**Research Article**

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# Optimization of Platelet-Rich Plasma Preparation for Use in Clinical Practice

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**INTRODUCTION** Development of effective biological products, based on human platelets, is very actual in regenerative medicine. The initial material for biological products' obtaining is platelet-rich plasma (PRP), but the method of PRP isolation has not yet been standardized.

**AIM** To assess the quality of platelets in PRP, harvested by different centrifugation modes.

**MATERIAL AND METHODS** For platelet study, venous blood was harvested from volunteer donors. We used 3 methods for PRP-preparation, each methods included 2-stage centrifugation: 5 min 300g and 17 min 700g (Group 1); 10 min 300g and 10 min 700g (Group 2); 15 min 300g and 5 min 700g (Group 3). Platelets were examined using morphofunctional method based on vital cell staining.

**RESULTS** In Group 1 and Group 2, the overall safety of biologically high-grade platelets in PRP was similar and estimated 55-60% of their total content in the blood. In Group 3 the safety of biologically high-grade platelets was only 30% ($p<0,05$).

**CONCLUSION** The centrifugation modes «5 min 300 g / 700 g 17 min» and «10 min 300 g / 10 min 700 g» allowed researchers to obtain equal quality PRP, while «10 min 300 g / 10 min 700 g» mode has a number of instrumental benefits.

**Keywords:** platelets, platelet-rich plasma, centrifugation, morphofunctional properties of platelets

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PRP – platelet-rich plasma
Dplgr – density of platelets with granules
Cplgr – concentration of platelets with granules

## INTRODUCTION

Today, treatment methods using human platelet-based drugs are becoming more common in many areas of clinical medicine. Human platelets contain a large number of growth factors, angiogenesis factors, and cytokines, which stimulate the processes of reparative regeneration [1–5]. The effectiveness of platelet preparations has been shown in a large number of studies; however, none of these preparations is fully standardized. The starting material for obtaining biological products is platelet-rich plasma (PRP). Methods for obtaining PRP are instrumentally and technically simple, and the use of autologous PRP in the clinical process has a small number of relative and absolute contraindications and is legally permitted. According to researchers, in order to achieve a clinical effect, the concentration of platelets in PRP should be at least 1000 thousand/mcL. There are various protocols for PRP production that allow obtaining the required concentration of platelets [6–9], special centrifuges and systems for PRP isolation have been developed, that maximally simplify the labor costs for the use of this technique in clinical practice [8, 9]. However, the actual quality of the resulting platelets, their structural and functional characteristics are not taken into account. Centrifugation can disrupt the native structure of platelets, cause their spontaneous aggregation, which ultimately can lead to the loss of the biological potential of platelets.

The **aim** of this work was to assess the morphological and functional status of PRP platelets isolated by different methods of centrifugation**.**

## MATERIAL AND METHODS

Our work was carried out with the approval of the Interuniversity Ethics Committee (protocol No. 02-21 dated February 18, 2021). We used venous blood samples from 15 volunteer donors. Venous blood was taken from the cubital vein into vacuum tubes with the anticoagulant EDTA (ethylenediaminetetraacetic acid). From each donor, 3 tubes of blood with a volume of 4 ml were harvested. Small aliquots (10–50 µl) were taken from the tubes to assess the initial quality of donor platelets, then the tubes were divided into three experimental groups, each group had one tube from each donor. In the experimental groups, double centrifugation was performed to isolate PRP. Initially, whole blood was centrifuged at 300 g to separate it into components and obtain primary plasma with platelets. The primary plasma was then centrifuged at 700 g to concentrate the platelets and obtain the final PRP. In Group 1, the duration of centrifugation at 300 g was 5 min, and at 700 g - 17 min; in Group 2 - 10 minutes and 10 minutes, in Group 3 - 15 minutes and 5 minutes, respectively. In all the cases, after the first centrifugation, the entire volume of supernatant plasma with platelets was transferred to a new tube. After centrifugation at 700 g, the primary plasma was divided into platelet pellet and platelet-poor plasma (platelet content less than 100,000/µl). Platelet-poor plasma was collected so that 0.5 ml of it remained in the test tube. This plasma volume was used to resuspend the platelet pellet. Plasma sampling and mixing in all the cases were carried out with disposable sterile medical syringes up to 10 ml in volume through disposable sterile 20 G spinal needles (Fig. 1). As a result, 0.5 ml of PRP was obtained from 4 ml of blood.

