RESEARCH ARTICLE

A Comparative Study of Concentration of Growth Factors in Lyophilized PRP with Fresh PRP at Different Storage Conditions

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ABSTRACT

Background: There is ongoing debate regarding platelet-rich plasma's (PRP) ability to effectively heal soft tissue injuries. Lyophilized platelet-rich plasma (L-PRP) was created and kept using the blood bank's standard separation procedures. The objective of study was to evaluate effects of lyophilization on PRP and its properties such as level of growth factors. Also, the effect of storage conditions on growth factors.

Methods: The PRPs were created through a two-step centrifugation process and lyophilized. After freeze-thawing to allow platelet growth factors to be released, levels of hepatocyte growth factor (HGF), transforming growth factor (TGF), fibroblast growth factor (FGF)-basic, platelet-derived growth factor (PDGF)-AB, insulin-like growth factor (IGF)-1, and vascular endothelial growth factor (VEGF) were measured each day during storage.

Results: After L-PRP preparation, levels of FGF-basic, VEGF, PDGF-AB, EGF and TGF- beta1 significantly raised and levels of HGF and IGF-1 in L-PRPs had light rise.

Conclusions: In comparison to F-PRP, platelet counts and seven growth factors increased during preservation in L-PRP. PRPs that have been stored may be injected several times using our method. L-PRP was efficacious and stable for 90 days when stored at 8^{o}C.

Keywords: Fibroblast growth factor, Lyophilized platelet-rich plasma, Lyophilization, Platelet counts.

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INTRODUCTION

The use of platelet-rich plasma (PRP) by experts in orthopedics, rheumatology, and physical therapy and rehabilitation has demonstrated promise in re-establishing the normal function of tissue after injury or degeneration. The growth factors and cytokines included in PRP are expected to promote tissue regeneration and repair when administered to the target location.[1,2] In contrast, PRP's effectiveness in treating soft tissue injuries is still in question.[3]

Many factors are at play in the disputed treatment efficacy. First, precise PRP injection is needed to provide growth factor-rich plasma to the culprit. Several techniques were employed, including anatomy guidance, arthroscopy guidance, and ultrasound guidance. It has been established that using ultrasound-guided injection is more accurate than using anatomical assistance.[4,5] Second, the patient parameters in the research were varied. Studies of PRP therapy for knee osteoarthritis revealed superior outcomes in younger persons with mild degree of cartilage deterioration.[6,7] It was difficult to combine studies of various populations to produce suggestions of high quality and supported by adequate confirmation. Thirdly, comparing the treatment effectiveness of different PRP preparation protocols and kits was impossible. The bulk of them were created by increasing volume and immediately injected in medical offices following creation. As a result, no standard dose regimens could be created because platelet or growth factor measurement wasn't done beforehand.[8]

The platelet concentrate is typically prepared in blood banks using PRP technique or buffy coat process. PRP includes platelet extract. In the PRP procedure, Red blood cells are first separated and removed from whole blood by spinning it at a low speed, and then, platelet concentration is achieved by rapidly spinning the supernatant.[9] The buffy coat technique[10] on the other hand requires rapidly centrifuging

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the entire blood to separate it into three layers: platelet-poor plasma on top, platelets and WBC in the middle, and red blood cells at the bottom. The fluffy coat's central layer has been retrieved. Vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, endothelial growth factor (EGF), fibroblast growth factor (FGF)-basic, hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF) were main growth factors (GF) discussed in reports on PRP growth factors.[11] Proangiogenic activity and chemotaxis are associated with VEGF, FGF-basic, and PDGF.[12] IGF-1 has a role in skeletal muscle growth and repair, chemotaxis, and the promotion of bone formation, whereas HGF acts as a mitogen for endothelial cells.[13,14] EGF and TGF can stimulate growth and migration of endothelial cells and the creation of tubules.[13,12]

Here, we prepared lyophilized PRP (L-PRP) in by using the standardized lyophilized process for preparing platelet concentrate[15,16] and comparatively assessed for observing the dynamic changes in concentrations of HGF, PDGF-AB, VEGF, IGF-1, EGF, FGF basic, and TGF- beta1 in L-PRPs and fresh PRP after 1, 7, 15, 30, 60 and 90th day by keeping the samples at room temperature, 8 and -80ºC and also the effect of agitation time and speed is studied to observe the level of concentrations of GFs released.[17]

MATERIALS AND METHODS

Study Subjects and Blood Collection

Six healthy volunteer peripheral blood donors (30 to 40 years old, 3 men and 3 women) were included in a main blood bag with the anticoagulant citrate-phosphate dextrose-adenine (CPDA)-1 was purchased from the blood bank of Acharya Vinoba Bhave Rural Hospital.

