Platelet-Rich Plasma Application During Closure Following Total Knee Arthroplasty

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Platelet treatment appears to improve several short-term outcomes following total knee arthroplasty.

Total knee arthroplasty (TKA) is one of the most common orthopedic procedures performed, restoring function and reducing pain in the arthritic knee. In general, results are excellent with reported survival rates as high as 90%-95% at 10-15 year follow-up. Complications are infrequent, with reoperations occurring in approximately 1% of patients per year. With an aging population, elective TKA rates are steadily increasing. In addition, there is a trend toward earlier hospital discharge during a more acute phase of recovery in an effort to reduce hospital costs. Consequently, there is great motivation for ensuring expedient postoperative recovery.

Postoperative wound healing is mediated by signaling proteins such as growth factors and cytokines. These messengers direct cellular chemotaxis, proliferation, and differentiation, resulting in the formation of extracellular matrix and the establishment of the appropriate environment for site-specific tissue regeneration, eg, skin, cartilage, fibrous tissue, bone, etc. Platelets play a fundamental role in the process through their participation in hemostasis as well as their secretion, on activation, of various growth factors and cytokines that set the pace of wound healing. Anticoagulated autologous blood drawn preoperatively may be processed to yield platelet-rich plasma, containing a platelet concentration in excess of baseline. Platelet-rich plasma, activated to a gel with thrombin and calcium chloride, may be placed at the wound site to enhance healing and also act as a hemostatic agent. A byproduct of platelet-rich plasma production is platelet-poor plasma, which, when activated in a similar manner as platelet-rich plasma, can be used as a hemostatic agent. Several commercial systems are available that collectively use from one unit of blood to as little as 45-60 mL, producing approximately 10% by volume of platelet-rich plasma with a platelet concentration ratio of 2-8x above baseline. Clinical use of platelet-rich plasma is most commonly reported in the periodontal and oral surgery literature, but other areas of application include maxillofacial, cosmetic and plastic surgery, spinal fusion, heart bypass, and the treatment of chronic skin and soft-tissue ulcers.

Use of platelet-rich plasma and fibrin sealant during TKA procedures has been reported. Mooar et al performed a controlled study of platelet-rich plasma use in TKA, finding that the group receiving platelet-rich plasma required significantly less narcotics, achieved a higher functional range of motion (ROM) two days earlier, and had a lower postoperative decrease in hemoglobin than the control population.

Levy et al studied the effects of a commercially available fibrin sealant used prior to closure following TKA on the need for blood transfusion afterward. The treatment group had less hemoglobin decrease and required less transfused blood than the control group. While these studies demonstrated benefit of the use of platelet-rich plasma and/or fibrin sealants in the immediate postoperative period, the outcomes reported were limited
in scope and follow-up was limited to a few days.

Despite widespread clinical use and the general consensus by most investigators that platelet-rich plasma application enhances healing, conclusive evidence of a positive effect has been hampered by at least two issues. First, many clinical studies lack controls. Therefore, despite excellent outcomes, it is difficult to reach a conclusion regarding platelet-rich plasma influence. Second, platelet product characteristics such as count, premature activation, and maintenance of viability during processing, all of which can influence the biological effect of platelet-rich plasma, often are unknown. One platelet concentration system that has been well characterized is the GPS Gravitational Platelet System (Biomet Biologics, Warsaw, Ind). The platelet concentration in the platelet-rich plasma produced by this system was approximately eightfold greater than baseline, which is in the upper range of fold-increase cited by others. Also, with this system platelets remained intact and were not prematurely activated during processing. On activation, growth factors released included transforming growth factor-1 (TGF-1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and endothelial growth factor (EGF).

We hypothesized that application of autologous platelet-rich and platelet-poor plasma, produced from a well-characterized system, would result in a significant enhancement of short-term (6-week) outcomes following TKA.

Materials and Methods

Study Design

This was a retrospective study, with data gathered through chart review. Patients were included if they had a primary diagnosis of osteoarthritis and no prior history of TKA. Both the control population (66 patients, 72 knees) and the treatment population (71 patients, 81 knees) received unilateral or bilateral TKA, the latter group also receiving platelet-rich and platelet-poor plasma during wound closure. Outcome data was gathered for six postoperative weeks, and the results obtained from the two groups were compared.

