), which can be applied exogenously to surgical wound sites during closure as a spray or gelatinous mass using syringe delivery technique. These gels provide numerous platelet-derived growth factors and peptides that act in sequential events to promote physiologic cascades including cell proliferation, differentiation, chemotaxis of various inflammatory cells involved in accelerating soft tissue wound healing and bone growth and angiogenesis.[3,8,9] Although recombinant growth factors had been used in the past, application of autologous PRP has the advantage of synergistically inducing various growth factors and

promoting mitogenesis of mesenchymal stem cells at the wound site.[10,11] Moreover, platelets had been identified to have analgesic properties by releasing protease-activated receptor 4 peptides.[12] PRP also contains an amount of differentiated and nonactivated leukocytes equivalent to two to four times greater than normal. These granulocytes and monocytes provide host defense inhibitory effect against bacteria and help in immunomodulatory activity in wound healing process.[3] *In vitro* studies have found that platelet-rich gel significantly inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*.[5] These biological adhesive properties that promote wound healing, tissue and bone forming, antiinflammatory and antibacterial properties made PRP the new "biological glue" in the surgical specialties.[5-7]

Use PRP or PLG has found multiple clinical applications as documented in various clinical studies in different surgical specialties. One of the first successful uses of PRP had been the reduction of superficial and sternal wound infections among patients who underwent cardiac surgery as reported by Trowbridge *et al.*[13] Since then PRP had found other applications including probably the one with best published result – a 93% reduction of pain after 1 year follow-up for patients with chronic lateral epicondylar tendinopathy treated with PRP.[14] This milestone had triggered multiple uses of PRP in sports medicine where patients desire a rapid return to their preinjury level of function. In particular, use of PRP in anterior cruciate ligament reconstruction has shown better autograft maturation, improved donor site morbidity, pain control and improved allograft incorporation.[15] Other uses include treatments for chronic tendinosis, acute tendon injury, muscle injury, and osteoarthritis. In orthopedic surgery, PRP application during incisional wound closure after TKA and among patients with chronic diabetic neuropathic foot ulcers provided improved wound healing.[16,17] Everts *et al.* reported superior postoperative range of motion ($P<0.001$), significantly less incidence of arthrofibrosis ($P<0.001$), less drop in hemoglobin ($P<0.001$), and shorter hospitalization time ($P<0.001$) among patients who underwent TKA and were treated with application of platelet gel and fibrin sealants compared with control group.[4]

Hip arthroplasty is often associated with significant amount of perioperative blood loss requiring blood product transfusions. Toy *et al.* reviewed records of 324 patients who underwent hip replacement in 6 hospitals and calculated the blood loss as 3.2 ± 1.3 units in primary procedures and 4.0 ± 2.1 units in revision procedures (mean $\pm$ SD). The maximum number of units given to 95% of the transfused patients was 4 for primary procedures and 6 for revision procedures.[1] The blood transfusion requirements during the duration of our study was kept to the minimum with the PRP group requiring only 0.39 units while 1.05 units
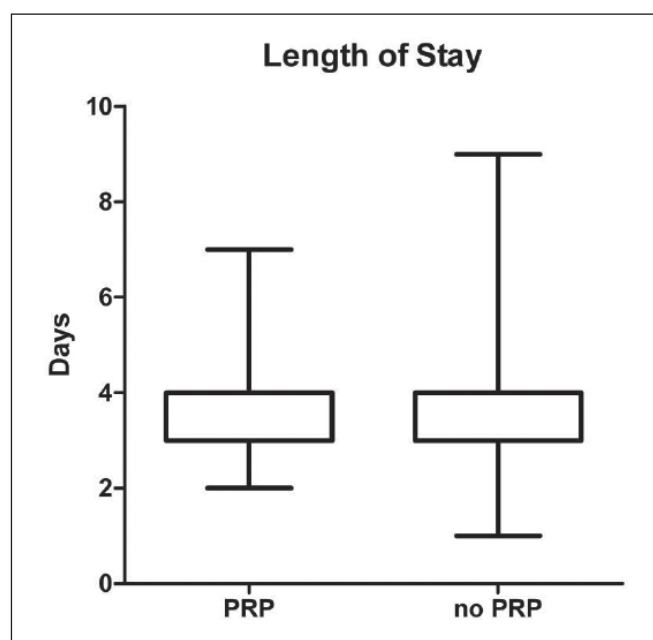

Figure 3: There was no significant difference in the length of hospital stay between the groups (P=0.68). The length of stay for PRP-treated and control groups were 3.56 and 3.51 days, respectively

were transfused to the untreated control.