Platelet Rich Plasma Preparation and Storage

The blood bank at Acharya Vinoba Bhave Rural Hospital, Sawangi (M), used the triple blood bag collection procedure to freshly collect 450 mL of whole blood in the primary bag. The method is called mother blood bag collection. The bag was kept at room temperature (ambient) 20 to 24ºC at a horizontal position for the components to settle down. The bag was then kept in a centrifuge for slow spin (1900 rpm at 20 to 24ºC) for 10 minutes. From the start, the machine takes approximately 30 minutes of de-acceleration speed to achieve the said RPM and again come back to stability. Once the machine is stopped the mother bag was removed and placed in the plasma expressor machine. The bag has three layers. The sedimented layer at the bottom is the PRC (Packed red cell), Buffy coat is the layer in the center, while PRP is the layer on top. With the use of light pressure, the PRP layer is removed and collected in a separate, empty bag. Once the platelet-poor plasma, or PPP, was transferred into a different bag, around 90 mL of PRP remained in the bag. This bag is added with an additive solution and given a second spin (3800 rpm at 20 to 24ºC) for 8 minutes. The second spin is given to remove any traces of blood cells from the PRP and also to discard the poor PRP layer at the top. This spin is required to ensure the highest purity of PRP. The final volume of PRP formed was approx. 85 to 88 mL. 5 mL of fresh PRP was stored at room temperature, 5 mL of fresh PRP was stored at 8ºC and 20 mL of fresh PRP was stored at -80ºC

Quantification of Growth Factors and Hematological Analysis

To quantify the growth factors and analyze the platelet counts in the fresh PRP sample of each volunteer at room temperature, 8 and 80ºC the aliquot of 2.5 mL from each storage condition of each volunteer was taken and was analysed in the hematological analyzer XE-5000 (Sysmex Corp) to count the platelets count. The growth factors were quantified by using the ELISA machine. We used Quantikine ELISA Kits (R&D Diagnostics; Minnesota, USA) to quantify HGF (DHG00), FGF basic (DFB50), PDGF-AB (DHD00C), IGF-1 (DG100), VEGF (Cat. DVE00), EGF (DEG00) and TGFbeta1 (DB100B).[17,18] Procedures were performed in accordance with the manufacturer's instructions at Central Research Laboratory of AVBRH Sawangi (M). The samples of Fresh PRP kept at room temperature were observed to be coagulated after 1 hours and stored at 8º C was coagulated at 8 hours.

Preparation of Processed PRP

The processed PRP was prepared using the lyophilization technique (MAC lyophiliser, freeze dryer. Cat No: MSW-137, Sr. No. 2511 Macro Scientific Works Pvt. Lmt). In 3 Sterile glass containers (petri dish) around 40 mL of fresh PRP of each volunteer was transferred and kept in the shelf of the lyophilizer to reach the temperature and vacuum. The temperature of the lyophiliser was set at - 35ºC at a vacuum of -897 mmhg gauge for 24 hours. Such 7 cycles were repeated for 7 days. After 7 days the dry, crystalline light-yellow powder was collected and stored in a dry air-tight container. L-PRP was reconstituted for the tests using sterile water for injection.[19]

Quantification of Growth Factor in Processed PRP

Based on the yield of the lyophilized sample taken from each volunteer, 60 mg of the lyophilized powder was reconstituted using 1.5 mL of sterile water for injection.[19] This reconstituted PRP solution was analyzed using a similar method as that used for fresh PRP for estimation of the growth factor in the LPRP.[17,18]

Quantification of Growth Factors in LPRP Stored at Different Storage Conditions of Temperature

Around 300 mg sample of LPRP was stored at 3 different storage conditions (room temperature, 8 and -80ºC). The stored samples were analyzed for quantifying the growth factors VEGF (DVE00), HGF (DHG00), FGF basic (DFB50), PDGF-AB (DHD00C), IGF-1 (DG100), EGF (DEG00), and TGFbeta1 (#DB100B).[17,18] The steps were completed per the manufacturer's instructions on 0, 7, 15 30, 60 and 90th day using the ELISA method described earlier. 1.5 mL of reconstituted sample was agitated for 30 seconds and 0.5 mL was withdrawn from it to quantify the GFs present. The remaining 1-mL was additionally agitated for 30 seconds

Table 1: Platelet yield and WBC reduction after fresh platelet-rich plasma preparation

