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Platelet-rich plasma as xenofree-growth factor for mesenchymal stem cells culture

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Abstract

Stem cell-based therapy is currently believed to be the future of medicine. The type of stem cell that is widely used in cell-based therapy is mesenchymal stem cells (MSCs). In stem cell-based therapy, the MSCs that will be used mostly need to be expanded first by culturing the cells. The serum that is often used in MSCs culturing is a fetal bovine serum (FBS). However, animal components should be avoided in clinical applications and Good Manufacturing Practices (GMP). FBS is not adhering to GMP, which compromises the safety and effectiveness of cell-based therapies. Therefore, other supplements are required for cultures without animal serum constituents. Platelet-rich plasma (PRP) is a plasma fraction derived from blood with a platelet concentration of 3-5 times higher than in blood. Several studies have shown that PRP contains various growth factors that can increase the proliferative and differentiation capacity of MSCs which is equivalent to or even better than FBS. PRP also has the advantage that it can be an autologous or allogeneic product from human peripheral blood, so it is free from animal substances. Therefore in the future PRP has great potential as an alternative to FBS both in culture expansion and in MSCs processing.

Keywords: Growth factors, mesenchymal stem cells, platelet-rich plasma, xenofree.

INTRODUCTION

Stem cells are currently thought to represent the future of medicine due to its significant therapeutic and technological advantages in treating progressive diseases like cardiovascular disease, diabetes, and neurological disorders (1).

Types of stem cells that are often used in cellular therapy in various medical fields are human mesenchymal stem cells (hMSCs) (2). Mesenchymal stem cells (MSCs) are undifferentiated cells that can be isolated from a variety of bodily tissues, including adipose tissue, cutaneous tissue, muscle-skeletal fluid, amniotic fluid, synovial fluid, tissue, and umbilical cord blood (3,4). Several reasons that cause MSCs to be widely used in clinical applications include MSCs being easily obtained from various sources, either through invasive procedures or from medical waste, such as umbilical cord. MSCs also have the rapid proliferative ability, high differentiation capacity, and the ability to migrate to damaged areas, so they are widely considered for clinical applications in cell-based therapies (5,6).

The multiplication of stem cells aims, among other things, to enhance the quality and number of cells so that they can be utilized in cell-based therapies (7). Moreover, cell culture plays an essential role in the in vitro research of human cells to establish disease mechanism simulation, vaccine discovery, and the toxicity of new therapeutic molecules. Historically, cell culture techniques have depended significantly on animal serum as the primary growth medium supplement (8). The animal serum supplement most commonly used in cell culture is fetal bovine serum (FBS), which is known to increase the proliferation, differentiation, and survival of numerous cell types (8,9). FBS has several advantages over other animal serums, including its high concentration of adhesion molecules, growth factors, micronutrients, and hormones that promote the attachment, development, and proliferation of mammalian cells. Additionally, FBS can boost cell growth and enhance the differentiation capacity of mesenchymal stem cells. FBS is also relatively inexpensive to utilize (2).

In conjunction with the expansion of regenerative medicine and stem cell-based therapy, the use of animal components in the cell preparation process has become a source of concern. There is a possibility of transmission of viruses, mycoplasmas, prions, and other unidentified zoonotic organisms when FBS is excluded from the principles of Good Manufacturing Practice, as it may compromise the safety and effectiveness of cell-based therapies (8,10). Alternative supplements are being investigated to replace FBS to avoid this issue.(2,8) As an alternative to FBS, human autologous and allogeneic products, such as platelet lysate, platelet-rich plasma (PRP), and poor platelet plasma (PPP), are being explored (8).

PRP is considered an autologous source for tissue engineering applications (11). PRP contains at least seven major growth factors namely platelet-derived growth factor, transforming growth factor, epidermal growth factor, insulin-like growth factor, basic fibroblast growth factor, and vascular endothelial growth factor that has been identified is present in platelets (12,13). This growth factor is known to regulate a series of molecular events leading to collagen synthesis, angiogenesis, mesenchymal stem cell mitogenesis, and chondrocyte growth and differentiation (12). Due to its potential, PRP is considered as a supplementary medium to replace FBS, especially in stem cell culture. In this paper, we will discuss further about PRP as a growth factor in MSCs culture.

