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Cell Multiplication in Platelet Rich Fibrin (PRF) and Platelet Rich Plasma (PRP) as Biofuels for Cartilage Tissue Engineering

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ABSTRACT

Cartilage injury remains a major orthopedic challenge due to its low repair capacity, with the tendency to cause osteoarthritis. In addition, the treatment method involving platelet rich plasma (PRP) and platelet rich fibrin (PRF) as cartilage engineering components greatly anticipates promising recovery results. This research is aimed at determining the number of cells in platelet rich plasma (PRP) and platelet rich fibrin (PRF), particularly inflammatory cells. Blood specimens were collected from 30 rabbits that were divided into three treatment groups. The samples were then processed into control, PRP and PRF. This was followed by determining the number of cells in the form of platelets, leukocytes, lymphocytes, neutrophils and monocytes. According to the blood test results, the number of platelets increased by 3.8 and 4.5-fold in the PRP and PRF groups, respectively. Furthermore, 2 times (2.1-fold) increment was observed in leukocyte concentration for PRF, but zero in PRP. Conversely, the number of lymphocytes, neutrophils and monocytes decreased to 82, 72 and 40%, correspondingly, while PRP still showed no change. Based on the overall results and discussion, the number of platelets in both PRP and PRF increased severally, while leukocyte only occurred in PRF. This multi-fold expansion accompanied by growth factors has the capacity to accelerate cartilage recovery. Furthermore, the presence of inflammatory cells enhances the healing potential already existing in PRF.

Keywords: *Cartilage engineering, Platelet rich fibrin, platelet rich plasma, Growth factor*

1. INTRODUCTION

Cartilage injury continues to remain a major orthopedic challenge. Low joint cartilage repairability instigates further complications, including osteoarthritis [1]. Treatment commences from a pharmacological approach, surgery and a combination with tissue engineering. Currently, the results vary depending on the severity of the injury and preferred corrective method [1,2].

Treatment with tissue engineering is widely adopted, due to its promising outcomes and is conducted by providing growth factor, scaffold and mesenchymal stem cell (MSCs) components [3]. In cartilage cases, tissue engineering using platelet rich plasma (PRP) and platelet rich fibrin (PRF) appears very predominant. This is because the PRP and PRF preparation processes are relatively simple, safe, relatively favorable results and are also performed with minimal facilities [3,4].

PRP was initially introduced as an alternative to fibrin glue in maxillofacial surgical reconstruction where bone grafting is required. PRP also contains platelets rich in growth factors and bioactive proteins known to accelerate the healing of cartilage injury [5,6]. Meanwhile, PRF presently has a wider application for tissue regeneration in bone, muscle and cartilage. This is not only attributed to the platelet contents, but also the numerous leukocytes. The combination of platelets, leukocytes and other growth factors are believed to accelerate cartilage recovery, although PRP and PRF are obtained from the blood centrifugation process in the tube to form a layer [7].

PRP and PRF contain diverse growth factors, such as transforming growth factor (TGF-β), platelet-derived

growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (ECGF), fibroblast growth factor basic (b-FGF), insulin growth factor (IGF1) and interleukin (IL-6). Circulating growth factors play an important role in tissue repair, including cell proliferation, migration, chemotaxis, cell differentiation and extracellular matrix synthesis [7,8].

The analysis of total cells in PRP and PRF has not been widely conducted. Inflammatory cells present in PRP and PRF also play a very significant role in the tissue healing process. Therefore, the objective of this research is to determine the number of cells in platelet rich plasma (PRP) and platelet rich fibrin (PRF), particularly inflammatory cells.

2. METHODS

2.1. Preparation of PRP and PRF

The research method was approved by the Ethical Committee of Hasanuddin University, Makassar. A total of 30 New Zealand white rabbits were divided into 3 treatment groups. In the control category, 5 cc of blood was collected from the first 10 animals, followed by counting the number of blood cells (complete blood count, CBC). In the second group, similar blood quantity and rabbit number were obtained and then processed with PRP, while the third group employed PRF.

In PRP preparation, 5 cc of blood was extracted and then placed in a tube with anti-coagulant, followed by the 2-stage centrifugation process. The first centrifugation occurred at 3000 rpm for 10 minutes, where the top layer of the buffy coat (middle) was removed. This isolated sample was re-centrifuged at 3000 rpm for 10 minutes to form 2 layers, and the top layer was platelet rich plasma [5]. The processing of platelet rich fibrin was based on Doohan standards, where 5 cc of blood was collected in a tube without anticoagulant, followed by centrifugation at 1500 rpm for 10 minutes in a vertical position. The tube is expected to form 3 layers, with PRF in the middle [9,10]. Furthermore, centrifugation was conducted using the Duo Quattro machine (process for PRF, Nice, France).