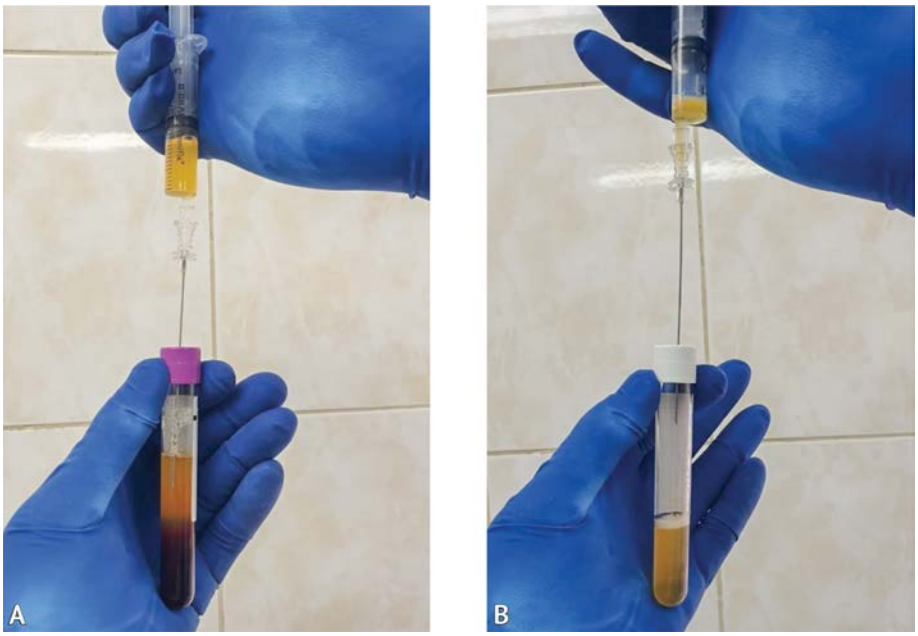

Fig. 1. A — selection of plasma with platelets from a test tube; B — transfer of primary plasma into a sterile tube before re-centrifugation

The volume of plasma obtained was measured with a graduated pipette. The concentration of platelets and leukocytes in the blood and plasma was determined on a hematological analyzer, and the total number of platelets in the sample was also calculated. Assessment of the morphofunctional status of human platelets included staining the cells with a fluorescent vital dye followed by their analysis using a fluorescence microscope [10, 11]. In the original blood, primary plasma and PRP, the level of biologically valuable platelets with granules was estimated, density of platelets with granules, Dplgr (%, the norm being 35–75%), the total number of platelets with granules in the sample was calculated, concentration of platelets with granules (Cplgr) (106). In addition, in each group, the overall survival of platelets and the safety of platelets with granules were calculated. To do this, the ratio of the number of platelets in the entire volume of the finished PRP to their number in the volume of the original blood was determined and expressed as a percentage. Similarly, the count for platelets with granules was performed.

When statistically processing the data, the median, the 1st and 3rd quartiles were determined, and the Wilcoxon signed-rank test for clustered data was used to evaluate differences. Results were considered statistically significant at p less than 0.05.