Procedural Details

Preoperatively, patients were counseled on the risks and benefits of TKA and platelet-rich/platelet-poor plasma application. Following spinal or general anesthesia, patients received unilateral or bilateral cemented, non-constrained, cruciate-retaining, primary knee prostheses (Richards Genesis II; Smith & Nephew, Memphis, Tenn), (Biomet Maxim; Biomet, Warsaw, Ind), or (Zimmer NexGen; Zimmer, Warsaw, Ind) through a medial parapatellar approach using intramedullary guides to make the osteotomies. All procedures were performed with use of a tourniquet to minimize bleeding. Patients in the treatment group received platelet-rich plasma and platelet-poor plasma during wound closure, while those in the control group did not. All surgeries were performed by the senior author (W.J.B.).

There were 71 patients (81 knees) in the treatment group and 66 patients (72 knees) in the control group. The surgery dates for control patients ranged from March 2002 to December 2002 while the dates for platelet-treated patients ranged from January 2003 to October 2003. Preoperative comparisons of these populations included age, gender, body mass index, preoperative ROM, and the presence of comorbidities (particularly rheumatoid arthritis, diabetes mellitus, kidney failure, and cardiovascular disease).

For patients in the treatment group, 55 mL of venous blood was drawn preoperatively and mixed with 5 mL of acid-citrate-dextrose-A (ACD-A) anticoagulant in a 60-mL syringe. The syringe contents were then transferred to the processing tube of the GPS Gravitational Platelet System. Following the manufacturer’s instructions, the tube, appropriately counterbalanced, was placed in the GPS centrifuge and spun at 3200 RPM for 15 minutes. The system operates on the principle that plasma, theuffy coat (that contains platelets and leukocytes), and red blood cells have different densities (increasing in that order). As these layers stratify, a pair of buoys move to a neutral density position, effectively trapping the buffy coat within a contained volume of approximately 6 mL of plasma (Figure 1). The tube was then vigorously shaken to suspend the platelets within this small plasma volume. The balance of the plasma (~30 mL), contained within a separate compartment in the tube, was deficient in platelets, ie, platelet-poor plasma. Ports connected to the platelet-rich plasma and platelet-poor plasma chambers allowed their respective contents to be drawn into separate syringes.
Activation solution was prepared by mixing 1000 units of topical bovine thrombin (Jones Pharma, St Louis, Mo) per milliliter of 10% CaCl\(_2\) solution. The thrombin directly activates platelets and participates in the coagulation cascade. The CaCl\(_2\) replenishes the ACD-A-bound calcium ion, also a critical element in the coagulation cascade. Activation solution was drawn into two 1-mL syringes. The treatments, ie, platelet-rich and platelet-poor plasma, were drawn into two 10-mL syringes, respectively. A treatment syringe (platelet-rich plasma or platelet-poor plasma) and an activation syringe were connected, in tandem, to a dual spray apparatus (Micromedics, St Paul, Minn) (Figure 2). This allowed both syringe plungers to be advanced in unison, mixing the two sprays in a 1:10 (activation:treatment) volume ratio. In this way, the treatments (platelet-rich plasma or platelet-poor plasma) were activated prior to reaching the wound bed. During closure, activated platelet-rich plasma was sprayed onto the cut bone surfaces, synovia, tendons, and the joint capsule (Figure 3). The activated platelet-poor plasma was then applied to the subcuticular surface prior to closure. Closure was performed using Vicryl (Johnson & Johnson, New Brunswick, NJ) sutures on the extensor mechanism and subcutaneous tissues, and staples for the skin. Postoperative drains were placed in the lateral gutter of the knees in both patient groups. Drainage was collected into an autosuction collection system (Stryker Kalamazoo, Mich). Drainage from the canister was filtered and reinfused during the first six postoperative hours. A complete blood count was performed each morning during the hospital stay. Patients with a hemoglobin concentration <10 g/dL received a transfusion of packed red blood cells. Immediately after surgery, patients were allowed weight bearing as tolerated. During hospitalization, patients underwent continuous passive motion and physical therapist-supervised ROM exercises and gait training. Afterwards, physical therapy consisted of three weeks of outpatient strength and ROM exercises, for three sessions per week.