S. No	*Volume (mL)*	*WBC (1000/ uL)*	*Platelet (1000/ uL)*	*Volume (mL)*	*WBC (1000/ uL)*	*Platelet (1000/ uL)*	*Yield (folds)*	*WBC Reduction (%)*
	Whole blood			*Fresh PRP*				
1	447	3.04	217	85	2.85	1231	5.6	83.0
2	451	5.53	213	85	2.91	952	4.4	90.6
3	430	6.31	220	85	0.40	353	1.5	98.7
4	437	4.41	278	85	0.14	828	3.0	99.3
5	446	5.00	235	85	3.85	977	4.0	86.0
6	453	6.41	277	85	4.56	896	3.1	87.3

A WBC reduction calculation: [(WBC of whole blood X Vol. of whole blood - WBC of platelet-rich plasma X volume of platelet-rich plasma)/ (WBC of whole blood x vol. of whole blood)] × 100.

Table 2: Platelet count (1000/uL) in lyophilized PRP at various storage conditions (1st to 90th day period)

Sample	*1st day*			*7th day*			*15th day*			*30th day*			*60th Day*			*90th day*		
	RT	*8°C*	*-80°C*	*RT*	*8°C*	*-80°C*	*RT*	*8°C*	*-80°C*	*RT*	*8°C*	*-80°C*	*RT*	*8°C*	*-80°C*	*RT*	*8°C*	*-80°C*
1		1201	1232	1150	1220	1251	1222	1235	1290	1276	1379	1367	1283	1374	1403	1272	1376	1456
2	942	945	953	953	999	801	997	1001	999	978	1000	989	980	989	1022	985	988	1040
3	349	341	354	378	355	332	379	383	378	383	411	421	392	465	490	389	476	439
4	801	790	829	760	803	956	780	880	1001	793	901	1235	799	1119	1298	1117	1123	1288
5	932	956	978	902	967	1028	920	998	1128	929	1156	1276	983	1153	1309	987	1158	1321
6	833	853	897	839	888	885	856	903	899	876	950	990	899	1001	1110	901	1007	1210
Average	830	847	873	830	872	875	859	900	949	872	966	1046	889	1016	1105	941	1021	1125

Table 3: Average GF in fresh PRP (0th day) prepared from 6 samples of whole blood.

Sample No.	*Day*	*VEGF(pg/uL)*	*FGF-basic(pg/ uL)*	*HGF(pg/uL)*	*IGF-1(pg/uL)*	*PDGF-AB(pg/ uL)*	*EGF(pg/uL)*	*TGF-beta 1(pg/uL)*
1	0	40.3	30.4	553	60.4	1763	111	20,570
2	0	52.6	6.0	632	61.4	1247	25	9489
3	0	86.0	4.8	600	88.0	3654	219	31,875
4	0	61.7	32.8	658	69.5	7939	350	80,563
5	0	70.7	23.8	576	46.5	5799	202	43,332
6	0	60.8	40.3	501	67.7	5163	280	52,358
Avg		62	23	586	65	4260	197	39697

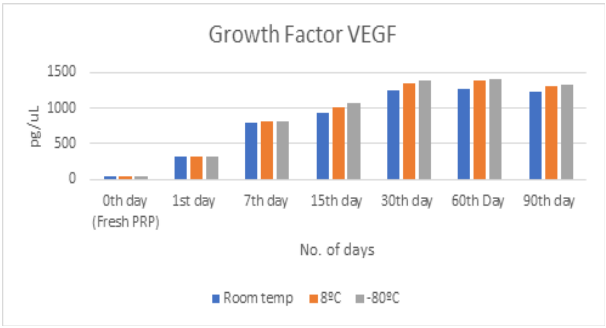

Figure 1: Vascular endothelial growth factor

(total agitation time 1-minute for this sample) and 0.5 mL was withdrawn from it for quantification of the GFs present. The remaining 0.5mL was again agitated for an additional 1-minute and was tested for quantification of GFs. The same was repeated for all storage conditions for all the days mentioned above. Three readings for each condition were taken to increase the statistical power and accuracy.

Statistical Analysis

Using the paired Student's t-test and ANOVA approach, data was statistically evaluated for changes in growth factor levels that were statistically significant.

RESULTS

Each of the six healthy individuals had their total blood volume drawn and processed at 423 to 457 mL. (Table 1). All of their platelet counts were greater than 150,000/ µL. The two-step centrifugation process produced 85 mL of PRP from each donor. Fresh PRP final concentrations increased by 1.6 to 5.7 times from their initial concentration (Table 1). After PRP preparation, all WBC decrease rates were more than 83.0%.