The criteria for blood transfusion requirements included:
1. Hgb < 7 g/dl in an asymptomatic patient.
2. Hgb < 10 g/dl in cases of increased risk of ischemia – pulmonary disease, coronary artery disease, cerebral vascular disease, etc.
3. Acute blood loss resulting in:
   a. estimated or anticipated blood loss > 15% of total blood volume (750 ml in 70 kg male)
   b. diastolic blood pressure < 60 mmHg
   c. systolic blood pressure decrease > 30 mmHg
   d. oliguria/anuria
4. Symptomatic anemia resulting in:
   a. tachycardia (> 100 beats/min)
   b. mental status changes
   c. electrocardiographic signs of cardiac ischemia
   d. angina
   e. shortness of breath, light headedness or dizziness with mild exertion.

The small amounts of transfusions may have been related to the improving surgical techniques and better bleeding control as the two studies were separated by more than a decade. However, the use of PRP did not make any significant difference in terms of blood loss as measured by the transfusion requirements (*P*=0.16). This is in contrast to the previous study done by Everts *et al.* showing significantly less drop of hemoglobin (*P*<0.001) among patients who underwent TKA and were treated with application of platelet gel and fibrin sealants compared with control group.[4]

Blood transfusion as a surrogate marker of clinical efficacy of bleeding control in orthopedic procedures had been used in multiple studies in the past including those involving use of PRP and related products. Blood transfusions do carry potential deleterious clinical effects including development of blood-borne diseases and febrile nonhemolytic transfusion reactions induced by leukoagglutinins.[7-9] They also have immunomodulatory effects leading to increased postoperative bacterial infection rates. However, it is a common observation that patients who receive allogeneic blood transfusions after orthopedic surgery have longer duration of hospital stay that cannot be explained by the more frequent incidence of infections in transfused patients.[2] In a study of 444 consecutive patients who underwent elective hip surgery by Weber *et al.*, 31% of transfused patients developed wound-healing disturbances versus 18% of the nontransfused group (*P*=0.05); allogeneic blood transfusion was the only significant predictor for development of minor wound-healing disturbances. Duration of hospitalization was also prolonged in transfused patients (12.3 versus 9.8 days, *P*<0.001).[2] Consistent with this study, our results showed a longer duration of hospital stay for patients who received transfusion (4.5 days) compared with those who were discharged without transfusions (3.05 days). However, subset analysis incorporating data on use of PRP in relation to the transfusions requirements and hospital stay did not yield any significant difference (*P*=0.68). Our study also did not look into the wound complications as clinical parameter in relation to duration of hospitalization. This is because there was a lack of agreement on the various features of wound complications and overall agreement on the presence or absence of wound complications was 'not significant'. We concluded that single observer determination of wound complication by inexperienced observers using imprecise or inconsistent definitions was an unreliable measure for our study.

As stated previously, multiple studies provided evidence of support to the beneficial effects of PRP use in terms of decreased blood loss, decrease transfusion requirements, better pain control by way of less oral and intravenous narcotic requirements and decreased length of hospital stay.[4,16,18] However, these cannot be generalized in all studies. In particular, some literature in maxillofacial and periodontics report failure to observe improvements in tissue healing, bone formation and maturation rates using PRP.[19] Everts *et al.* reviewed 28 human studies and found 7 studies showing no benefit or negative effects of PRPs.[20] Peerbooms *et al.* reported that application of platelet gel to wound site after TKA did not promote wound healing and had no effect on pain, knee function, and hemoglobin values.[19] The results of our study can be added to the growing list of literature showing absence of clinical benefit of the use of PRP in orthopedic procedures.

The conflicting results related to its clinical application can be attributed to the many variations in the preparations of PRP, not to include the fact that its autogenous nature makes its composition differ from every patient. Being a relatively new technology, PRP lacks suitable standardization and definition of different preparations. Many commercial systems and products employ varying protocols and techniques in handling and administration of PRPs. As such, different terminologies had been used interchangeably including platelet leukocyte gel, platelet rich plasma gel, platelet concentrate, blood plasma technology, etc. Variations in some key properties including amount of blood drawn, platelet concentration, speed and number of centrifugations, use of anticoagulant in sample containers, type of clot activator, the leukocyte and growth factor content can influence the different biological properties of PRP.[20,21] Analysis of published reports needs thorough evaluation of the differences in these key elements that render diverse biologic effects. For instance, the actual quantity of platelets needed to achieve improved outcome is

