

A Simplified Technique for Producing Platelet-Rich Plasma and Platelet Concentrate for Intraoral Bone Grafting Techniques: A Technical Note

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A method to produce platelet-rich plasma and platelet concentrate using a double centrifuge technique in combination with the fibrin adhesive Tisseel is described. This technique constitutes the basic mixture for augmenting and improving an inadequate bone site. Also described is a procedure by which autologous bone or bone substitute is added to this mixture to increase the volume of grafting material. Platelet concentrates cause growth factors to be delivered to graft sites in an intense form, while Tisseel serves as a standardized, pharmaceutically manufactured fibrin adhesive. (INT J ORAL MAXILLOFAC IMPLANTS 2000;15:879-882)

Key words: alveolar ridge augmentation, factor XIII, fibrin tissue adhesive, plasmapheresis, platelets

Platelet-rich plasma (PRP) and platelet concentrates (PC) made from autologous blood are used to deliver growth factors in high concentrations to the site of a bone defect or a region requiring augmentation.¹ The extraction of platelet concentrates through plasmapheresis is a process by which only PRP is taken from the patient and the remaining components of blood are delivered back into the body. This technique causes PRP to be produced at a concentration of 300% of normal blood levels.¹ For practical economic reasons, this procedure is generally only suitable within larger clinics or hospitals.

Platelet-rich plasma is usually mixed with ground autologous bone for optimum results. It is then delivered to the recipient bed along with bovine thrombin (the thrombin being previously diluted with 10% calcium chloride to nullify the citrate effect) and placed in the graft site. The PRP mix-

ture is typically applied in a layered fashion to establish contour. The fibrinogen present in the PRP is activated and becomes cross-linked to form a fibrin network.^{2,3} Thus, the graft is solidified and adheres within the defect. This technique is also advantageous when used with granulated bone substitutes, such as bovine bone, hydroxyapatite,^{4,5} or beta-tricalcium phosphate granulates. The advantages, disadvantages, and actual compatibility of the numerous available materials and their mixture ratios have been previously reported.⁶

Nevertheless, quantitative and qualitative measurements have shown that autologous bone grafts treated with PRP mature within two-thirds of the non-PRP graft's time, have a 1.6- to 2.6-fold higher radiopacity, and are 70% more mature than untreated, naturally occurring bone at the site.¹

A simple variation of this method for filling extraction sites and to improve the quality of bone for subsequent placement of dental implants is to draw 5 mL of blood in a citrate vacuole. This is centrifuged at 160g for 6 minutes. The PRP is then pipetted out and mixed with calcium chloride (50 µL, Eppendorf pipette). After 15 minutes the coagulum has solidified and is introduced into the extraction socket as a graft to improve bone quality on healing.⁷

A further variation of this technique for more extensive graft sites, such as the maxillary sinus, will be described here.

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Fig 1 Electronically controlled centrifuge.

DEFINITION OF TERMS

After a first centrifugation the following can be differentiated:

- Platelet-poor plasma (PPP): Top level of the serum, which contains autologous fibrinogen and is poor in platelets.
- Platelet-rich plasma (PRP): Second level of the serum, which contains autologous fibrinogen but is rich in platelets.
- Demarcation line: A whitish layer on top of the red-colored blood cell fraction, which is rich in platelets and white blood cells.
- Blood cells: The red-colored fraction of the second level, containing mainly red blood cells and platelets. The upper 6 to 7 mm are very rich in fresh, young platelets; below this, the platelet concentration decreases.

After a second centrifugation, the final fractions develop and are referred to as:

- Platelet-poor plasma (PPP): A top level of clear yellow serum with fibrinogen and a very low concentration of platelets.
- Platelet concentrate (PC): A small amount of very concentrated platelets at the bottom of the centrifugation tube.