The concentration of growth factors in fresh PRP quantified on 0th day of preparation at room temperature from all the 6 samples collected was found to be in the normal range as mentioned in the literature (Tables 1 and 2). For the convenience of further processing of data and comparison, the average values of all 7 growth factors for all 6 samples

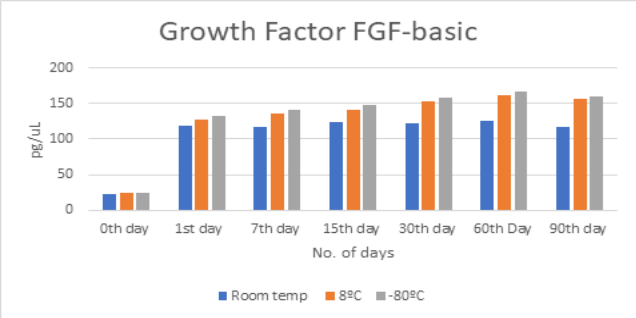

Figure 2: Fibroblast growth factors (FGF)-basic.

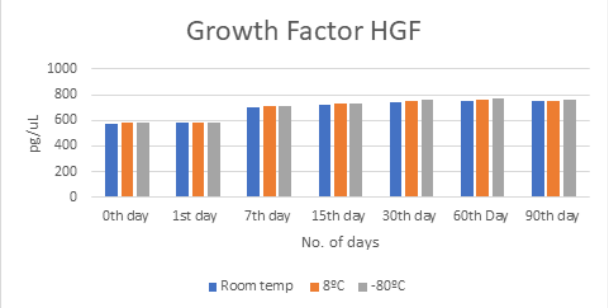

Figure 3: Hepatocyte growth factor (HGF).

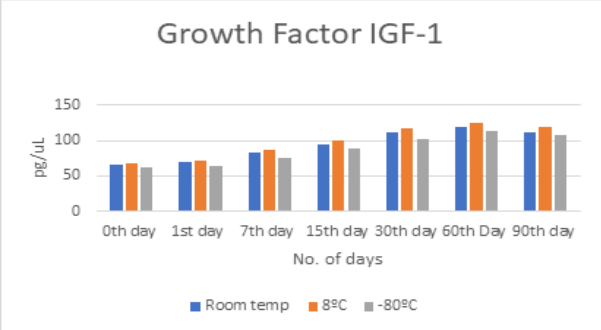

Figure 4: Insulin-like growth factor (IGF)-1.

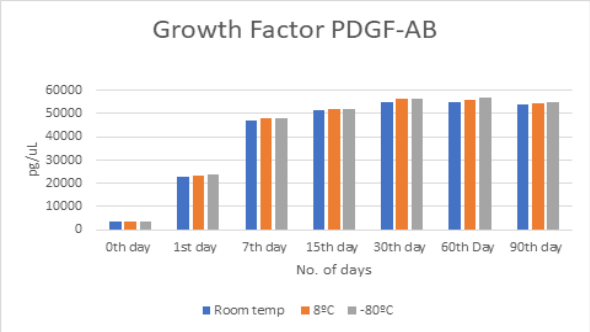

Figure 5: Platelet-derived growth factor (PDGF)-AB.

were calculated (Table 3). It was observed that the fresh PRP when kept at room temperature gets coagulated and rendered useless for further studies. As a result, no more growth factor measurements could be done at room temperature. Similar to this, it was shown that the PRP stored at 8ºC coagulated after 3 to 4 hours. The fresh PRP kept at -80ºC was evaluated for 10 days to quantify the GFs. It was observed that for the 1st–7th day, the PRP had good properties. The growth factors when evaluated were found to be increased significantly till day 7. From day 8, the appearance of PRP was deteriorated and even the concentration of growth factors was found to be declined and variable. Hence the sample was discarded on day 10.

Vascular Endothelial Growth Factor (VEGF)

The first whole blood samples' plasma VEGF concentrations (day 0) ranged from 40.3 to 86.0 pg/mL. Figure 1, the mean VEGF level of PRPs dramatically increased to 324 pg/mL on day 1 following centrifugation and lyophilization (t-test, p 0.001). There were statistically significant differences (rANOVA, p 0.023) across storage days, demonstrating that VEGF levels increased statistically between days 1 and 7, 15 and 30, as well as between days 60 and 90. It was observed that the concentration of release of VEGF stored at -80ºC was not significantly higher than that stored at 8ºC.

Fibroblast Growth Factors (FGF) Basic

Plasma FGF-Basic levels in the initial whole blood samples (day 0) varied from 4.8 to 40.3 pg/mL. (Figure 2). During centrifuge preparation and lyophilization, the mean FGF-Basic level of PRPs significantly rose from 119.0 to 132 pg/mL on day 1 (t-test, p <0.001). There were no discernible differences (rANOVA, p = 0.146) across storage days. It was observed that the concentration of release of L-FGF stored at -80ºC was not significantly higher than that stored at 8ºC.