Platelet-Rich Plasma

Platelet-Rich Plasma (PRP) is a plasma fraction derived from blood with a platelet concentration 3-5 times higher than in the blood. Platelets are small nucleated cell fragments with a diameter of 2 to 3 m that are released from megakaryocytes in the bone marrow. Platelets contain abundant protein, growth factors, and cytokines which are stored in cytoplasmic granules (14). PRP contains at least seven major growth factors, namely platelet-derived growth factor (PDGF), transforming growth factor (TGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) (9,12,14). This growth factor is recognized to play a vital part in a range of molecular processes that contribute to tissue regeneration, including collagen synthesis, angiogenesis, mitogenesis, and cell growth and differentiation (12).

Mechanically, in cartilage and tendons, PRP improves the repair of wounded tissues by blocking cell death and lowering the inflammatory response, as well as by boosting cell proliferation and collagen formation (13–15). Dense granules in platelets carry critical components for tissue homeostasis, including adenosine diphosphate (ADP), adenosine triphosphate (ATP), calcium ions, histamine, serotonin, and dopamine (14). There are several types of platelet concentrates based on the manufacturing process or production, namely(11,14,16):

a. Pure platelet-rich plasma (P-PRP), contains no leukocytes and exhibits a low-density fibrin mesh upon activation.
b. Leukocyte and platelet-rich plasma (L-PRP), contains leukocytes and exhibits a low-density fibrin net upon activation.
c. Pure platelet-rich fibrin (P-PRF), containing no leukocytes and with a high-density fibrin mesh. Unlike P-PRP and L-PRP, this product is non-injectable and takes the form of a gel when activated.
d. Leukocyte and platelet-rich fibrin (L-PRF), containing leukocytes and with a high-density fibrin mesh.

Each type is distinguished based on the content of leukocytes and fibrin (Figure 2).

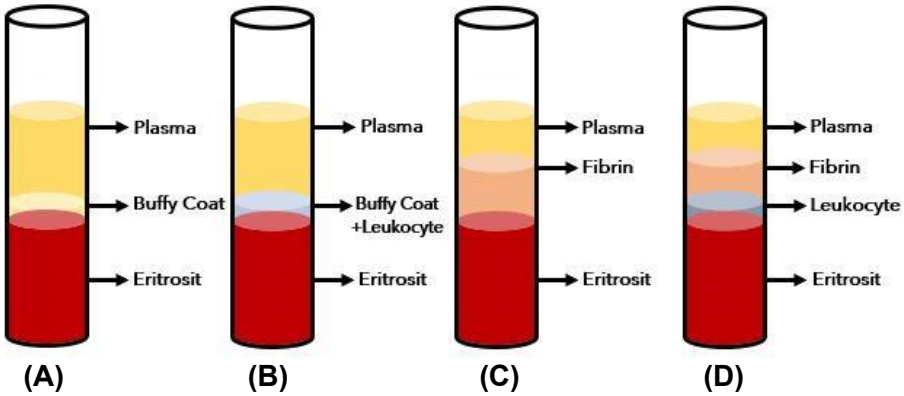

Figure 1. Type of Platelet Concentrates
(A) Pure platelet-rich plasma (P-PRP), (B) Leukocyte and platelet-rich plasma (L-PRP), (C) Pure platelet-rich fibrin (P-PRF), (D) Leukocyte and platelet-rich fibrin (L-PRF)

Isolation Methods of Platelet-Rich Plasma

PRP isolation is typically prepared by drawing autologous whole blood from the patient, which is then centrifuged in two steps to separate the plasma from the red blood cells and leukocytes. Patients donor must undergo laboratory examination of complete blood cell counts to ensure that the patient donor does not have thrombocytopenia. The platelet count must be above 100,000/mm^3, for platelet aggregation to occur (17). The blood used is obtained from a vein puncture in an anticoagulant tube which contained citric acid dextrose or sodium citrate solution (15,18,19). The minimal volume of the blood which can be used for PRP isolation was 3.5 mL with results in platelet count was 1.222 ± 166 x 10^3 as reported in previous study (20). The isolation method is affected by the centrifugation speed. This factor plays a role in determining the final concentration of platelets and leukocytes in PRP (18,21). Centrifugation of the blood that has been taken with a light rotation to separate all blood components into three layers, namely the supernatant that is compatible with the acellular plasma, the buffy coat in the middle which contains platelet concentrate and at the bottom, there is a pellet rich in red blood cells (Figure 3) (14,22,23). Several PRP isolation methods have been developed to obtain optimal PRP product results (Table 1).

