PLATELET-RICH PLASMA: HARVESTING WITH A SINGLE-SPIN CENTRIFUGE

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KEY WORDS

Platelet-rich plasma
Centrifuge

This paper discusses the results recently seen by practitioners who use platelet-rich plasma, as well as the practical considerations that they must make when considering whether to adapt their practice for its use. In this study, an average of a 358% increase in platelet concentration was achieved.

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INTRODUCTION

The ability to speed up initial healing of soft tissue with autologous platelet concentrate derived from whole blood has been well documented.[1–5] The short- and long-term effects of speeding up and enhancing grafted bone healing are under study and show a lot of promise. However, one of the drawbacks of instituting the use of platelet-rich plasma (PRP) in a private general dentistry office is the initial cost of the commercially-available automated double-spin centrifuge and the necessary disposable component parts that are required for each patient. The initial investment can be as high as $8000, with each patient requiring a disposable component of approximately $250. For a small implant practice that places 150 implants or fewer per year, this is a sizable investment to add to the expense of maintaining an implant surgical and restorative practice.

However, the use of PRP offers the promise of faster soft-tissue healing and potentially faster graft maturity. A minimal initial investment of about $800 appears reasonable to make this relatively new technique available for most offices. This appears possible with the equipment and techniques described herein. Anecdotal experience with this technique has shown soft-tissue healing in 7 to 10 days that used to require 3 weeks, prior to using PRP. This study offers an economic alternative that permits the use of PRP.

MATERIALS AND METHODS

According to Kevy and Jacobson,[6] the maximum potential harvest from a single-spin centrifuge typically yields 56% (±4%) of the total platelets from whole blood. This could potentially yield a maximum of a 520% to 600% increase in the platelet concentration in 1 mL of PRP harvested from 10 mL of whole blood. The potential maximum of percentage platelet concentration fluctuates depending on the ratio of whole-blood volume to the target volume of PRP.

This case study was conducted in the writer's private practice and is based on 32 blood draws from 32 different persons. Local clinical pathologists used a coulter counter to measure initial blood platelet count against the harvested PRP platelet count. An average of a 358% increase in platelet

concentration was achieved. (The percentage reflects how the coulter counter reads clumped platelets as single platelets.)

A Clinaseal centrifuge purchased from Salvin Dental Specialties (Charlotte, NC) was used to concentrate platelets in the plasma referred to as PRP. The target was 7 mL of PRP. The amount of blood drawn was approximately 65 mL. Eight mL of whole blood was kept intact with its anticoagulant citrate dextrose (ACD) additive in a 10-mL yellow-topped Vacu-Tube. Six additional 10-mL Vacu-Tubes were filled with the individual patient's whole blood and spun in the Clinaseal centrifuge for 10 minutes. Using a blunt 16-gauge catheter, all but approximately 0.8 mL of the platelet-poor plasma (PPP) (the clear yellow fluid) was drawn off the top of each Vacu-Tube. The blunt catheter was then placed in the Vacu-Tube in the remaining PPP, and approximately 1.2 mL of fluid was removed from each tube. The layers removed included the PPP, the layer of platelets, the additional cells spun out of the red blood cells (RBCs) (including at least 3 growth factors: platelet-derived growth factor, transforming growth factor beta 1, and transforming growth factor beta 2), and approximately 0.3 mL of the top layer of RBCs.[7,8] A total of 6 to 7 mL of fluid was harvested from the 6 Vacu-Tubes and tested for platelet count. This harvest was labeled PRP. The original whole-blood tube was tested for platelet count, and the PRP platelet count was compared with the whole-blood platelet count.

TECHNIQUE USED FOR HARVESTING PRP AND PPP

The technique used to harvest platelets and plasma is the critical part of this procedure. It is legal to use a standard single-stage centrifuge to harvest PRP in your office.