Hepatocyte Growth Factor (HGF)

The initial whole blood samples' plasma HGF concentrations (day 0) ranged from 501 to 658 pg/mL (Figure 3). On day 1 following lyophilization, the mean HGF level remained at a level similar to that of the initial plasma (t-test, p=0.9609). Though, it was observed that the concentration of release of L-HGF increased significantly from day 7th to day 60th and a slight decline was observed with further storage till day 90th. Also, the concentration of release of L-HGF stored at -80ºC was not significantly higher than that stored at 8ºC.

Insulin-like Growth Factor (IGF)-1

Plasma IGF-1 levels in the initial whole blood samples (day 0) varied from 46.5 to 88.0 pg/mL (Figure 4). The average IGF level has a pattern that is comparable to that of HGF. However, it was observed that the concentration of release of L-IGF-1 increased significantly from day 7th to day 60th and a slight decline was observed with further storage till day 90th. Also, the concentration of release of L-IGF-1 stored at -80ºC was not significantly higher than that stored at 8ºC.

Platelet-derived Growth Factor (PDGF)-AB

The initial whole blood samples' plasma PDGF-AB levels (day 0) ranged from 1247 to 7939 pg/mL (Figure 5). It was observed that the concentration of release of PDGF-AB increased significantly from day 1st to day 90th after lyophilization (t-test, p<0.001). There was no decline observed with further storage till day 90th. Also, the concentration of release of L-IGF-1 stored at -80ºC was not significantly higher than that stored at 8ºC.

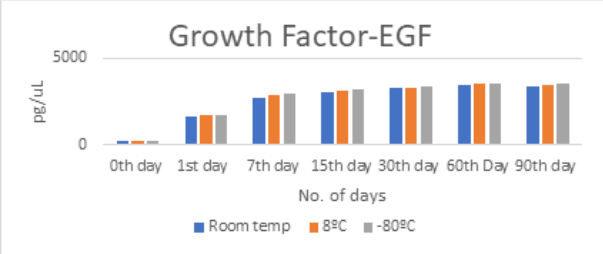

Figure 6: Epidermal growth factor (EGF).

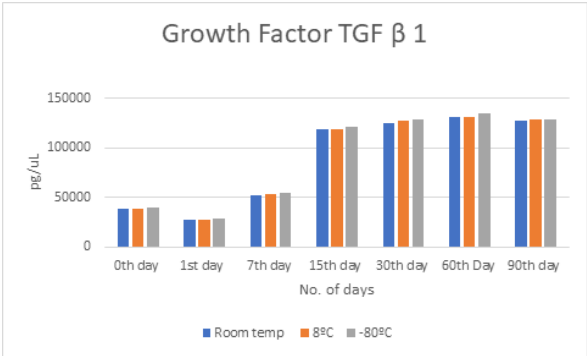

Figure 7: Transforming growth factor (TGF)-beta1.

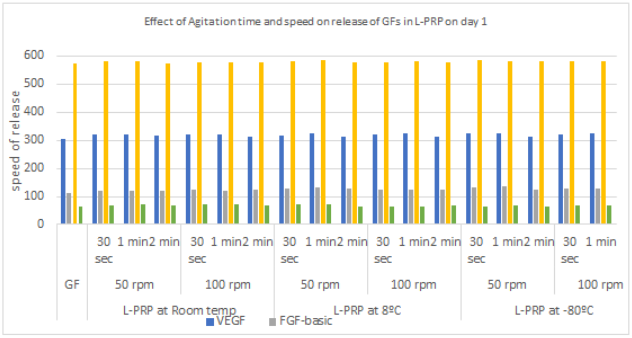

Figure 8: Effect of Agitation on release of GFs in L-PRP.

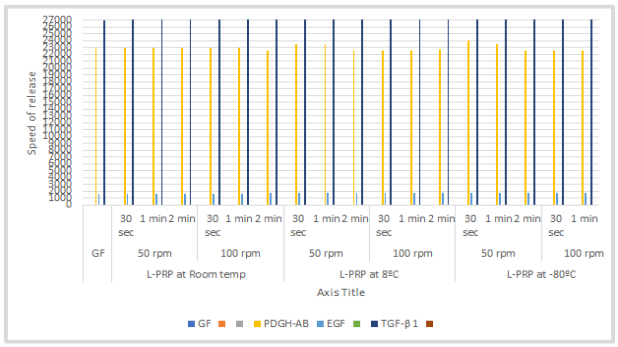

Figure 9: Effect of Agitation time and speed on release of GFs in L-PRP on day 1.