MATERIALS AND METHODS

Adhesive

Tisseel fibrin adhesive (Baxter Healthcare Corporation, Deerfield, IL) has been available for more than

2 decades. This tissue adhesive is used for a variety of purposes in surgery (eg, vascular endoscopic surgery). The product was developed in the early 1970s by Matras.² Publications confirm its effectiveness, safety, and compatibility, as well as its simple use and application.^{2,3} Tisseel adhesive is produced from human serum and consists mainly of 2 components: a concentrate of fibrinogen, enriched with factor XIIIa, and thrombin, to which calcium chloride is added. The adhesive is available in 2 different forms:

1. Deep-frozen, as Tisseel Duo Quick. This consists of prefabricated fibrinogen and thrombin, each packaged in separate syringes within an applicator system. Tisseel Duo Quick adhesive must be stored at -18°C .
2. Lyophilized. It is recommended that this product be processed using a mixing and temperature-controlled device. This Tisseel lyophilized kit must be stored in a refrigerator (at 4°C).

Extraction of PRP and PC

Depending on the size of the defect, 3 to 8 vacuettes of citrated blood, each consisting of 6 mL, are drawn from the patient and centrifuged for 20 minutes at 1,200 rpm (160g) using a standard electronically controlled bench-top centrifuge (Hettich Universal 32, Tuttlingen, Germany) (Fig 1). The centrifuge can hold up to 16 vacuettes of blood. This results in a red, opaque lower fraction—the *blood cell component* (BCC), consisting of red and white blood cells and platelets—and a second, upper straw-yellow turbid fraction with plasma and platelets, called the *serum component* (SEC) (Fig 2).

To maximize the platelet concentration, a point is marked 6 to 8 mm below the dividing line between these 2 phases, within the BCC, with a waterproof permanent marker. The entire SEC and BCC up to this point is pipetted out and into a fresh, sterile vacuette without citrate. This pipetted material is again centrifuged for 15 minutes at 2,000 rpm (400g), and the top yellow SEC is removed. The remaining substance, approximately 0.5 mL in quantity, is the available PC (Fig 3).

Detailed measurements have shown that the platelet content after the first centrifugation, starting from the top limit of the SEC and measured in 250- μL intervals, has a concentration of 22,000 to 24,000 platelets. From a point 6 mm below the upper limit of the BCC, the platelet count increases to 37,000 to 45,000 per 250 μL . Within the first 6 mm of the BCC, the platelet count increases to 90,000, and at 9 mm into the BCC, the platelet concentration drops to 53,000.

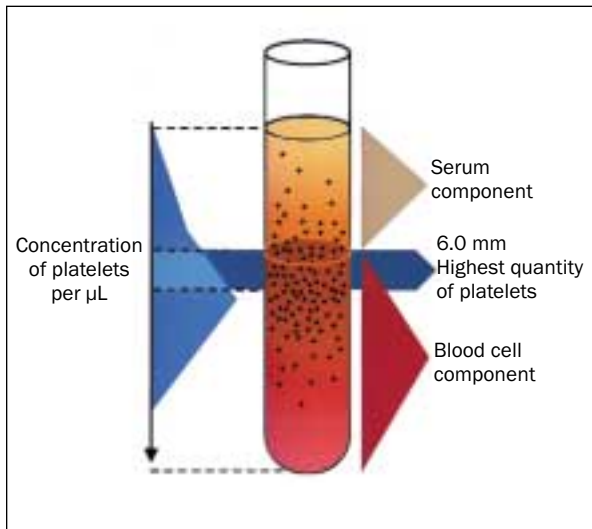


Fig 2 First centrifugation.

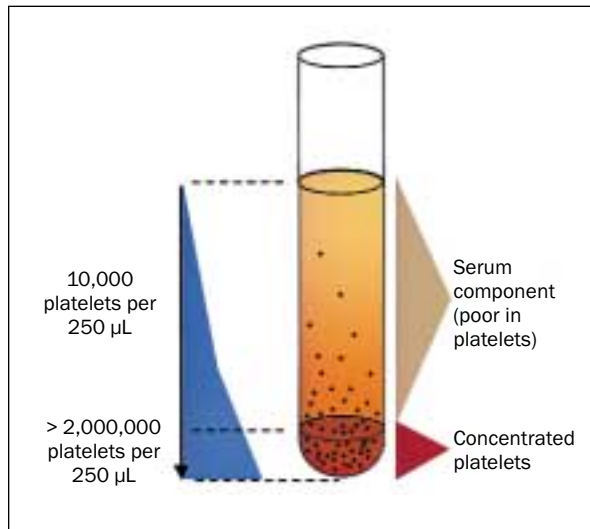


Fig 3 Second centrifugation.

