Original Article

Assessment of Quality of Platelet-Rich Plasma Produced With Desktop Centrifuge and Comparison With Standardized Commercially Available Platelet-rich plasma

Ramesh Srinivasan V1, M. Rekha1, Effie Edsor1, S. Pradeep Raja1, T. Dinesh Kumar2, S. Kalaiselvan3

1Department of Oral and Maxillofacial Surgery, RVS Dental College and Hospital, Coimbatore, Tamil Nadu, India, 2Department of Oral and Maxillofacial Pathology, RVS Dental College and Hospital, Coimbatore, Tamil Nadu, India, 3Department of Oral and Maxillofacial Surgery, CSI Dental College, Madurai, Tamil Nadu, India

Aims: The aim of this study was to assess the quality of platelet-rich plasma (PRP) produced by in-house desktop centrifuge method and compare it with that of standardized commercial PRP. Materials and Methods: REMI desktop centrifuge was used to prepare PRP and to compare with standardized commercial PRP by calculating the quantity of platelets using Beckman Coulter cell counter in 10 PRP samples and assessing the morphological quality of platelets using JEOL JEM transmission electron microscope (TEM). Statistical Analysis Used: The t test for platelet count in desktop PRP with the test value of therapeutic PRP was 12.618. The P value was <0.001, which was significant statistically. The data followed a normal distribution in normal Q-Q plot for platelet count in desktop centrifuge. So the test samples were not much deviated. Results: The platelet count was lesser than that of standardized commercial PRP. When viewed under JEOL JEM transmission electron microscope, the α granules in platelets were intact and the morphological quality of the PRP was good. Conclusions: With this study, we have determined that the morphological quality of PRP produced by the in-house desktop centrifuge method is comparable to that of standardized commercial PRP. Though the quantity of platelets was less than 1 million cells/μL, the clinical results were good with desired bone formation, thereby providing good avenue for further research.

Keywords: Beckman Coulter counter, JEOL JEM transmission electron microscope, platelet-rich plasma

INTRODUCTION

Platelet-rich plasma (PRP) was introduced by Whitman and Berry in 1997.[1] According to Marx et al.[2] in 1998, mixing PRP with autogenous bone resulted in the release of growth factors, producing denser bone regeneration and radiographic maturation within a short time. Since then quality and efficacy of various PRP preparations have been analyzed by various authors using different methodologies.[2-4] The α granules of the platelets contain growth factors[5] such as vascular endothelial growth factor, platelet-derived growth factors, and transforming growth factor-β.[6]

Since 2002, we have developed a technique based on vacutainer system as described by Sonnleitner et al.[7] in 2000, to make PRP and have used it successfully in many cases. The standard techniques for evaluating the quality of PRP are by depicting the growth factor...
levels in PRP\textsuperscript{[5,6]} and quantity by assessing the platelet concentration\textsuperscript{[8]} and platelet count using cell counters.\textsuperscript{[8]}

**MATERIALS AND METHODS**

This study was aimed to evaluate the quality of desktop PRP by calculating the amount of platelets present in the collected plasma and assessing the morphological quality of $\alpha$ granules in the platelets. We have used JEOL JEM 100 SX (JEOL, Akishima, Tokyo, Japan) Transmission Electron Microscope (TEM) and Beckman Coulter cell counter Ac-T 8 (Beckman Coulter, Pasadena, CA) series for the analysis.

Blood samples were taken from 10 patients who were healthy and need PRP gel\textsuperscript{[9]} or PRP sheets to fill the bony cavities as in case of cyst, multiple impacted teeth orthognathic, surgeries etc.\textsuperscript{[10-15]} PRP samples were also taken from 10 volunteers. The PRP obtained by this procedure is analyzed for quality and quantity.

**Harvesting platelet-rich plasma**

Autogenous venous blood was centrifuged using desktop centrifuge [Figure 1] at 1200 rpm for 10 min [Figure 2], which resulted in three layers: the blood cell concentrate, buffy coat, and supernatant layer of platelet-poor plasma (PPP) [Figure 3]. The buffy coat or PRP\textsuperscript{[16]} extends 6–8 mm into blood cell component layer. Using Eppendorf micro pipette, the supernatant layer and buffy coat including its extensions into the blood cell component layer were pipetted into another test tube [Figure 4] and centrifuged again. The PPP was pipetted out and the PRP was isolated [Figure 5]. The PRP was mixed with calcium chloride resulting in the formation of PRP sheet [Figure 6] and PRP gel [Figure 7]. PRP gel was then mixed with autologous bone graft and placed into the surgical defect.\textsuperscript{[9]} The cell counter used for counting platelets is Beckman Coulter.
Coulter cell counter Ac-T 8 series. The plasma samples were then processed and the ultrathin sections obtained were viewed at 60 or 80 kV using JEOL JEM 100 SX TEM.

RESULTS

The transmission electron microscopic study showed that the \( \alpha \) granules in platelets of desktop PRP obtained with REMI table top centrifuge using Sonnleitner et al. technique were intact without loss of growth factors.