Epidermal Growth Factor (EGF)

Plasma EGF levels in the initial whole blood samples (day 0) varied from 25 to 350 pg/mL (Figure 6). The EGF level shows increased significantly from 1658 to 3569 pg/uL from day 1st to day 60th and a slight decline was observed with further storage till day 90th. Also, the concentration of release of L-IGF-1 stored at -80ºC was not significantly higher than that stored at 8ºC.

Transforming Growth Factor (TGF)-Beta1

Mean TGF-beta1 levels in PRPs after centrifugation and deep freezing exhibited the same trend as FGF basic (Figure 7). Plasma TGF-beta1 levels in initial whole blood samples (day 0) varied from 9689 to 80,563 pg/mL. After lyophilization, the TGF-beta1 level of L-PRPs significantly rose from 27025 pg/mL to a plateau level on day 30 (t-test, p 0.001). Again, showing a significant rise in TGF-beta 1 levels from day 60th to 90th. No discernible change between storage days was statistically significant (rANOVA, p=0.924). Also, the concentration of release of TGF-beta 1 stored at -80ºC was not significantly higher than that stored at 8ºC.

Transforming Growth Factor (TGF)-Beta1

Figure 8 shows effect of agitation on release of GFs in L-PRP. The results of the investigation demonstrate that the concentration of GFs in L-PRP held at room temperature has not changed much (although it has slightly increased), 8 and -80ºC even if agitated for 2 minutes at 100 rpm.

Figure 8 shows that there is no significant change (slight rise) in the concentration of GFs VEGE, FGF- basic, HGF and IGF-1 in lyophilized-PRP stored at room temperature, 8 and -80ºC even if agitated for 2 minutes at 100 rpm.

Figure 9 shows that there is no significant change (slight rise) in the concentration of GFs PDGH- AB, EGF and TGF-β1 in lyophilized-PRP stored at room temperature, 8 and -80ºC even if agitated for 2 minutes at 100 rpm.

DISCUSSION

The main objective of study was to assess how lyophilization affected PRP and its characteristics, such as the number of growth factors. Also, the effect of storage conditions on growth factors were studied. This will be very useful in understanding and deciding the effectivity of lyophilized PRP for longer.[20-22] It was observed that the lyophilized PRP when kept at room temperature, 8 and -80ºC was stable. As per over observations, the concentration of growth factors in lyophilized PRP was also as per the trend reported in the earlier studies. But it was observed that the increase in the GFs in fresh PRP stored at -80ºC was much higher than that of lyophilized PRP stored at same condition. This may be because of more stable physical condition of lyophilized PRP, the release of GF is slow when the Lyophilized PRP is kept undisturbed. Many studies measure GFs of PRP after activating the platelets by various techniques. Hence there is huge difference in the observations of various studies for estimating the concentrations of the GFs in PRP.[23-25] Our data is more towards accuracy as the concentration of the growth factors are measured immediately after reconstitution and the readings for each sample at each storage condition is taken thrice and the mean value is used for further calculations.

Our results suggest that platelet-released factors during the freeze-thawing of L-PRP included EGF, VEGF, PDGF-AB, FGF-basic, and TGF-beta1. HGF and IGF-1 were categorized as plasma factors because they maintained steady levels following

preparation by centrifugation and the lyophilization procedure. Paola *et al.*[26] and Barry *et al.*[24] only sometimes identified IGF-1 as a plasmatic factor, possibly because there were no obvious differences in IGF-1 levels between PRP and PPP. Hesham *et al.* demonstrated that L-PRP had much higher levels of IGF-1 than the whole original blood.

L-PRP was reconstituted by adding sterile water for injection. The reconstitution was carefully carried out to make the volume of LPRP exactly equal to that of fresh PRP which was lyophilized. The LPRP stored at room temperature was directly reconstituted, but the PRP stored at 8 and -80ºC will allow thaw to room temperature before reconstitution. The thawing process required approx. 0.5 hours for L-PRP stored at 8ºC and 2.40 hours for L-PRP stored at -80ºC. Even the time and agitation speed required for reconstitution of the L-PRP stored at -80ºC was relatively more than that required for reconstituting the L-PRP stored at room temperature and 8ºC. Also, the concentration of GFs obtained from the L-PRP kept at room temperature less as that observed from the L-PRP kept at 8ºC. Surprisingly, the concentration of GFs observed for L-PRP kept at -80ºC was almost the same as that kept at 8ºC. Even though the time required for reconstitution and agitation rate was much higher for L-PRP stored at -80ºC.[21]