The equipment used consisted of the following:

- Clinaseal centrifuge (Figure 1)
- 6 yellow-topped 10-mL Vacu-Tubes (citrate additive included) (Figure 2)
- 6 red-topped 10-mL Vacu-Tubes
- A tube rack to hold the 10-mL Vacu-Tubes
- 17- or 18-gauge butterfly or blood collection system
- 3-inch 16-gauge blunt catheter
- 30-mL syringe to collect the PPP
- 10-mL syringe to harvest the PRP
- 1-mL syringe (Calcium chloride [CaCl]/thrombin − 1000 units of bovine thrombin with 1 ml of 10% CaCl)

Use a blood-collection kit to draw blood directly into the 6 yellow-topped Vacu-Tubes, using at least a 19-gauge needle (Figure 3). Gently rock each tube back and forth to incorporate the whole blood and ACD. Place the 6 tubes into the centrifuge and spin them for 10 minutes.

If you are planning on intravenous (IV) sedation, you may draw the whole blood through a 17- or 18-gauge butterfly needle into a 60-mL syringe. First, draw 8 mL of ACD into the 60-mL syringe. Next, using standard venous blood collection techniques, draw approximately 52 mL of whole blood into the 60-mL syringe. Keep the tourniquet/blood pressure cuff tight and inflated during the blood withdrawal to avoid collapse of the vein. Attach the butterfly to the IV line. Release the arm tourniquet/blood pressure cuff and start the IV solution flowing. After rocking the 60-mL syringe gently back and forth to mix the anticoagulant and the whole blood, place 9 mL of the whole blood, with ACD, into the 6 10-mL red-topped Vacu-Tubes (red-topped to indicate that there are no additives in the tube). The tubes are placed into the centrifuge and spun for a total of 10 minutes (Figure 4).

Using a 30-mL syringe with a sterile 16-gauge blunt catheter, draw off the PPP from each Vacu-Tube by placing the 16-gauge catheter in the yellow top layer, leaving approximately 0.8 mL of the fluid (Figure 5). Next, using the same blunt 16-gauge catheter attached to a 10-mL syringe, draw off approximately 1.2 mL from the top of the remaining fluid in each tube (Figure 6). This will be composed of approximately 0.8 mL of PPP, the platelets, the cytoplasmic granules, and approximately 0.3 mL of the very top of the RBCs (Figure 7). This will yield a total of 6 to 7 mL of fluid in the 10-mL syringe harvested from the 6 Vacu-Tubes. This is PRP (Figure 8).

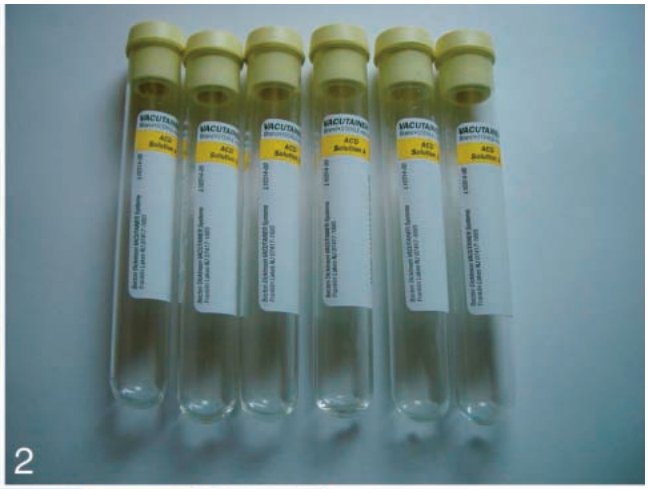
A total of approximately 7 mL of PRP should now be available. When gently mixed, the resultant PRP will become pink to red. The goal is to have a small amount (>15%) of the very top of the RBCs in the mixture with the buffy coat layer. The top 10% of the RBCs holds a large percentage of the less mature platelets.[7]

When the surgeon is ready to use the PRP, add approximately 0.1 mL of the CaCl/thrombin to each milliliter of PRP you wish to activate (initiate clotting). You may place some PRP mixed with the CaCl/thrombin into a glass container. In about 30 minutes, the mixture becomes a gel-like biologic membrane that can be used to cover the graft material and the collagen or vicryl membrane that you may use to contain and isolate the graft material from soft-tissue invasion. An additional use of the PRP is to mix it with particulate graft material, which produces a gel-like consistency that is easy to handle.