The results of PRP isolation can potentially be impacted by changes in pH, temperature, and time (within hours) after the vein was punctured. The growth factor will remain stable between plasma and PRP at the proper temperature and pH. PRP will quickly transform into a natural fiber scaffold that can trap additional platelets and slow down the rate of growth factor oxidation following activation (24). In addition, the time from the blood collection also crucial to be considered The Food and Drug Administration (FDA) in the United States, does not recommend the use of platelets that are more than 5 days after collection, due to a higher risk of bacterial contamination during vein puncture (18).

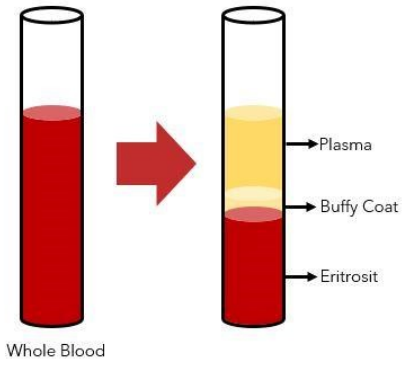

Figure 2. Platelet-Rich Plasma separation from the whole blood

Table 1. Isolation Methods of PRP

Centrifugal Condition	Anti-coagulant	Activated PRP	Platelet and Growth Factor	Ref
First spin 160 x g for 10 minutes at room temperature second spin 250 x g for 15 minutes at room temperature	Acid-citrate dextrose solution- A	10% $CaCl_2$ 22.8 mM	Platelet concentration ± 1300 x 10^9/l PDGF-AB concentration ± 40 ng/ml TGF-β1 concentration ± 130 ng/ml	25
First spin 472 x g for 10 minutes at 22-25° C second spin 984 x g for 5 minutes 22-25° C	Acid-citrate dextrose solution- A	$CaCl_2$ (0.1% at the final concentration)	Platelet count 6 x 10^6 /µl PDGF-BB concentration ± 2000 pg/ml	24
First spin 3000 rpm for 3 minutes second spin 4000 rpm for 15 minutes	Acid-citrate dextrose solution- A + 1 µg of PGE1 diluted in 0.05mL of saline prostaglandin E1 (PGE1) as a platelet aggregation suppressant.	20 µL of 8.5% $CaCl_2$	Platelet count 6,4 x 10^6 /µl PDGF-BB concentration 22171,7 pg/ml	27
First spin 200 g for 10 minutes at 4° C second spin 1550 g for 10 minutes at 4° C	-	1 ml of precipitated platelets and bottom-level plasma from the second centrifugation were used. It was then transferred to a fresh glass tube and incubated at 37 ° C for 15 minutes.	Platelet concentration ± 1156 x 10^9/l PDGF-AB concentration ± 600 pg/ml PDGF-BB concentration ± 1200 pg/ml TGF-β concentration ± 1000 pg/ml	28

Growth Factor within Platelet-Rich Plasma

The platelets in PRP need to be activated prior to application in order to have the growth factors effect. In the use of PRP for cell culture, heparin is used to prevent blood clotting before being added to the medium (25). Thrombin and calcium chloride are known to induce platelet aggregation, which aims to activate platelets and stimulate degranulation, causing the release of growth factor (18,26). If PRP is to be applied to a wound, activating factors such as thrombin can be added to the final platelet concentrate to stimulate platelet degranulation and exocytosis of factors.

Platelets contain a variety of bioactive molecules, including growth factors, cytokines, chemokines, and pro-inflammatory mediators such as prostaglandins, prostacyclins, histamine, thromboxane, serotonin, and bradykinin (27,28). Upon activation, platelets release various growth factors in high

concentrations, including members of the transforming growth factor b superfamily (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factors (IGF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). It also secretes hormones and cytokines associated with tissue repair, angiogenesis, and regeneration (25).