The platelet count in the PRP produced using REMI desktop centrifuge, counted using Beckman Coulter cell counter Ac- T 8 series, was less with a mean value of 0.45 million cells/\( \mu \)L.

DISCUSSION

The healing of both soft and hard tissues was accelerated by PRP. However, the clinical efficacy of PRP varies depending on the preparation method and the present methods to evaluate efficacy of PRP systems have several limitations.

According to Marx, platelet membrane integrity is of paramount importance because fragmented platelets may spill more growth factors and that their tertiary structure is altered resulting in decreased clinical efficiency.

The morphological quality assessment of the PRP produced by our method was carried out using TEM, which showed that the platelets were intact and \( \alpha \) granules were also intact [Figure 8]. So the PRP produced by desktop centrifuge method was of good quality.
According to Marx,[4] therapeutic PRP should contain 1 million platelet cells/µL or 400% of the peripheral blood platelet count as measured with Beckman Coulter cell counter.[4]

The platelet count in 10 PRP samples counted using Beckman Coulter cell counter showed the PRP count was lesser than therapeutic range [Graph 1]. The statistical analysis showed that the t test [Table 1] for

Table 1: The t test for platelet count in desktop PRP with the test value of therapeutic PRP was 12.618; the P value was < 0.001, which was statistically significant

| N | Mean  | Std deviation | Std.Error mean |
|---|-------|---------------|---------------|
| Platelet count in desktop PRP | 10 | 440400.00 | 140242.013 | 44348.418 |

One-sample test

Test value = 1000000

| T   | DF | Sig (2-tailed) | Mean difference | 95% Confidence interval of the difference |
|-----|----|----------------|-----------------|----------------------------------------|
| Platelet count in desktop PRP | 12.618 | 9 | .000 | -559600.00 | -659923.09 | -459276.91 |

Table 2: In the normal Q-Q plot for platelet count in desktop centrifuge, the data followed a normal distribution. So the test samples were not much deviated

| Statistic | Std. Error |
|-----------|------------|
| Platelet count in desktop PRP | 440400.00 | 44348.418 |

| Mean | Lower bound | Upper bound |
|------|-------------|-------------|
| 440400.00 | 340076.91 | 540723.09 |

| 5% Trimmed mean | Median | Variance | Std. Deviation | Minimum | Maximum | Range | Interquartile range | Skewness | Kurtosis |
|------------------|--------|---------|----------------|---------|---------|-------|---------------------|----------|----------|
| 439888.89 | 420000.00 | 19667822222.222 | 140242.013 | 264000 | 626000 | 362000 | 274500.00 | .063 | .687 |
| -1784 | 1.334 |

Graph 1: The platelet count in desktop platelet-rich plasma was lesser than therapeutic range
platelet count in desktop PRP with the test value of therapeutic PRP is 12.618. The P value was significant statistically (< 0.001) [Table 2]. In the normal Q-Q plot for platelet count in desktop centrifuge, the data followed a normal distribution and the test samples were not much deviated [Graph 2].

The platelet count was less when compared with the commercial PRP [Graph 3] produced with Smart-Prep, PCCS,Curasan[22] or Placon type of centrifuge as given by Marx,[6] Weibrich et al.,[22-24] and Eby.[25]

The reason for this may be the cell counter what we used was not as effective as that used in counting commercial PRP. We have used Beckman Coulter counter Ac-T 8 series instead of Cell-dyn 3700 where clumps of platelets may have been counted as individual platelet. But the morphological quality was good when viewed under JEOL JEM TEM. The platelets and α granules were intact, which showed that there was no spillage of growth hormone during the preparation, which may reduce the effectiveness of PRP. Tsay.C.R (2005) shows PRP prepared with thrombin results in a large, immediate release of growth factors, which could be lost into the interstitium in vivo. While thrombin receptor activator peptide(TRAP) and bone substitutes are more efficacious in sustaining growth factors and bone formation.[21] With this study, we have determined that the quality of PRP produced by the in-house desktop centrifuge method is comparable to that of standardized commercial PRP. Though the quantity of platelets as assessed by the Cell Dyn 3700 is more accurate than the Beckman Coulter cell counter Ac-T 8 series, the clinical results were good, providing good avenue for further research. The morphological quality of the alpha granules assessed using JEOL JEM TEM is a good method for evaluation and standardization of in-house PRP as it is important to preserve the platelet membrane integrity which relates to its ability to release growth factors. Fragmented platelets may spill more growth factors into solution and therefore their effectiveness are lessened.

**CONCLUSION**

Tsay.C.R (2005) shows PRP prepared with thrombin results in a large, immediate release of growth factors, which could be lost into the interstitium in vivo. While thrombin receptor activator peptide(TRAP) and bone substitutes are more efficacious in sustaining growth factors and bone formation.[21] With this study, we have determined that the quality of PRP produced by the in-house desktop centrifuge method is comparable to that of standardized commercial PRP. Though the quantity of platelets as assessed by the Cell Dyn 3700 is more accurate than the Beckman Coulter cell counter Ac-T 8 series, the clinical results were good, providing good avenue for further research. The morphological quality of the alpha granules assessed using JEOL JEM TEM is a good method for evaluation and standardization of in-house PRP as it is important to preserve the platelet membrane integrity which relates to its ability to release growth factors. Fragmented platelets may spill more growth factors into solution and therefore their effectiveness are lessened.

