

## LETTER TO THE EDITOR

# Where have all the platelets gone? A simple solution to the suboptimal performance of PRP tubes that contain a thixotropic gel separator

To the Editor,

The interest in platelet-rich plasma (PRP) within dermatology has been increasing despite modest evidence supported by randomized controlled clinical trials. Recently, Sadick et al. reported on the poor platelet counts observed in USA FDA-approved PRP kits that utilize a thixotropic gel separator.<sup>1</sup> As we observed similar results in our laboratory, we sought to understand the root cause underlying the suboptimal platelet counts. One of the issues identified was that during the process of preparing PRP from whole blood, a large portion of platelets would get trapped in the thixotropic gel separator, a common component of many PRP devices on the market. To investigate this issue, we evaluated how using an inversion versus a patented agitation method affects platelet counts during whole blood PRP preparation. Five healthy volunteers consented to having their PRP prepared using three different FDA cleared devices (A: PROGEN PRP™; Crown Aesthetics, B: REGENKIT® BCT; Regenlab USA, and C: Selphyl® PRFM; Factor Medical). All samples were prepared according to the manufacturer instructions (Table 1), but

instead of inverting the tube several times per manufacturers instructions, we applied an agitation method covered under US patent no. 11654428.<sup>2</sup> In this method, tubes are agitated by hand along the long axis in 5 s intervals for up to 25 s with the goal of releasing the platelets from the gel, resulting in higher platelet counts and platelet capture efficiency (Video S1). Compared with the standard inversion method (Figure 1A), the agitation method resulted in a darker PRP lysate with the presence of foam (Figure 1B). The overall average platelet count was  $263 \pm 15.8$  K/ $\mu$ L for whole blood, and  $278.3 \pm 18.0$  K/ $\mu$ L for PRP with inversion. Compared with inversion, platelet counts from PRP with agitation were significantly higher ( $371.13 \pm 23.0$  K/ $\mu$ L;  $p < 0.01$ ). The fold increase of platelet concentration using inversion versus whole blood was only  $1.06 \pm 0.03$ , which significantly increased with agitation to  $1.42 \pm 0.04$  ( $p < 0.01$ ). In addition to an increase of platelet counts, we observed a concomitant increase in the amount of white blood cells in suspension. This is a notable finding as it suggests that due to their similar density profiles white blood cells are released together the denser platelets

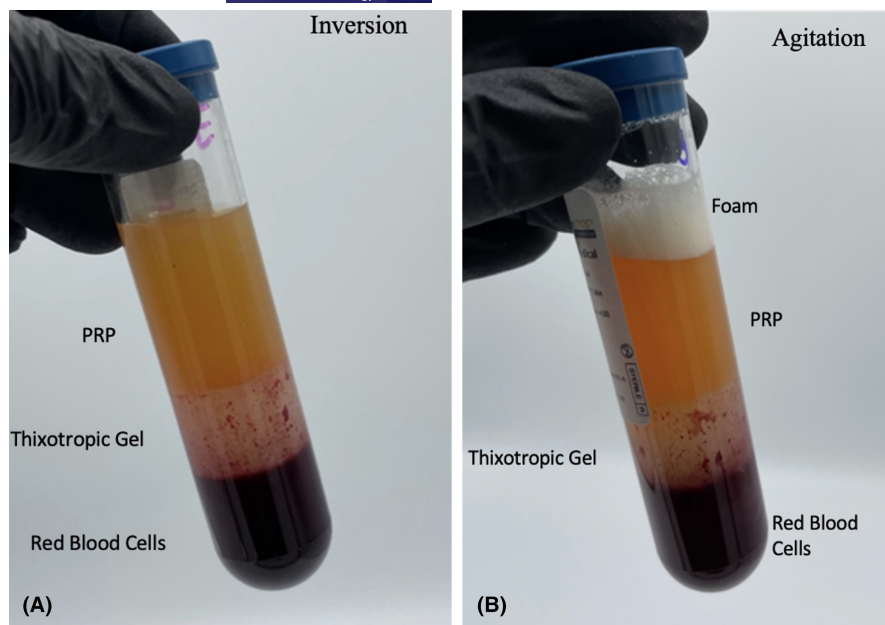
**TABLE 1** Platelet concentration, factor increase in platelet concentration, platelet capture efficiency, white blood cell and hematocrit in whole blood, platelet plasma inversion, or agitation.