still unknown. Weibrich *et al.* demonstrated that the number of platelets needed to obtain optimal bone regeneration has to be between 503,000 and 1,729,000 platelets/μL of PRP and that lower platelet concentrations can lead to suboptimal effects on peri-implant bone regeneration, while higher concentrations might have a paradoxically inhibitory effect.[21] The use of platelet activators to release growth factors also vary among many commercial systems. Some use endogenous or exogenous thrombin to activate PRP, which had been considered to cause potential adverse immune reactions. Others used calcium chloride to prevent this potential side effect.[22] The timing between the PRP retrieval and application also vary from among studies. Concern about diminished efficacy and reparative potential has been raised if there has time delay in its application.[23] PRP application techniques also vary and can come in form of injection, gel, PRP scaffold, and PRP fibrin membrane. Gel formulation has been said to decrease absorption and diffusion to the application site compared with the liquid injection. Likewise, the presence of leukocytes may also affect the biological properties of PRP. While leukocytes may confer antimicrobial activity, they also express matrix-degrading enzymes such as matrix metalloproteinases-8 (MMP-8) and MMP-9 and release reactive oxygen species that may increase tissue damage.[22]

Furthermore, multiple studies involving PRP had different applications and endpoints. There had been numerous basic science studies, animal studies, and small case reports regarding PRP-related products but there were only few controlled, clinical studies that provide high level of medical evidence regarding the potential benefits of PRP. Majority of cited studies are anecdotal based on small case series. Taylor *et al.* reported a systematic review of evidence-based outcomes of use of PRP for treatment of tendon and ligament injuries and found only 13 human *in vivo* studies of the 32 retrieved. They found three prospective, randomized double- blind studies, three prospective cohort studies, and seven case reports and case control studies.[23] The paucity of randomized controlled trials and small sample sizes of most studies makes it difficult to draw meaningful conclusions and generalization of findings.

Although the autogenous nature of PRP deem its use safe, uncertainties exist regarding its systemic effect and concerns had been raised about possible local and systemic carcinogenic effect related to high fraction of growth factors based on studies on transgenic mice of the role of growth factors on promotion of proliferation and division of mutated cells.[23,24] However, there are no evidence at this time of neoplastic transformation from clinical application of PRP.[23]

This study presents some other limitations and weaknesses. The population sample is small and did not reach the estimated number of patients to power statistical significance. A future prospective study involving a larger number of subjects is recommended. Also this study, while performed by the same surgeon, surgeries were done in three different hospital settings, precluding a good comparative analysis in terms of population demographics, which may affect the overall outcome measures.

Ideally, the amounts of platelet and the quantity of activated growth factors that were present in the PRP applications would have been measured and documented. Our study suffers from lack of technology to determine these. It is recommended that future studies explore into defining these standards. Another likely reason for the nonsignificant outcome of our study is the possibility that the larger operative field of the hip surgeries may require a higher dose of PRP gel application. This is considering that other studies had centrifuged as much as 350 ml of autologous blood while we used only 50 ml consistent with majority of the studies done on knee arthroplasty. Use of larger amount of PRP may also produce a more immediate hemostasis. A second application of PRP may also be considered in future studies.

As mentioned in the "methods" section, all patients received standard postoperative pain protocol including intraoperative site instillation of methylprednisolone, morphine, and bupivacaine (cocktail). It is possible that the positive and beneficial effects of PRP were compromised by this intervention, which potentially contributed to the nonsignificant outcome. There were also many other clinical parameters that were looked for in other studies that were not included in our study, including functional outcome parameters effects on range of motion, fibrotic and infectious complication rates, and different quality of life measures.

CONCLUSION

In summary, our study demonstrated lack of clinical efficacy of use of PRP in terms of lowering bleeding complications, pain control, and length of hospital stay when applied during hip replacement surgeries. A prospective study involving a larger population is recommended to establish its clinical use before its further application in hip replacement surgeries.

REFERENCES

1. Toy PT, Kaplan EB, McVay PA, Lee SJ, Strauss RG, Stehling LC. Blood loss and replacement in total hip arthroplasty: A multicenter study. The preoperative autologous blood donation study group. Transfusion 1992;32:63-7.

2. Weber EW1, Slappendel R, Prins MH, van der Schaaf DB, Durieux ME, Strümper D. Perioperative blood transfusions and delayed wound healing after hip replacement surgery: Effects on duration of hospitalization. Anaesth Analg 2005;100:1416-21.