After the surgical site is sutured, it is helpful to take a saline-soaked 2 × 2 gauze and firmly hold the area together for several minutes to finish shaping and setting the grafted site. A final covering of the surgical site with PRP helps create a fibrin clot over the wound.

TECHNIQUE FOR USE OF CACL AND THROMBIN TO CLOT THE PRP AND PPP

The materials required for this portion of the procedure are 5000 units of bovine thrombin powder (requiring 5000



FIGURES 1–3. FIGURE 1. Single-spin centrifuge. FIGURE 2. Vacu-Tubes with anticoagulent citrate dextrose (ACD). FIGURE 3. Blood collection system.

units stored between 2.2°C and 25°C) at an approximate cost of $45 and 5 mL of 10% CaCl at an approximate cost of $1 (25 10-mL vials for $25). Calcium chloride and bovine thrombin can be purchased at most university hospital pharmacies or ACE surgical specialties (Figure 9).

Hydrate the 5000 units of bovine thrombin powder in its 10-mL vial with 5 mL of 10% CaCl. You now have 1000 units of bovine thrombin mixed with 1 mL of 10% CaCl for every 1 mL of CaCl you draw out of the thrombin vial. Using a 1-mL syringe, add approximately 0.15 mL of thrombin/CaCl to each milliliter of PRP you wish to activate. Clotting takes between 5 and 30 seconds.