A glycoprotein called PDGF is produced at the site of damage when platelets degranulate. Target cells' cell membrane receptors are activated by PDGF, and these receptors subsequently activate signaling proteins to start actions unique to that target cells. These specific processes include macrophage activation, angiogenesis, and mitogenesis. In the meantime, platelets and macrophages release TGF-β, which inhibits the proliferation of healthy epithelial cells. PRP is also known to encourage fibroblast activation, migration, and growth. Cells produce the signaling protein VEGF, which promotes angiogenesis and vasculogenesis. The term vascular permeability factor is sometimes used. By promoting the migration, proliferation, differentiation, and stability of endothelial cells in new blood vessels, the VEGF content in PRP is known to contribute to the revascularization of injured tissue. An essential step in the recovery and/or regeneration of damaged tissue is neovascularization, or the development of new blood vessels. Although epidermal growth factor (EGF) is a growth factor that promotes epithelial cell growth, proliferation, and differentiation by binding to its receptors (16,22).

Table 2. The Main Growth Factor within PRP

Growth Factor	Function	Ref
PDGF	To stimulate MSCs proliferation, osteogenic differentiation of BMSCs, and stimulate angiogenesis	23, 35
TGF-β	To stimulate MSCs proliferation and osteogenic differentiation of ADSC	35
VEGF	Migration and mitosis of endothelial cells, to stimulate angiogenesis	21, 26
EGF	Stimulate angiogenesis, to stimulate re-epithelialization	21, 23
IGF	Promote cell proliferation, stimulate MSCs proliferation and osteogenic differentiation	21, 35
FGF	To stimulate MSCs proliferation, support BMSCs expansion, timulate BMSCs differentiation into bone, and stimulate angiogenesis	26, 35

PRP as an Alternative to Fetal Bovine Serum

Serum from fetal bovine (FBS) is the most frequent serum used in vitro for cell culture. FBS works as a broad-spectrum booster of the processes of cell adhesion, growth, and proliferation (2,9). FBS is typically recovered from fetal bovine blood obtained under sterile conditions following the slaughter of pregnant cows. After freezing, the cellular components were separated by centrifugation, to get rid of any possible bacterial and virus contamination, it is then filtered.

Until now, FBS is still the supplementary medium of choice in various cell culture protocols (2,29). FBS enables the expansion of human cell culture in vitro and supports the osteogenic, adipogenic, and chondrogenic differentiation potential of hMSCs. However, there are drawbacks to using FBS. The use of FBS as a supplement in cell culture carries the risk of transmission of pathogenic agents such as viruses, mycoplasmas, prions, and other unknown zoonotic agents. In addition, FBS cell culture showed an accumulation of bovine protein in the cells, which can be recognized as an antigenic substrate after transplantation into the host. This can trigger an immunological reaction thereby increasing its immunogenicity potential (2,8). For this reason, various attempts have been made to find substitutes for animal serum (30).

Efforts to find an alternative to FBS are very important, along with advances in regenerative medicine and stem cell-based therapy. Several candidates are available, including serum-free media and platelet derivatives. Alternatives to FBS and serum-free media, which can be used for cell therapy, are human serum (hS), platelet-rich plasma (PRP), and human platelet lysate (hPL) (2). The advantage of serum-free media is that it reduces the risk of contamination from pathogens. In serum-free media such as PRP, the advantages

are that it does not trigger an immunological reaction and contains growth factors for tissue regeneration purposes (31,32). However, the available commercial products of serum-free media are less affordable. This may become an additional burden for MSCs expansion as part of the preparation phase for clinical application.