## RESULTS AND DISCUSSION

In the original blood, the platelet concentration was 249 [223; 284] thousand/μl, their total number - 964 [888; 1136], Dplgr — 50 [45; 60]%, leukocyte concentration — 7 [6; 10] thousand/μl. Plasma parameters after the first centrifugation are presented in Table 1. Compared to the original blood, the concentration of platelets in the plasma increased, while in Group 3 the concentration of platelets was statistically significantly lower compared to Group 1 and Group 2. The concentration of leukocytes, on the contrary, statistically significantly decreased in all the groups, but in Group 1 it was 3–5 times higher than in Group 2 and Group 3 ($p<0.05$). The volume of plasma obtained after the first centrifugation in Group 1 was 1.30 (1.00; 1.50), in Group 2 – 1.40 (1.40; 1.60), and in Group 3 - 1.60 (1.40; 1.80). As a result, the total number of platelets obtained after the first centrifugation in all the samples was statistically significantly less than in the original blood. The losses in Group 1 were 43%, in Group 2 — 38%, and in Group 3 — 47% of the initial total number of platelets. Thus, the first centrifugation for more than 10 minutes is impractical. Longer centrifugation leads to excessive sedimentation of platelets and increases their loss.

*Table 1*

**Characteristics of plasma samples isolated from the blood of patients during centrifugation at 300 g**

| Morphofunctional parameters: median (Q1; Q3) | Sample type | | | |
|---|---|---|---|---|
| | Whole blood | Primary plasma, after the first 300 g centrifugation | | |
| | | Group 1 (5 minutes) | Group 2 (10 minutes) | Group 3 (15 minutes) |
| Platelet concentration, $\times 10^3$/μl | 241 (223; 284) | 447 (336; 511)* | 462 (341; 574)* | 332 (254; 438)*, ** |
| Leukocyte concentration, $\times 10^3$/μl | 7 (6; 10) | 0.8 (0.5; 2.5)* | 0,3 (0.1; 0.5)*, ** | 0.2 (0.1; 0.8)*, ** |
| Total number of platelets in the sample, $\times 10^6$ | 952 (848; 1156) | 548 (429; 682)* | 591 (429; 742)* | 506 (342; 615)* |
| Total number of platelets with granules, $\times 10^6$ | 501 (400; 674) | 282 (216; 353)* | 375 (280; 445)* | 306 (263; 380)* |

Notes: * — statistically significant as compared to control ($p<0.05$); ** — statistically significant as compared to Group 1 ($p<0.05$)

After the second centrifugation with an acceleration of 700 g and subsequent resuspension in a smaller volume of plasma, the total platelet concentration in Groups 1 and 2 averaged 1000 thousand/μl and above, which corresponded to the recommended platelet concentration in PRP. In Group 3, the target platelet concentration could not be achieved. In these samples, the content of platelets was lower than in Groups 1 and 2 by an average of 1.8 times. Platelets of Groups 1 and 2 retained their morphofunctional status, while the relative content of platelets with granules after double centrifugation did not statistically significantly change compared to that in the original blood. On the contrary, in PRP samples of Group 3, a statistically significant decrease in Dplgr and other morphofunctional parameters of platelets ($p<0.05$) were noted. Numerous platelet conglomerates, in which platelets retained granules, were observed in many samples of this group. Small aggregates formed by cells without granules could also be seen. The brightness of the cytoplasm in such platelets was markedly reduced. All this indicates spontaneous activation of platelets. The total number of obtained platelets with granules in PRP is shown in Fig. 2.

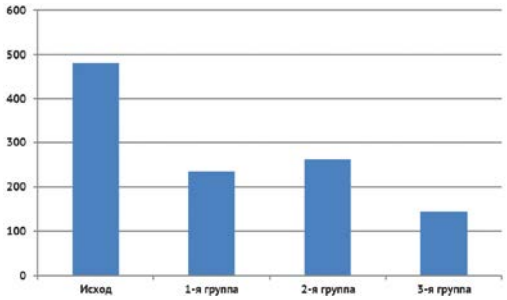


Fig. 2. The total number of platelets with granules in the initial blood sample and in the platelet-rich plasma of the studied groups

In Groups 1 and 2, the total number of fully functioning platelets averaged 55–60%, while in Group 3 – only 30%. Comparative analysis of PRP samples obtained by different methods is presented in Table 2. It can be concluded that the PRP production mode “15 min at 300 g / 5 min at 700 g” is ineffective.