Financial support and sponsorship
Nil.

**Conflicts of interest**
There are no conflicts of interest.

**REFERENCES**

1. Whitman HD, Berry RL. A technique for improving the handling of particulate cancellous bone and marrow grafts using platelet gel. J Oral Maxillofac Surg 1998;1217-8.
2. Marx RE, Carlson ER, Eichstaedt RM. Platelet-rich plasma: growth factor enhancement for bone grafts. OOO Endod 1998;85:638-46.
3. Landesberg R, Burke A, Pinsky D, Katz R, Vo J, Eisig SB, et al. Activation of platelet-rich plasma using thrombin receptor agonist peptide. J Oral Maxillofac Surg 2005;63:529-35.
4. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg 2004;62:489-96.
5. Weibrich G, Kleis WK, Hafner G, Hitzler WE. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. J Craniomaxillofac Surg 2002;30:97-102.
6. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation future sites for implants. Int J Oral Maxillofac Surg 1999;14:529-35.

7. Sonnleitner D, Huemer P, Sullivan DY. A simplified technique for producing platelet-rich plasma and platelet concentrate for intraoral bone grafting techniques: a technical note. Int J Oral Maxillofac Implants 2000;15:879-82.

8. O’Neill EM, Zalewski WM, Eaton LJ, Popovsky MA, Pivacek LE, Ragno G, et al. Autologous platelet-rich plasma isolated using the haemonetics cell saver 5 and haemonetics MCS+ for the preparation of platelet gel. Vox Sang 2001;81:172-5.

9. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg 1997;55:1294-9.

10. Bhanot S, Alex JC. Current applications of platelet gels in facial plastic surgery. Facial Plast Surg 2002;18:2733.

11. Carlson NE, Roach RB. Platelet-rich plasma, clinical application in dentistry. JADA 2002;133:1383-6.

12. Choi BH, Zhu SJ, Kim BY, Huh JY, Lee SH, Jung JH. Effect of platelet-rich plasma (PRP) concentration on the viability and proliferation of alveolar bone cells: an in vitro study. Int J Oral Maxillofac Surg 2005;34:420-4.

13. Cohen MA. Recombinant human platelet-derived growth factor gel speeds healing of acute full-thickness punch biopsy wounds. J Am Acad Dermatol 2001;45:857-62.

14. Durgrillon A. Autologous concentrated platelet-rich plasma (PRP) for local application in bone regeneration. Int J Oral Maxillofac Surg 2002;31:615-9.

15. Gerard D, Carlson RE, Gotcher JE, Jacobs M. Effect of platelet rich plasma on the healing of autologous bone grafted mandibular defects in dogs. J Oral Maxillofacial Surg 2006;64:443-51.

16. Zbigniew R. A novel technique for preparing improved buffy coat platelet concentrates. BCMD 1995;21:25-33.

17. Schmitz JP. The biology of platelet rich plasma. J oral Maxillofac Surg 2001;59:1119-21.

18. Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. Plast Reconstr Surg 2004;114:1502-8.

19. Keegan T, Heaton A, Holme S, Owens M, Nelson E, Carmen R. Paired comparison of platelet concentrates prepared from platelet-rich plasma and buffy coats using a new technique with 11In and 51Cr. Transfusion 1992;32:113-20.

20. Landesberg R, Roy M, Glickman RS. Quantification of growth factor levels using a simplified method of platelet-rich plasma gel preparation. J Oral Maxillofac Surg 2000;58:297-300; discussion 300-1.

21. Tsay RC, Vo J, Burke A, Eisig SB. Differential growth factor retention by platelet-rich plasma composites. J Oral Maxillofacial Surgery 2005;63:521-8.

22. Weibrich G, Kleis KGW, Hafner G. Growth factor levels in the platelet-rich plasma produced by 2 different methods: curasan-type PRP kit versus PCCS PRP system. Int J Oral Maxillofac Implant 2002 Mar-A (2):184-90.

23. Weibrich G, Kleis WK, Buch R, Hitzler WE, Hafner G. The harvest smart PRePTM system versus the Friadent-Schütze platelet-rich plasma kit. Clin Oral Implants Res 2003;14:233-9.

24. Weibrich G, Kleis WK, Hitzler WE, Hafner G. Comparison of the platelet concentrate collection system with the platelet-rich-in-growth-factors kit to produce platelet-rich plasma: a technical report. Int J Oral Maxillofac Implants 2005;20:118-23.

25. Eby BW. Platelet-rich plasma: harvesting with a single-spin centrifuge. J Oral Implantol 2002;28:297-301.