Alternatively, the PRP, gives advantages such as it does not trigger an immunological reaction and contains growth factors that crucial for cell growth and differentiation which support tissue regeneration purposes (31,32). The current progression of PRP applications have shown a broad yet safe applications in musculo-skeletal cases , including osteoarthritis , wound healing , and also COVID-19 (17,33–36). Those clinical applications have supported the safety of PRP content. Regarding immunogenicity of PRP, animal study has shown that injection of allogeneic PRP intra muscular did not significantly change the CD4+ and CD8+ lymphocytes subpopulation nor the ratio of CD4+/CD8+ (37). Moreover, studies also shown the immunomodulatory properties of PRP which potentially facilitate the less immunogenic of PRP (38). In addition, studies have revealed the benefits of PRP compared to FBS, especially in MSCs culture. PRP prominently showed the benefit in promoting MSCs proliferation and differentiation . These effects were superior or at least comparable to FBS. Thus, PRP provides potential source of xenofree serum for variety sources of MSCs culture to support clinical applications (31,32,39).

Table 3. Comparison between PRP and FBS

	PRP	FBS	Ref
Advantage	Xenofree Low risk of immunological rejection No pathogen contamination Contain high level of growth factor	Contain many benefits for in vitro cell growth and its suitable for all cell types	32, 34
Disadvantage	Variability of growth factor concentration from each individual due which is individuals with chronic diseases, can have limited growth factor content of PRP	Risk of contamination (virus, bacteria, and fungi) Ethical problem for using bovine fetus Increasing its immunogenicity potential	32, 34
Cell proliferation	Adipose derived mesenchymal stem cell (AD-MSCs) cultures supplemented with PRP had 13.9 times better proliferative ability than cell cultures supplemented with FBS, without changing the phenotype, capacity, differentiation and chromosomal status of the AD-MSCs.		37
Cell Differentiation	MSCs cultures supplemented with PRP had better osteogenic differentiation ability than MSCs with FBS.		4, 33

PRP as a growth factor in MSCs culture

For cell culture, basic components are generally required such as growth factors, nutritional sources, antibiotics, and antifungals to prevent contamination (25,40). With the development of stem cell-based therapies and regenerative medicine, MSC has become one of the most commonly used stem cell types. MSCs can be cultivated in vitro using a dish or flask for cell growth. However, FBS is still commonly employed for cell multiplication and processing. This will severely restrict the clinical applicability of MSCs in relation to the animal components of FBS. Therefore, as an alternative to FBS, experiments have been conducted to use platelet derivatives as growth factors in in vitro culture of MSCs (41).

Growth factors secreted by activated PRP can increase stem cell proliferation. Some studies have demonstrated the beneficial positive effect of PRP in many mesenchymal stem cell cultures (30). PRP as a substitute for FBS can eliminate the risks associated with the use of xenogenic supplements in the MSCs cell preparation process.

PRP is known to enhance the short-term and long-term proliferative ability of various MSCs derived from bone marrow, adipose tissue, and muscle tissue, both in the presence and absence of FBS. Study by Fukuda et al, was shown that the proliferative activity of a group of

bone marrow MSCs (BM-MSCs) cultured with 10% PRP was higher on day six than 10% FBS. Then on the eighth day also obtained the same results where the proliferative activity of BM-MSCs was higher in the 10% PRP group compared to the 10% FBS group (25).

Another study also showed, in the umbilical cord MSCs (UC-MSCs) group, those cultured with PRP 10% has higher proliferative activity than MSCs with PRP 5 % and 10% FBS in monolayer culture. Not only the proliferative activity but also the osteogenic differentiation capacity of these UC-MSCs, as indicated by stronger staining of Alkaline Phosphatase (ALP) and Alizarin red in the group of UC-MSCs cultured with 10% PRP compared to those cultured with 10% FBS group (4).

From these studies, it was shown that culturing MSCs with PRP could not only increase the number of doubling of the cell population and reduce the time required to duplicate the cell population but also increase the differentiation capacity of MSCs when compared to cultures with FBS (4,18,42). Based on the above studies, PRP is a promising supplement for in vitro and ex vivo expansion of MSCs (4,42).