Patients were followed for six weeks postoperatively, documenting a variety of outcomes, ie, days to discharge, discharge location (home or rehabilitation), active ROM, number of units of transfused packed red blood cells used, hemoglobin level, pain (1-10 point scale with greater scores indicating greater pain), patient-controlled analgesia, and cellulitis.

**Statistical Analysis**

Treatment and control means were statistically compared using the two-tailed \(t\) test. Nominal data was compared using the Chi-square or two-tailed Fischer exact test. Ordinal data was compared using the Mann-Whitney rank sum test. Differences between means were considered to be significant for \(P<0.05\).

**Results**

The preoperative comparison of the control and treatment populations is summarized in Table 1. The only significant preoperative difference between the two groups was that there were significantly more women in the control population.
For both populations, morphine was the sole postoperative patient-controlled analgesia used by approximately 90% of the patients. During inpatient stay, the balance received a combination of morphine, meperidine, nalbuphine, and/or hydromorphone. In one case, no patient-controlled analgesia was required. For comparison purposes, the amounts of these analgesics were expressed in “morphine equivalents” based on the relative doses required. For instance, it was estimated that 1 mg of meperidine, nalbuphine, and hydromorphone were roughly equivalent (in effect) to 0.1 mg, 1 mg, and 5 mg of morphine, respectively.41-43

Table 2 summarizes the postoperative parameters collected for the two groups of patients.

Patients receiving the platelet-rich/platelet-poor plasma treatment were discharged from the hospital sooner, had greater postoperative active ROM through six weeks (with the exception of postoperative day 3), received fewer
units of transfused packed red blood cells, and had less hemoglobin decrement compared to baseline on the first two postoperative days than the control patients. There was no influence of platelet treatment on the location to which patients were discharged (home versus rehabilitation) or on the incidence of cellulitis. There were no deep infections in either group over the follow-up period. On average, treated patients had less pain on the first postoperative day and used less patient-controlled analgesia during inpatient stay; however, these averages were not significantly different than those of the control patients. Figure 4 shows the ROM values, Figure 5 shows the change in hemoglobin levels compared to the preoperative baseline, and Figure 6 shows the pain score on the first postoperative day, patient-controlled analgesia through the entire inpatient period in morphine equivalents, length of the inpatient stay, and the number of units of packed red cells used per patient. To fit the data in Figure 6 on the same axes, the values for a given type of measurement were normalized to the average respective control value.

Discussion

There are several reasons for the current clinical interest in using platelet concentrates to enhance wound healing. First, the immediate natural response of the body to tissue damage is the accumulation of large numbers of activated platelets at the injury site. These platelets, activated by contact with biomolecules (e.g., collagen) that become exposed on tissue damage, become sticky and form a platelet plug. For small vascular breaches, this may be sufficient to effect hemostasis. For large vascular defects, however, a clot is required. The activated platelets interact at several levels within the coagulation cascade, quickly forming a clot comprised of a fibrin mesh with entrapped platelets and red and white blood cells. Consequently, administration of a supraphysiological dose of platelets would be expected to improve hemostasis. Second, on activation, the -granules contained within the platelets fuse with the platelet plasma membrane and release their contents, a cocktail of over 30 bioactive proteins (growth factors, cytokines, and chemokines), outside of the cell. These proteins, which include PDGF-, TGF-, TGF-, and TGF-, as well as TGF-1 and TGF-2, collectively set the stage for tissue healing by attracting macrophages, mesenchymal stem cells, osteoblasts, and other cells that are responsible for the removal of necrotic tissue and regeneration of site-specific tissue. The bioactive proteins released by platelets act as chemotactic agents, morphogens, and mitogens. Secretion of presynthesized proteins occurs within 10 minutes of platelet activation; >95% are secreted within the first hour. The platelets then continue to synthesize and secrete these proteins for the balance of their 5-10 day lifespan. Thus, as with hemostasis, a supraphysiological dose of activated platelets might theoretically increase the rate of healing. Third, technology has become available that makes it convenient for the surgeon to remove a small amount (55-60 mL) of venous blood from the patient for processing to platelet-rich and platelet-poor plasma. Due to the small volume removed, the residual packed red blood cells do not require infusion back into the patient.
of controlled human clinical studies have shown an enhancement of wound healing. For example, Marx performed histomorphometry on core biopsies taken from the mandibles of 44 patients following bone augmentation for dental implants. He reported that there was a significant improvement in graft maturation and trabecular bone area after six months compared to grafts in which platelets were not used. Margolis et al performed a meta-analysis of >25,000 cases of neuropathic diabetic foot ulcers treated with or without platelet therapy. Ulcers treated with platelets were 14%-59% more likely to heal than those treated without. Pietrzak and Eppley summarized several other controlled, clinical studies that support the use of platelet-rich plasma.