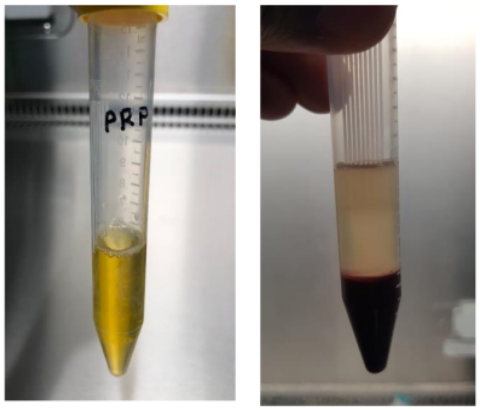
Subsequently, PRP and PRF were calculated with the complete blood count (CBC) to ascertain the number of platelets, leukocytes, lymphocytes, monocytes and neutrophils. Also, the CBC was performed with a Sysmex XN-550 machine (Sysmex Corporation, Kobe, Japan), based on flow-cytometry.

3. RESULT AND DISCUSSION

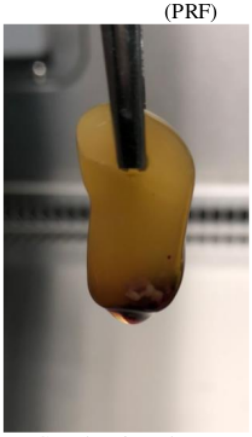
3.1 Results

3.1.1 Platelet rich Plasma (PRP) and Platelet Rich Fibrin (PRF)

The centrifugation process was conducted immediately after the blood samples were collected from the rabbits, either in the PRP or PRF.



A. Platelet Rich Plasma (PRP) B. Platelet Rich Fibrin (PRF)



C. Tube-ejected PRF

Figure 1. Processed Platelet rich plasma (PRP) and Platelet rich fibrin (PRF).

3.1.2 Cell number on PRP and PRF

The examination for total cell count was obtained from the middle layer of PRP and PRF. In PRF, the material was collected on a buffy coat, which was 0.5 cm from the bottom of the middle layer (Figure 1).

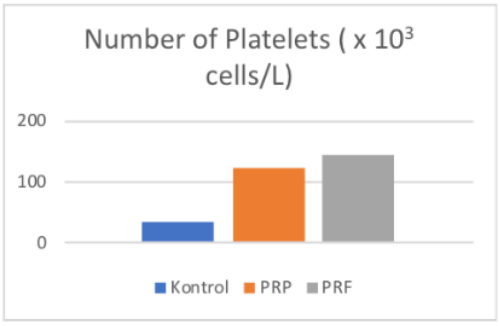


Figure 2. Number of platelets on control, PRP and PRF.

Based on complete blood count (CBC), the average number of platelets in whole blood, PRP and PRF were 32 x 10^3, 122 x 10^3 and 143 x 10^3 cells/L, respectively (Figure 2).

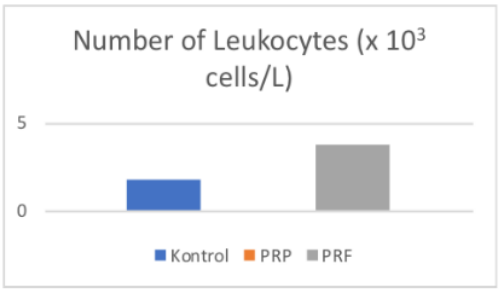


Figure 3. Number of Leukocytes on control, PRP and PRF.

Based on complete blood count (CBC), the average number of leukocytes in whole blood sample, PRP and PRF were 1.8 x 10^3, 0 and 3.8 x 10^3 cells/ L, respectively (Figure 3).

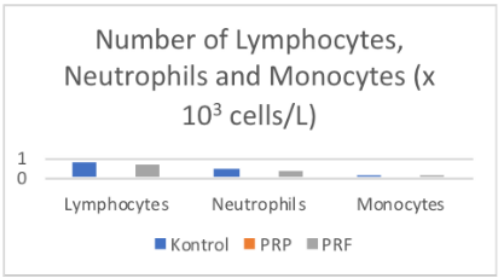


Figure 4. Number of Lymphocytes, Neutrophils and Monocytes on control, PRP and PRF.

Based on the cell count, the average lymphocytes in whole blood, PRP and PRF were 8.2 x 10^2, 0 and 6.4 x 10^2 cells/L, respectively. Furthermore, the average number of neutrophils in whole blood, PRP and PRF were 4.3 x 10^2, 0 and 3.1 x 10^2 cells/L, while the average number of monocytes in whole blood, PRP and PRF were 50, 0 and 20 cells/L, correspondingly (figure 4).