	Platelet concentration (K/ $\mu$ L)	Factor increase in platelet concentration	Platelet capture efficiency (%)	White blood cells (K/ $\mu$ L)	Hematocrit (%)
Whole blood	$263 \pm 15.8$	–	–	$7.68 \pm 0.4$	$42 \pm 0.74$
Mean Inversion	$278.3 \pm 18.0$	$1.06 \pm 0.03$	$55.8 \pm 0.45$	$0.45 \pm 0.1$	$0.04 \pm 0.01$
Mean Agitation	$371.1 \pm 23.0$	$1.42 \pm 0.04$	$77.7 \pm 2.6$	$2.64 \pm 0.4$	$0.11 \pm 0.02$
PROGEN Inversion	$285 \pm 36.5$	$1.1 \pm 0.04$	$56.1 \pm 1.58$	$0.9 \pm 0.1$	–
PROGEN Agitation	$408 \pm 50.7$	$1.53 \pm 0.03$	$84.2 \pm 1.93$	$3.62 \pm 0.11$	–
REGEN BCT Inversion	$259 \pm 34.8$	$0.97 \pm 0.04$	$58.3 \pm 4.31$	$0.22 \pm 0.05$	–
REGEN BCT Agitation	$335.8 \pm 35.9$	$1.3 \pm 0.07$	$82.6 \pm 4.31$	$3.7 \pm 0.13$	–
SELPHYL Inversion	$291 \pm 27.0$	$1.13 \pm 0.05$	$52.9 \pm 3.56$	$0.6 \pm 0.08$	–
SELPHYL Agitation	$369.6 \pm 32.6$	$1.4 \pm 0.06$	$66.4 \pm 3.15$	$0.6 \pm 0.08$	–

Note: Manufacturer "A, PROGEN PRP™; Crown Aesthetics": 30 mL whole blood, ACD-A anticoagulant, no activator, 1600g for 10 min using swing bucket centrifuge. Manufacturer "B, REGENKIT® BCT; Regenlab USA": 10 mL whole blood, ACD-A anticoagulant, no activator, 1500g for 10 min using fixed bucket centrifuge. Manufacturer "C, Selphyl® PRFM; Factor Medical": 9 mL whole blood, sodium citrate anticoagulant, Calcium Chloride activator, 1100g for 6 min using swing bucket centrifuge.

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**FIGURE 1** (A) Horizontal swing bucket centrifugation using inversion for PRP. Inversion leads to fewer platelets, no foam and a lighter color. (B) Horizontal swing bucket centrifugation using agitation for PRP. Agitation protocol results in the presence of foam, darker color and higher platelet count.

that tend to have a higher payload of growth factors.<sup>3</sup> Hematocrit levels remained very low with either inversion or agitation methods (inversion  $0.04 \pm 0.01\%$  vs. agitation  $0.11 \pm 0.02\%$ ). In sum, we present a simple method to increase the yield of platelets in PRP preparation when using FDA-approved devices containing thixotropic gel separators. Improved platelet concentration, together with the presence of white blood cells that are thought to be conducive to the regenerative properties of the PRP preparation,<sup>4</sup> can help unlock the true potential and impact of PRP in various dermatologic applications. We encourage all manufacturers to independently evaluate and adopt these methods. Future studies are warranted to assess if higher platelet counts lead to better clinical outcomes with PRP prepared with agitation as compared to traditional inversion.

#### CONFLICT OF INTEREST STATEMENT

None disclosed.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

#### ETHICS STATEMENT

Written informed consent was obtained from the patients.

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