Our study showed that TKA patients treated with platelet-rich and platelet-poor plasma had significantly shorter hospital stay after surgery, had improved ROM for six weeks, required fewer units of transfused packed red cells, and had an improved hemoglobin profile compared to patients receiving no platelet-rich/platelet-poor plasma. All of these improved outcomes can be rationalized by the known mechanisms by which platelets (and platelet-poor plasma) influence hemostasis and wound healing.

Mooar et al performed a similar study in 85 individuals, finding that TKA patients receiving platelet gel experienced a significantly lower reduction in hemoglobin levels (2.68 g/dL versus 3.12 g/dL), demonstrating a hemostatic effect, and achieved a significantly higher ROM (79.7° versus 72.1°) earlier (4.35 days versus 6.38 days) than did patients that did not receive platelet therapy.

Wang et al used a non-autologous fibrin sealant following TKA (22 patients) and compared results with a control cohort (24 patients). The decrease in hemoglobin level on the first postoperative day in fibrin sealant-treated patients was 28.9% less than in the control group (P=.0005).

Levy et al used a commercial fibrin sealant following TKA in a prospective, controlled, randomized clinical study. Fibrin-treated patients required significantly less transfused blood than did the control patients (P<.001) and exhibited less hemoglobin decrease (P<.001). Collectively, these studies corroborate many of our positive findings on the influence of platelet-rich plasma and platelet-poor plasma on outcomes following TKA.
Figure 6: Pain score on the first postoperative day, patient-controlled analgesia in morphine equivalents (morph eq), length of inpatient stay (days), and the number of units of transfused packed red cells. All scores are normalized to respective control values. Average±SD.

These positive findings suggest that the platelet-rich/platelet-poor plasma application can have a beneficial effect on TKA outcome as early as the day of surgery as shown by reduced transfusion requirement, to at least the sixth postoperative week as suggested by improved ROM. Certainly the hemostatic effect would be expected to be achieved quickly, within minutes of application, based on the speed with which the coagulation cascade reacts to form the clot. As stated above, the majority (>95%) of the presynthesized bioactive proteins are released within one hour of clot formation. Conceptually, the additional bolus release provided by the administered platelet-rich plasma could increase the rate of precursor cell migration and proliferation within hours and days. The ability of activated platelets to continue to produce and secrete these protein factors for 5-10 days can provide the opportunity to amplify healing within this period. To the extent that knee ROM is reflective of tissue healing around the implant, this suggests that the state of healing remained more advanced in the patients treated with platelet-rich and platelet-poor plasma at six weeks.