*Table 2*

**Comparison of platelet rich plasma samples obtained by different methods**

| Morphofunctional parameters: median (Q1; Q3) | Method for PRP production | | |
|---|---|---|---|
| | 5 min at 300 g / 17 min at 700 g (Group 1) | 10 min at 300 g / 10 min at 700 g (Group 2) | 15 min at 300 g / 5 min at 700 g (Group 3) |
| The level of platelets with granules, % | 45 (40; 51) | 47 (43; 59) | 40 (37; 45)* |
| Total number of platelets in PRP, $\times 10^6$ | 545 (479; 665) | 657 (582; 782) | 315 (274; 405)* |
| Total number of platelets with granules in PRP, $\times 10^6$ | 305 (223; 352) | 275 (199; 341) | 142 (118; 172)* |

Note: * — statistically significant as compared to Group 1 ($p<0.05$)

Centrifugation modes 5/17 and 10/10 make it possible to produce PRP that is optimal in terms of its qualitative and quantitative characteristics, capable of manifesting regenerative potential.

## CONCLUSION

The study showed that the well-known mode "5 min at 300 g / 17 min at 700 g" [7] makes it possible to obtain PRP with platelets of satisfactory quality. The centrifugation mode "10 min at 300 g / 10 min at 700 g" is comparable to the recommended mode in number and quality of platelets (Table 2), moreover, it has a lot of instrumental advantages. First, in the "10 min at 300 g / 10 min at 700 g" mode, the primary plasma contains far fewer leukocytes, which can be critical in the manufacture of platelet preparations. Leukocytes contain a large number of pro-inflammatory cytokines, which, in combination with platelet components, can enhance many pathophysiological reactions, and therefore most researchers recommend producing PRP without leukocytes [2, 3, 6]. Secondly, in some cases, 5 minutes at 300 g may be insufficient to obtain the required volume of original plasma. Increased blood and plasma viscosity is observed in patients with arterial hypertension, massive blood loss, extensive burns, poisoning, and in many pathologies accompanied by dysproteinemia [12–14]. Besides, even in healthy people, the duration of plasma release can be extended due to low values of the erythrocyte sedimentation rate [15]. Thus, blood centrifugation at 300 g for 10 min increases the reliability of obtaining PRP with the standard cell concentration while maintaining their morphological and functional properties. Samples of PRP obtained in this way also contained a sufficient number of platelets with granules, which is required to achieve a therapeutic effect previously demonstrated both in vitro and in clinical studies [5, 16].

## CONCLUSIONS

The proposed mode of producing platelet-rich plasma – 10 min at 300 g / 10 min at 700 g – seems to be effective for obtaining a platelet concentrate with the required quality of 47% (43; 59) ($p<0.05$) and quantitative composition of 657 (582; 782) thousand/μl 106 ($p<0.05$) and is recommended for use in clinical practice, including traumatology and orthopedics.