This may be because the L-PRP kept at 8ºC underwent freeze-thawing as soon as it was removed from the fridge and kept at room temperature. Due to freeze thawing process that took place naturally, the agitation time required to reconstitute the L-PRP was much less and the release of GFs was much higher than the L-PRP stored at room temperature.[21] As the maximum GFs are present inside the platelet structure, the L-PRP shows higher concentration of GFs than fresh PRP. The increase in GF in L-PRP may also be due to the alteration of the structure of platelets that took place during the process of lyophilization. The storage of L-PRP in lower temperature and subsequent thawing also aided the release of GFs of L-PRP which was also confirmed by the difference in the concentration of GFs of L-PRP stored at room temperature, 8 and -80ºC.

The increase in concentration of GFs was not significantly different in L-PRPs stored 8ºC and -80ºC. Also as mentioned above the time and agitation rate required to reconstitute the L-PRP stored at -80ºC was much more than that stored at 8ºC. We suggest that storing lyophilized PRP at 8ºC or in normal fridge is sufficient to have effective growth factor concentration. This method is also cost-effective easy to access and less time-consuming. Because of limited sample size the study on effect of agitation speed and time could be performed for only one reading for various storage conditions, speed and time. The statistical power should be increased by using more samples.

Our findings indicated that platelets release FGF-basic, VEGF, EGF, PDGF-AB, and TGF-beta1. Further research is needed to determine whether and how these growth factors assist tissue regeneration following PRP injection.

CONCLUSION

PRP is an effective and emerging technique for medical procedures for treating diabetic wounds, tendon injury, osteoarthritis, cosmetics, ENT, Dental procedures etc. All these treatments demand multiple injections of PRP for better results. Our data demonstrate that the lyophilized PRP has the potential to be effective as it maintains high level of growth factors required, on storage at 8ºC and is reconstituted every time with sterile water for injection by applying agitation of 50 rpm for 1-minute. Hence multiple injections of same sample of PRP could be possible. The study shows that the L-PRP can release greater amount growth factors with more stable formulation than fresh PRP for 90 days. This is because the process of lyophilization causes the alteration in the structure of platelets which reflects in the release of growth factors. The best storage condition to maintain the stability of L-PRP is at 8ºC which is economical and easily available at any clinic. This also results in low-cost treatment for the patient.

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REFERENCES

1. Thompson M, Hulley P, Noble A, Willett K. The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery a review of the literature. The Journal Of Bone And Joint Surgery. 2009;91(8):987–996. Available from: https://doi.org/10.1302/0301-620X.91B8.22546
2. Alsousou J, Ali A, Willett K, Harrison P. The role of platelet-rich plasma in tissue regeneration. 24(April 2012), Platelets. 2013;173–182. Available from:https://doi.org/10.3109/09537104.2012.684730
3. Khan M, Bedi A. Cochrane in CORR: Platelet-rich Therapies for Musculoskeletal Soft Tissue Injuries (Review). Clin Orthop Relat Res. 2015
4. Gilliland AC, Salazar DL, Borchers RJ. Ultrasound Versus Anatomic Guidance for Intra-articular and Periarticular Injection:A Systematic Review, The Physician and Sportsmedicine. 2011;121-131.
5. Bookman AJS, Pereira DS. Ultrasound guidance for i ntra-articular k nee a nd s houlder i njections : a r eview. Bulletin of the NYU Hospital for Joint Diseases. 2014.
6. Kon E, Buda R, Mandelbaum B, Filardo G, Delcogliano M, Timoncini A, Fornasari MP, Giannini S, Marcacci M. Platelet-Rich Plasma Intra-Articular Injection Versus Hyaluronic Acid Viscosupplementation as Treatments for Cartilage Pathology: From Early Degeneration to Osteoarthritis, Arthroscopy: The Journal of Arthroscopic and Related Surgery. 2011;1490-1501.
7. Kon E, Buda R, Filardo G, Di A, Timoncini A, Cenacchi A, Maria P. Platelet-rich plasma : intra-articular knee injections produced favorable results on degenerative cartilage lesions.