Some of the outcomes of the current study were statistically unaffected by the platelet-rich/platelet-poor plasma treatment. Although average pain on the first postoperative day and overall patient-controlled analgesia use was less for the treated patients than for the control patients, the differences were not significant. Also, there was no significant difference in the incidence of cellulitis between the two groups. Mooar et al36 reported TKA patients receiving platelet gel required significantly less intravenous and oral narcotics than did control patients. In that study, however, platelet gel was produced from 500 mL of autologous blood. It is possible that a greater amount of platelets were administered compared to our study, and this may account for the significant reduction in analgesia use. Advocates of the use of platelet concentrates believe that such treatment can decrease postoperative infection.49 Although there is little evidence for this, and this was not demonstrated in the current study, some potential mechanisms can be proposed. The buffy coat is comprised of not only platelets, but leukocytes as well. Thus, leukocytes are concentrated in platelet-rich plasma over baseline values. Their increased number may help to diminish infection. Additionally, platelet-induced migration of neutrophils and macrophages to the wound site might provide additional benefit.

As described above, patients treated with platelet-rich and platelet-poor plasma exhibited a significantly lower drop in hemoglobin levels during the first two postoperative days, suggesting a hemostatic effect of this treatment. Another method by which hemostasis might have been demonstrated is comparison of the collected wound drainage between the treatment and control cohorts. An attempt was made to perform such a comparison in this study until it was determined that errors incurred in the reporting of this data made the analysis unreliable. Prior studies have shown that collected wound drainage is significantly less following fibrin sealant-treatment in TKA patients. For example, Wang et al48 measured the collected drainage volume using wound drains. They found that within 12 hours postoperatively, patients treated with a commercial fibrin sealant (no platelet-rich plasma) yielded about half the volume of collected drainage as the control population (185.5 mL versus 408.3 mL), a significant difference. Levy et al37 also used drains and calculated blood loss through direct measurement, as well as by a calculation that uses the maximum postoperative decrease in hemoglobin adjusted for the height and weight of the
patient to account for extravasation of blood into the tissues. Mean measured postoperative blood losses were 360 mL for the fibrin sealant-treated patients and 878 mL for the control group ($P<.001$). Calculated means were 1063 mL and 1768 mL ($P<.001$), respectively.

There were several limitations to this study. First, its retrospective nature limited the comparisons that could be made between the two groups since the study design was not optimized prior to its performance. For instance, many factors may affect postoperative ROM, including preoperative, operative, and postoperative factors. Preoperative factors include gender, which was not equivalent between the two groups, with the control population having a significantly greater proportion of females. Operative factors include implant design. While a variety of implants were used, all shared many features in common (cemented, non-constrained, cruciate-retaining, primary knee prosthesis). In a similar study, Levy et al.\textsuperscript{37} also used a variety of total knee prostheses in their comparison of the effects of fibrin sealant on outcomes following TKA. Second, patients were followed only to the sixth postoperative week. From a healing standpoint, six weeks was felt to be sufficient for both cohorts to demonstrate substantial healing and return of function, as was seen. However, wound remodeling will continue for months and years after surgery, and longer follow-up, perhaps to 12 months or beyond, would be required to determine whether the ROM of the control patients would eventually become equivalent to that of the platelet-rich/platelet-poor plasma--treated patients.\textsuperscript{3} Third, more complete patient assessments, such as the Knee Society clinical rating system, SF-36, or Western Ontario and McMaster Osteoarthritis Index may have enabled a more critical comparison of outcomes between the two groups.\textsuperscript{51,52} Despite these limitations, several beneficial effects of platelet-rich and platelet-poor plasma on outcomes following TKA were demonstrated, and corroborated and extended results from prior studies.

The optimal use of platelet-rich and platelet-poor plasma is likely procedure- and site-specific and may be also specific to the equipment used in its production. Future work should include a prospective, randomized, blinded clinical study with follow-up to at least one year, to more fully determine the benefit of platelet-rich and platelet-poor plasma in TKA.

**Conclusion**

In this retrospective 6-week study, TKA patients treated with platelet-rich and platelet-poor plasma during wound closure demonstrated shortened hospital stay, improved ROM, improved hemoglobin profile, and reduced need for blood transfusion compared to control patients who received no platelet-rich or platelet-poor plasma treatment. On average, treated patients had reduced pain and used less narcotics than untreated control patients, but these differences were not significant. There was no difference in the incidence of cellulitis between the two groups.

**References**


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