## REFERENCES


1. Kushida S, Kakudo N, Morimoto N, Hara T, Ogawa T, Mitsui T, et al. Platelet and growth factor concentrations in activated platelet-rich plasma: a comparison of seven commercial separation systems. *J Artif Organs*. 2014;17(2):186–192. PMID: 24748436 https://doi.org/10.1007/s10047-014-0761-5
2. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med*. 2013;41(2):356–364. PMID: 23299850 https://doi.org/10.1177/0363546512471299
3. Lacci KM, Dardik A. Platelet-rich plasma: support for its use in wound healing. *Yale J Biol Med*. 2010;83(1):1–9. PMID: 20351977
4. Yassibag-Berkman Z, Tuncer O, Subasioglu T, Kantarci A. Combined use of platelet-rich plasma and bone grafting with or without guided tissue regeneration in the treatment of anterior interproximal defects. *J Periodontol*. 2007;78(5):801–809. PMID: 17470012 https://doi.org/10.1902/jop.2007.060318
5. Makarov MS, Storozheva MV, Konyushko OI, Borovkova NV, Khvatov VB. Vliyanie kontsentratsii trombotsitarnogo faktora rosta na proliferativnuyu aktivnost' fibroblastov cheloveka. *Cell Technologies in Biology and Medicine*. 2013;(2):111–115. (In Russ.)
6. Etulain J, Mena HA, Meiss RP, Frechtel G, Gutt S, Negrotto S, et al. An optimised protocol for platelet-rich plasma preparation to improve its angiogenic and regenerative properties. *Sci Rep*. 2018;8(1):1513. PMID: 29367608 https://doi.org/10.1038/s41598-018-19419-6
7. Amable PR, Carias RB, Teixeira MV, da Cruz Pacheco I, Corrêa do Amaral RJ, Granjeiro JM, et al. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. *Stem Cell Res Ther*. 2013;4(3):67. PMID: 23759113 https://doi.org/10.1186/scrt218
8. Degen RM, Bernard JA, Oliver KS, Dines JS. Commercial Separation Systems Designed for Preparation of Platelet-Rich Plasma Yield Differences in Cellular Composition. *HSS J*. 2017;13(1):75–80. PMID: 28167878 https://doi.org/10.1007/s11420-016-9519-3
9. Croisé B, Paré A, Joly A, Louisy A, Laure B, Goga D. Optimized centrifugation preparation of the platelet rich plasma: Literature review. *J Stomatol Oral Maxillofac Surg*. 2020;121(2):150–154. PMID:31299341 https://doi.org/10.1016/j.jormas.2019.07.001
10. Khubutiya MSh, Makarov MS, Khvatov VB, Vysochin IV, Kobzeva EN, Borovkova NV, et al. *Sposob otsenki morfofunktsional'nogo statusa trombotsitov cheloveka*. Patent No 2485502C1; decl. 17.02.2012; publ. 20.06.2013. Bull. No 17. (In Russ.). Available at: https://patentimages.storage.googleapis.com/db/51/6f/194ae2d17f6f8b/RU2485502C1.pdf [Accessed Apr 21, 2023]
11. Makarov MS, Kobzeva EN, Vysochin IV, Borovkova NV, Khvatov VB. Use of Vital Staining in Stored Human Platelets Morphofunctional Analysis. *Almanac of Clinical Medicine*. 2014;(30):83–87. (In Russ.)
12. Kozinets GI (ed.) *Prakticheskaya transfuziologiya*. Moscow: Prakticheskaya meditsina Publ.; 2005. (In Russ.)
13. Ragimov AA. (ed.) *Transfuziologiya*. Moscow: GEOTAR-Media Publ.; 2012. (In Russ.)
14. Muravyov AV, Mikhailov PV, Tikhomirova IA. Microcirculation and Hemorheology: points of interaction. *Regional blood circulation and microcirculation*. 2017;16(2):90–100. (In Russ.) https://doi.org/10.24884/1682-6655-2017-16-2-90-100
15. Maksimovich NE, Khodosovskiy MN, Troyan EI, Lelevich AV, Rukan TA, Maksimovich EN. *Patologicheskaya fiziologiya*. 2nd ed., exp. and rev. Grodno: GrGMU Publ.; 2014. (In Russ.)
16. Borovkova NV, Malygina MA, Makarov MS, Sakharova OM, Ponomarev IN. *Sposob lecheniya patsientov s perelomom sheyki plecha*. Patent No 2681753 S1; decl. 21.05.2018; publ. 12.03.2019. Bull. No 8. (In Russ.)



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