When pipetted and measured in 250- μ L portions, the second centrifugation provides fraction values between 8,000 and 11,000 platelets in the upper yellow SEC. When the red component is measured, the platelet cell count indicates that the measurable limit of 2,000,000 has been exceeded. In the zone of transition into the red phase (buffy coat), the proportion of lymphocytes is high. This is of interest, because lymphocytes also release growth factors² and should be used in the mixtures for this very reason.

Processing

It is best to centrifuge the serum as freshly as possible and to prepare the graft product in the operating room. If the described chairside technique is too time-consuming for the operator, the required quantity of platelet concentrate may be prepared in advance in a blood laboratory. It can then be processed with the graft material in the operating room. However, the question arises as to how long it takes for the alpha granules of the platelets to be degranulated and for growth factors to be lost.

Once the PC is produced, it is mixed with the preferred augmentation material, and then the fibrinogen of the Tisseel adhesive is added, so that a readily malleable transplant material is obtained. This filler is introduced to the site in layers, with thrombin dripped over it for the purpose of consolidation. Alternatively, it can be molded to form outside the oral cavity and then applied and fixed with thrombin adhesive. Measurements of the needed quantity of augmentation volume have previously been published.⁸ A sinus graft procedure requires 4

to 5 Vacuettes of blood for each sinus, combined with 1 to 2 mL of the Tisseel adhesive and adequate quantities of autologous bone or bone substitute material.

A second option is to mix PRP with the fibrinogen component alone in a 1:1 ratio. This mixture is allowed to flow onto a glass plate or a small flat cup and is consolidated by coating with thrombin. This creates a flat, membrane-like structure. It is elastic and silicone-like in consistency and can be shaped with a scalpel. This product is used like a membrane to cover fenestrations and small defects, or it can be used to fill small bone cavities (eg, at the donor site, extraction site, or sinus membrane). To fill a defect in a single dental region, 2 to 3 vacuettes with 0.5 mL adhesive are required. After the membrane-like material is applied, about 1 minute is allowed to pass before primary wound closure is achieved.

DISCUSSION

The application of fibrin adhesive as a carrier for pharmaceuticals has been reported.^{9,10} The use of Tisseel with augmentation material and PC is described in this article. This combination creates a very stable and dense fibrin network, which is more compact, as if fabricated with autologous fibrinogen, because of the fact that fibrinogen and factor XIII are concentrated in the Tisseel adhesive. The successful use of Tisseel fibrin glue in tissue remodeling has been reported previously.¹¹⁻¹⁴ Also, the honey-like consistency of the fibrinogen in the Tisseel adhesive makes application easy. The choice

between fast and slow processing additives extends its range of application.

Tisseel is a product approved in the European Union and the United States and has a wide variety of surgical uses. It has been approved by the FDA for use as an adjunct to hemostasis in surgeries involving cardiopulmonary bypass and treatment of splenic injuries resulting from blunt or penetrating trauma to the abdomen, when control of bleeding by conventional surgical techniques, including suture, ligation, and cautery, is ineffective or impractical. Tisseel has also been shown to be an effective sealant as an adjunct in the closure of colostomies. The majority of its current use would be classified as off-label.

The use of standard 6-mL vacuettes for drawing blood is a patient-friendly and common standard for procuring reasonable quantities of blood because it presents a closed system. Furthermore, it provides a uniform level of safety for the operator. Equipment required for this technique is readily available from commercial medical suppliers, and the centrifuge has a footprint of 36 in², which facilitates placement in the average-sized operator.

CONCLUSION

A simplified technique utilizing commercially available blood procurement products and a pharmaceutically available, clinically proven, widely used tissue adhesive has been described. This technique has demonstrated increased efficiency for handling PC graft materials. It provides a less costly alternative to other previously described augmentation techniques and also presents a patient-friendly and operator-safe alternative.

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