3.2 Discussion

Based on this research, the platelet rich plasma (PRP) and platelet rich fibrin (PRF) were known to increase the platelet concentration by 3.8 and 4.5 fold, respectively, compared to whole blood. The number of cycles and centrifugation interval tends to influence the platelet count. In another research with centrifugation at 2,700 rpm for 12 minutes, the number of platelets and leukocytes decreased to 97 and 50%, correspondingly, compared to whole blood. Centrifugation was further conducted at 1,300 rpm for 8 minutes, leading to an increment in the number of platelets by 1.6 fold. This was subsequently referred to as an advanced platelet rich plasma [11].

The research method that involved changing the PRF reading on the tube also showed a significant increase in platelet count. Taking PRF at 0.3-0.5 cm at the bottom of the PRF layer has a high tendency to enhance platelet count. This region is typically called the buffy coat, where the number of platelets and leukocytes expands between 10-20 folds, compared to whole blood [12]. Furthermore, PRF materials are not only present in the coagulation pathway in cartilage healing, due to their ability to release a broad spectrum of cytokines, chemokines, growth factors, and other mediators, but also has the capacity to release several molecules such as von Willebrand factor, P-selectin, fibronectin, VEGF, platelet-derived endothelial growth factor (PDEGF), vitronectin and fibrinogen [13,14].

In this research, 2.1 fold increase was observed in the number of leukocytes for PRF, but zero for PRP. This increment is always in harmony with the growth in the number of platelets during PRF manufacture. The zero-leukocyte count in PRP was due to rapid centrifugation that was performed twice, with nothing left in the layer. However, low centrifugation on PRF allows leukocytes to remain in its layer. The presence of these leukocytes is considered a PRF advantage, where a faster healing appears obtainable [13].

Platelet rich plasma and platelet rich fibrin are tissue engineering technologies widely applied in healing cartilage injuries. In addition, platelets as the dominant components, represent the main cells responsible for the biological activity during the recovery process. The PRF composition includes the concentrate of white blood cells, platelets and fibrin [13]. Regardless of the significant roles of these cells in blood clot formation, various platelet-derived protein molecules involved in the cartilage healing cascade are inherent contents [13].

However, to determine the amount of growth factor in PRP, PRF and Advance PRF, PRP is expected to release significantly higher proteins at an earlier period.

Meanwhile, in PRF and advance-PRF, a continuous and steady release of growth factors for over 10 days was recorded [8]. In another research, TGF-b1, VEGF in CGF and CD34 positive cells were discovered in the PRF. These protein components jointly help promote tissue healing [15].

In platelet rich fibrin, the number of lymphocytes decreased to 82%, while neutrophils declined to 72% and eventually to 40%. However, the number of lymphocytes, neutrophils and monocytes were all zero in the platelet rich plasma, with no traces of inflammatory cells. This was because the high and repeated cycles caused the cells to be more concentrated in the lowest red blood cell layer [13].

Monocytes, lymphocytes and neutrophils are the main cells for the healing of cartilage tissues. The monocytes migrate into the inflamed region after the neutrophil's influx, where both then become macrophages with a crucial role in early inflammation. Meanwhile, neutrophils and other components such as granulocytes are capable of travelling deeper into the clot matrix of the clot, due to their sizes. With an average diameter between 8.5–10 lm, neutrophilic granulocytes appear remarkably smaller, compared to monocytes (15–20 lm) and therefore are more prone to intense clot penetration during centrifugation [14].

PRP has been analyzed for over 10 years with varying results. Several research in osteoarthritis patients generated satisfactory outcomes. Research on intra-articular injection with PRP showed that the results were dependent on the injection timing. The duration of the therapeutic effect is based on the action interval of the growth factor and the 9 months estimated median interval of the PRP effect [16]. Conversely, research regarding the PRF benefits on cartilage appear relatively recent, compared to PRP. Various attempts generated a greater potential for PRF to heal cartilage injuries than PRP, although more investigations on the PRF benefits for joint cartilage are highly necessary [7,16].

4. CONCLUSION

Based on the overall results and discussion, several increases in the number of platelets (3 to 4-fold) were reported in both platelet rich plasma (PRP) and platelet rich fibrin (PRF), while leukocytes only occurred in PRF. This multi-fold enhancement accompanied by growth factors are believed to accelerate cartilage healing. Furthermore, the presence of inflammatory cells, including lymphoists, neutrophils and monocytes also has the capacity to increase the healing potential already present in PRF.

AUTHORS' CONTRIBUTIONS

Ahmad Taufik S: Data collection, manuscript writing
Adnanto Wiweko: Data collection, literature review writing
Bayu Tirta Dirja: Manuscript review, proof-reading.
Devi Rahmadona: Laboratory supervisor, manuscript writing
Dyah Purnaning: Data collection, literature review writing.
Mohammad Rizki: Laboratory supervisor, manuscript writing.

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