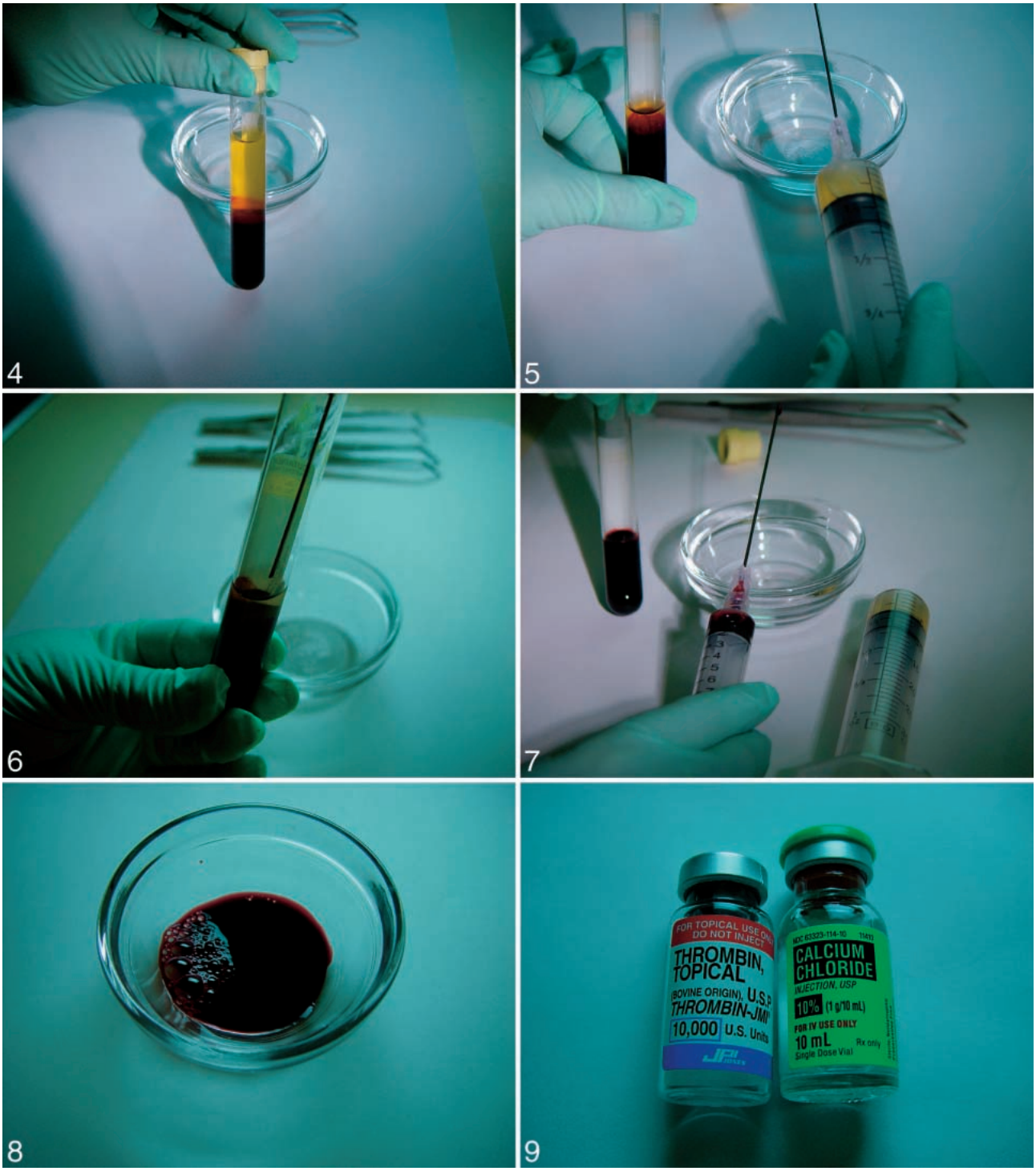
DATA RESULTS

In this study there was a variety of harvest yield of PRP, with a variation of 268% to 462% increase in platelet count and with an average yield of 356%, above the original whole-blood platelet count. In Marx et al's[7] recent study, the Clinaseal centrifuge, using only 27 mL of whole-blood volume with an initial whole-blood platelet count of 258 000, yielded a harvest of 7.6 mL of PRP that showed an average platelet concentration of 401 000, which is a 150% increase. Mathematically extrapolating from these data, it can be estimated that 7 mL of PRP harvested from 50 mL of whole blood would have given a harvest of 874 180, or an increase of 339%, in platelet concentration. Marx et al[7] concluded that both the SmartPReP and Clinaseal centrifuge delivered viable platelets for clinical use. Personal experience with the Clinaseal centrifuge suggests that the 10-mL Vacu-Tubes work best in the centrifuge, using 2, 3, 4, or 6 of them. Depending on how much PRP practitioners require, the initial collection of whole blood averages 16, 24, 36, or 54 mL.

DISCUSSION

An autologous source of growth factors to enhance soft-tissue healing and graft maturation is possible through the use of PRP.

In private dental offices, the expense of harvesting PRP has prevented



FIGURES 4–9. FIGURE 4. Blood separation. FIGURE 5. Platelet-poor plasma. FIGURE 6. Harvesting platelet-rich plasma (PRP). FIGURE 7. Collected PRP. FIGURE 8. PRP. FIGURE 9. Thrombin and calcium chloride.

some practitioners from incorporating this natural source of healing enhancement into grafting procedures. The presented technique appears to provide a reasonable source of PRP for each patient, with minimal staff training and cost. The centrifuge and supporting equipment is an initial investment of about $800.

The centrifuge used in this study has the capacity to process from 18 to 54 mL of whole blood in less than 20 minutes. Except for the initial blood draw, this process can be done almost exclusively by a trained assistant.

Summary

The use of a single-spin centrifuge appears to be warranted for those practitioners who are able to develop a technique to achieve a consistent PRP platelet concentration of approximately 300% above whole-blood levels. More clinical studies will be needed to see if the healing efficacy of an automated dual-spin centrifuge is superior to that of a single-spin centrifuge.[9]

Note

The author of this paper is a private practitioner with no financial interest or affiliation with Salvin Dental or any other company mentioned in this article and has received no remuneration for this research.

The following is a list of suggested reading material:

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