

# Platelet Concentrate Preparation: A Comparison of the Smartprep 2® with the B.T.I. PRGF System

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## INTRODUCTION

Platelet concentrate (PC) is an autologous source of growth factors obtained by concentrating freshly drawn blood. Platelet alpha granules release these growth factors in specific ratios to one another. Growth factors act locally to (1) recruit undifferentiated cells to the site of injury 1 and (2) trigger mitosis in these cells.<sup>2,3</sup>

Following the initial report of Marx et al, its use to promote tissue healing had become popular in oral and maxillofacial surgery.<sup>1</sup> Clinically the platelet concentrate is most often applied as a concentrated platelet gel following the addition of 10% calcium chloride or bovine thrombin solution (1000U/ $\mu$ l). This results in the release of growth factors such as PDGF-AB, TGF-B1, VEGF, and basic fibroblast growth factors.

Recently, PC has been applied clinically to facilitate bone and tissue healing. Trials have demonstrated that using PC during the placement of dental implants promotes osseointegration and bone regeneration.<sup>4,5</sup> The use of PC in maxillofacial reconstruction and periodontal regenerative therapy has been proven very successful.<sup>6,7</sup> Marx reported that PC enhanced the bone maturation rate and bone density 1.62 to 2.16 fold using clinical, histological and histomorphometric methods.<sup>1</sup> The mean platelet concentrate was 3.4x baseline. The effect is not limited to osteoblasts. Gfatter et al. demonstrated the efficacy of activated platelets bound on fibrinogen in the mitosis of fibroblasts, which (effect) is a response to growth factors secreted by platelets.<sup>8</sup>

It is important that the process for producing a PC is capable of concentrating as many platelets from the blood sample as possible and that these platelets can release the desired growth factors. The more growth factors that can be delivered to the injury site, the greater the potential to enhance the healing process. The quality of a PC must also be evaluated by two in vitro tests; platelet aggregation and P- selectin. The later test has been demonstrated to correlate with in vivo platelet survival.

## OBJECTIVE

The study was designed to compare the platelet concentrate (PC) prepared by the Smartprep 2 (Harvest Technologies, Plymouth, MA) with the PRGF System (Biotechnology Institute).

## MATERIALS AND METHODS

Blood was collected from normal donors utilizing the anticoagulant recommended for each of the systems. Processing was performed according to the manufacturer's instructions. The blood samples obtained from each donor were collected both in sodium citrate for the BTI system and ACD for the Harvest Smartprep 2 system. The comparison was made using 20 ml volumes of whole blood. This would require the use of 4 of the sodium citrate tubes provided in the BTI- PRGF kit.

## RESULTS

Table 1 lists the centrifuge protocols used as well as the number of sterile barrier entries and nurse/technologist time. Since both systems require an identical blood draw this is not accounted for in the table. The BTI system requires the use of 4 sodium citrate tubes, which can require up to 36 sterile barrier entries into an open vial. The Smartprep 2 results in a total of 5 sterile barrier entries that are aseptically prepared according to standard hospital protocol.