Knee Surg Sports Traumatol Arthrosc. 2010;472–479. Available from: https:// doi.org/10.1007/s00167-009-0940-8
8. Dhurat R, Sukesh MS. Principles and Methods of Preparation of Platelet-Rich Plasma : A Review and Author' s Perspective. Journal of Cutaneous and Aesthetic Surgery. 2014;7(4):189–198. Available from:https://doi.org/10.4103/0974-2077.150734
9. Ehrenfest DMD, Andia I, Zumstein MA, Zhang C, Pinto NR, Bielecki T. Classification of platelet concentrates (Platelet-Rich Plasma-PRP , Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine : current consensus , clinical implications and perspectives. Muscles, Ligaments and Tendons Journal. 2014;4(1):3–9.
10. Ehrenfest DMD, Bielecki T, Mishra A, Borzini P, Inchingolo F, Sammartino G, Rasmusson L, Everts PA. In Search of a Consensus Terminology in the Field of Platelet Concentrates for Surgical Use: Platelet-Rich Plasma (PRP), Platelet-Rich Fibrin (PRF), Fibrin Gel Polymerization and Leukocytes. Current Pharmaceutical Biotechnology. 2012;1131–1137.
11. Lubkowska A, Dolegowska B, Banfi G. Growth factor content in PRP and their applicability in medicine, J. Biol. Regul. Homeost. Agents. 2012;26(2 Suppl. 1):3s–22s.
12. Gobbi G, Vitale M. Platelet-Rich Plasma Preparations for Biological Therapy: Applications and Limits, Operative Techniques in Orthopedics, 2012;10-15
13. Martínez CE, Smith PC, Alvarado VAP. The influence of platelet-derived products on angiogenesis and tissue repair : a concise update. Frontiers in Physiology. 2015; 6(8):1–7. Available from: https://doi.org/10.3389/fphys.2015.00290
14. Ramzy MM, Essawy TA, Shamaa A, Sayed S, Mohammed A. Evaluation of the Effect of Platelet Rich Plasma on Wound Healing in the Tongue of Normal and Streptozotocin-induced Diabetic Albino Rats : Histological , Immunohistochemical , and Ultrastructural Study, Macedonian Journal of Medical Sciences. 2020;8:666–689.
15. Fung KM, Grossman JB, Hillyer DC, Westhoff MC, AABB Technical Mannual, 18TH Edition 2014.
16. Kumar RD, Vasanth PM, Ramesh T, Ramesh T. Formulation and Evaluation of Lyophilized Antibacterial Agent, International Journal of PharmTech Research. 2013;5(4):1581-1589.
17. Silva A. Platelet-rich plasma lyophilization enables growth factor preservation and functionality when compared with fresh platelet-rich plasma. Regenarative Medicine. 2018.
18. Hafner G. Comparison of platelet, leukocyte, and growth factor levels in point-of-care platelet-enriched plasma, prepared using a modified Curasan kit , with preparations received from a local blood bank. Clin. Oral Impl. Res. 1998:357–362.
19. Huber S. Freeze-dried versus fresh platelet-rich plasma in acute wound healing of an animal model. Regenerative Medicine 2019;14:525–534.
20. Kieb M, Sander F, Prinz C, Adam S, Mau-möller A, Bader R, Peters K, Adam S, Mau-mo A. The American Journal of Sports Medicine 2016;XX(x). Available from: https:// doi.org/10.1177/0363546516674475
21. Wen Y, Lin W, Lin C, Sun Y, Chang P, Wang H, Lu J, Yeh W, Chiueh T. Clinica Chimica Acta Sustained or higher levels of growth factors in platelet-rich plasma during 7- day storage. Clinica Chimica Acta. 2018;483(4):89–93. Available from: https://doi.org/10.1016/j.cca.2018.04.027
22. Silva A. Platelet-rich plasma lyophilization enables growth factor preservation and functionality when compared with fresh platelet-rich plasma. Regenerative Medicine. 2018.
23. El-sharkawy H, Kantarci A, Deady J, Hasturk H, Liu H, Alshahat M, Dyke, TE Van. Platelet-Rich Plasma : Growth Factors and Pro- and Anti-Inflammatory Properties. J. Periodontol. 2007):661–669. Available from:https://doi.org/10.1902/ jop.2007.060302
24. Eppley BL, Woodell JE, Higgins J. Platelet Quantification and Growth Factor Analysis from Platelet- Rich Plasma: Implications for Wound Healing. Plastic and reconstructive surgery 2003:1502–1508.
25. Kleinheinz J, Jung S, Wermker K, Fischer C, Joos U. Release kinetics of VEGF 165 from a collagen matrix and structural matrix changes in a circulation model. Head & Face Medicine. 2010:1–7.
26. Amable PR, Bizon R, Carias V, Vinicius M, Teixeira T, Pacheco C. Platelet-rich plasma preparation for regenerative medicine : optimization and quantification of cytokines and growth factors. Stem Cell Research & Therapy. 2013:1–13.