3. Everts P, Overdevest E, Jakimowicz J, Oosterbos CJ, Schönberger JP, Knape JT, *et al*. The use of autologous Platelet-Leukocyte gels to enhance the healing process in surgery, a review. Surg Endosc 2007;21:2063-8.

4. Everts P, Devilee R, Oosterbos C, Mahoney CB, Schattenkerk ME, Knape JT, *et al*. Autologous Platelet gel and fibrin sealant enhance the efficacy of total knee arthroplasty: Improved range of motion, decreased length of stay and a reduced incidence of arthrofibrosis. Knee Surg Sports Traumatol Arthrosc 2007;15:888-94.

5. Bielecki T, Gazdzik T, Arendt J, Szczepanski T, Krol W, Wielkoszynski T. Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances: An In Vitro study. J Bone Joint Surg Br 2007;89:417-20.

6. Woolf A, Pfleger B. Burden of major musculoskeletal conditions. Bull World Health Organ 2003;81:646-56.

7. Pietrzak W, An Y, Kang Q. Platelet-rich and Platelet-poor Plasma: Development of an animal model to evaluate hemostatic efficacy. J Craniofac Surg 2007;18:559-67.

8. Slater M, Patava J, Kingham K, Mason, RS. Involvement of platelets in stimulating osteogenic activity. J Orthop Res 1995;13:655-63.

9. Tabata Y. Tissue Regeneration Based on Growth Factor Release. Tissue Eng 2003;9(Suppl 1):S5-15.

10. Lynch S, Nixon J, Colvin R, Antoniades HN. Role of platelet-derived growth factor in wound healing: Synergistic effects with other growth factors. Proc Natl Acad Sci USA 1987;84:7696-700.

11. Brown R, Breeden M, Greenhalg D. PDGF and TGF-alpha act synergistically to improve wound healing in the genetically diabetic mouse. J Surg Res 1994;56:562-70.

12. Asfaha S, Cenac N, Houle S, Altier C, Papez MD, Nguyen C, et al. Protease-activated receptor 4 - A Novel mechanism of inflammatory pain modulation. Br J Pharmacol 2007;150:176-85.

13. Trowbridge C, Stammers A, Woods E, Yen BR, Klayman M, Gilbert C. Use of Platelet Gel and its Effects on the Infection in Cardiac Surgery. J Extra Corpor Technol 2007;37:381-6.

14. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med 2006;34:1774-8.

15. Lopez-Vidriero E, Goulding K, Simon D, Sanchez M, Johnson DH. The use of platelet-rich plasma in arthroscopy and sports medicine: Optimizing the healing environment. Arthroscopy 2010;26:269-78.

16. Everts P, Devilee R, Brown-Mahoney C, Van Erp A, Oosterbos CJM, Stellenboom M, et al. Platelet gel and fibrin sealant reduce allogenic blood transfusions in total knee arthroplasty. Acta Anaesth Scand 2006;50:593-9.

17. Margolis D, Kantor J, Santanna J, Strom BL, Berlin JA. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. Diabetes Care 2001;24:483-8.

18. Gardner MJ, Demetrakopoulos D, Klepchick PR, Mooar PA.. The efficacy of autologous platelet gel in pain control and blood loss in total knee arthroplasty. Int Orthop 2007;31:309-13.

19. Peerbooms JC, de Wolf GS, Colaris JW, Bruijn DJ, Verhaar JA. No positive effect of autologous platelet gel after total knee arthroplasty. Acta Orthop 2009;80:557-62.

20. Everts PA, Knape JT, Weibrich G, Schönberger JP, Hoffmann J, Overdevest EP, Box HA, et al. Platelet-Rich Plasma and Platelet Gel: A Review. J Extra Corpor Techno 2006;38:174-87.

21. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone 2004;34:665-71.

22. Anitua E, Sanchez M, Nurden A, Nurden P, Orive G, Andia I. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol 2006;24:227-34.

23. Taylor DW, Petrera M, Hendry M, Theodoropoulos JS. A Systematic review of the use of Platelet-Rich Plasma in sports medicine as a new treatment for Tendon and Ligament Injuries. Clin J Sport Med 2011;21:344-52.

24. DiGiovanni J, Bol DK, Wilker E, Beltrán L, Carbajal S, Moats S, et al. Constitutive expression of insulin-like growth-1 in epidermal basal cells of the transgenic mice leads to Spontaneous tumor promotion. Cancer Res 2000;60:1561-70.