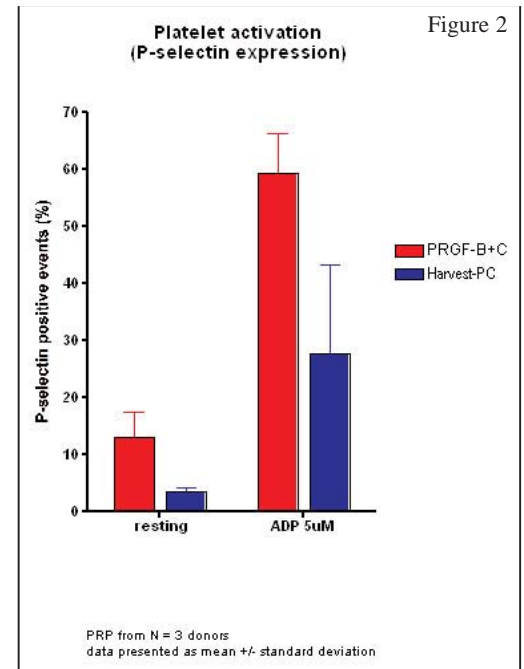
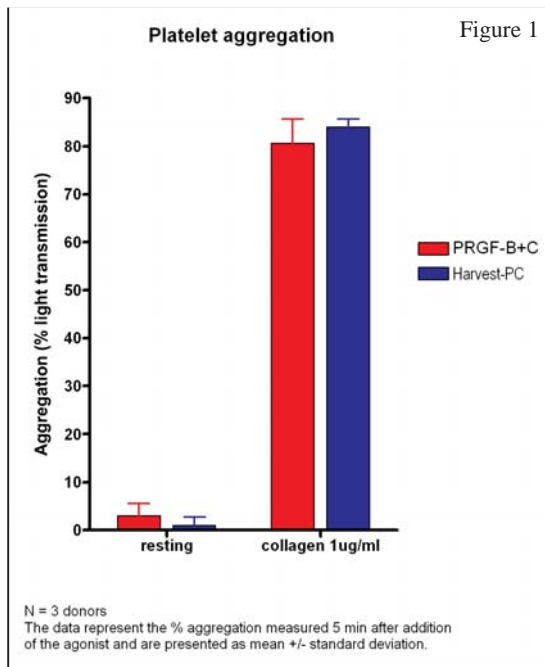
| Protocol             | Centrifuge Spins/time (min)<br>G force | Whole Blood Volume Processed (ml) | Sterile Barrier Entries | Technologist Time (min) | Process Time (min) | Total Time (min) |
|----------------------|--|-----------------------------------|-------------------------|-------------------------|--------------------|------------------|
| Smartprep 2Automated | Automated                              | 20                                | 5                       | 3                       | 14                 | 17               |
| BTISingle Spin       | 1 460g 8min                            | 20<br>4 tubes                     | 36                      | 15                      | 8                  | 23               |

Table 2 summarizes and compares the platelet products produced by both the systems. As expected the volume of the concentrates (3.0 - 3.5 ml) were identical for both systems. The Smartprep 2 produces a greater platelet yield. This is reflected in the increased levels of growth factors (Table 2). The level of VEGF in the Smartprep2 concentrate is almost 6 times that of PRGF. This reflects the increased concentration of granulocytes in the Smartprep 2 concentrate. VEGF is the most potent angiogenic growth factor.

**Table 2 - A Comparison Analysis of Growth Factor Release**

| Device                | PRP  |                  |                                |             |               |              |            |
|-----------------------|--|------------------|--------------------------------|-------------|---------------|--------------|------------|
|                       | Baseline Plt Concentration 10 <sup>3</sup> /μl | Product Vol (ml) | Plt Conc X 10 <sup>3</sup> /μl | Plt Yield % | PDGF-AB ng/ml | TGF-β1 ng/ml | VEGF pg/ml |
| Smartprep 2 Automated | 238 ± 87                                       | 3.5              | 774 ± 284                      | 63.2 ± 3.3  | 88.5 ± 22.5   | 137 ± 27     | 368 ± 74   |
| BTI N=3               | 198 ± 94                                       | 3.5              | 320 ± 35                       | 34.3 ± 10.1 | 22.5 ± 9.6    | 42 ± 9       | 65 ± 36    |

The two in vitro tests of platelet viability/functionality are platelet aggregation and P-selectin. The former is the binding of platelets to other platelets following stimulation by an agonist utilizing an optical aggregometer. The normal values obtained using collagen 200 μg/ml are 81 ± 12 %. The alpha-granule membrane protein, P-selectin, is sequestered on the internal membrane of the alpha granule in resting platelets. When platelets are activated the alpha granule fuses with the platelet membrane, which allows P-selectin to be detected on the platelet surface. A freshly prepared platelet concentrate will have a ±10% value which increases to 20% after 5 days storage. There is a three-fold increase with the addition of ADP.



The results of the platelet aggregation studies are shown in Figure 1. The data compares the concentrate prepared from 3 donors and is presented as a mean ± standard deviation.

The results of the P-selectin expression for the same three donors are shown in Figure 2. The baseline levels for the PRGF are slightly higher than that of the Smartprep 2, but within normal limits. This could be a reflection of the very high pH (7.4) of the sodium citrate based anticoagulant.

### CONCLUSION AND SUMMARY

The PRGF technique is essentially an open system, which is not desirable for any surgical procedure. The sterility of platelet concentrates prepared by the Smartprep 2 immediately following and 8 hours post-processing has been demonstrated using the culture method specified by the FDA-CBER for platelets prepared for transfusion.

The BTI-PRGF system is labor intensive and yields low levels of both platelets and growth factors compared to the Smartprep 2 product.

Both systems can be clotted with either a bovine thrombin solution or 10% calcium chloride.

### References

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