Growth factors in PRP can contribute to tissue regeneration by stimulating cell migration, cell proliferation, and differentiation. The growth factors PDGF and FGF-2 act on cells that are in the early G0 or G1 phase of the cell cycle, to initiate DNA replication. While growth factors, EGF and IGF-I allow cells to progress through the pre-replication phase of the cycle, encouraging cells to enter the S, G2, and M phases. PRP is rich in these growth factors, so in other words, PRP can accelerate the cell cycle. thereby increasing cell proliferation. A study states that the increase in cell proliferation by PRP also involves the expression of cyclins. Cyclins are factors that bind to and activate cyclin-dependent kinases (CDKs). Cyclins and kinases are known to control the cell cycle by binding to each other in different combinations. Expression hADSCs is known to increase with the cell cycle transition from G1 to S phase (43).

Study by Lai et al, demonstrated that adding PRP to human adipose stem cells (hASCs) activates the JNK, ERK1/2, and Akt signaling pathways. Platelet-derived adenosine diphosphate in PRP is a critical mediator of cell proliferation. In addition, growth factors in PRP increase DNA synthesis and cell proliferation in hASCs by activating JNK or Akt. By activating ERK1/2, growth hormones such as FGF-2, EGF, or VEGF stimulate the proliferation of hASC. Given that PRP contains a high concentration of PDGF-BB, FGF-2, EGF, and VEGF, interactions between these growth factors can drive cell proliferation via many signaling pathways. In other words, PRP can stimulate the proliferation of human adipose stem cells (hASC) via activating the ERK1/2, Akt, and JNK signaling pathways (43).

Growth factors in PRP also have a role in cartilage regeneration by stimulating chondrocyte proliferation and attachment and MSCs differentiation. PRP can increase the expression of VEGF and PDGF and stimulate stem cell differentiation. Studies have shown that PRP can stimulate integrin-dependent cell-extracellular matrix, which plays a role in cell differentiation, to enhance chondrogenic differentiation through TGF-β3 stimulation. In addition, PRP can also regulate the expression of Col-2, Sox-9, and AGC, which are cartilage-specific gene expressions, thereby stimulating chondrogenic differentiation of stem cells (32). Thus, the growth factors contained in PRP have a role not only in stimulating proliferation but also in the differentiation of stem cells.

Although PRP has a lot of potential as a supplement for MSCs culture, it also has limitations. One of them is that PRP comes from different individual blood sources, so there are individual variations in terms of its growth factor. Each individual can have a growth factor that is different from other individuals such as in its concentration. In addition, other factors can influence, such as the individual's medical history. In individuals with chronic diseases, such as diabetes mellitus, vascular disease, or chronic infections, the growth factor content of PRP can be very limited. Another limiting factor of PRP is the variation in isolation methods. The time required for each method can vary, so some methods require a long time for the PRP isolation process (43). With all of the above limitations, up to now PRP still has potential as an alternative to animal serum such as FBS for cell culture, especially stem cell culture.

CONCLUSION

Along with the development of regenerative medicine and stem cell-based

therapy, it is necessary to prepare everything following good manufacturing products to be safe for patients. One of them is to avoid all animal elements in the processing and preparation of stem cells. Platelet-rich plasma (PRP) is currently being investigated for its potential as a supplementary medium to replace FBS, especially in culturing mesenchymal stem cell from variety sources. PRP has a great potential as it is shown to be rich in growth factors and cytokines, which can be used as a supplement in MSCs culture which shown a positive effect on proliferative activity and increasing differentiation capacity. Moreover, the benefits of less immunogenic of PRP has support its superiority to FBS. Therefore, in the future PRP is a potential xenofree supplement to substitute the FBS for MSCs expansion and clinical applications.

REFERENCES

1. Alatyyat SM, Alasmari HM, Aleid OA, Abdel-maksoud MS, Elsherbiny N. Umbilical cord stem cells : Background, processing and applications. Tissue Cell [Internet]. 2020;65(March):1–10.
2. Guiotto M, Raffoul W, Hart AM, Riehle MO, Summa PG. Human platelet lysate to substitute fetal bovine serum in hMSC expansion for translational applications : a systematic review. J Transl Med. 2020;18:1–14.
3. Sumarwoto T, Suroto H, Mahyudin F, Novembri D, Tinduh D, Basuki H, et al. Role of adipose mesenchymal stem cells and secretome in peripheral nerve regeneration. Annals of Medicine and Surgery. 2021;67(April):1–10.
4. Wen Y, Gu W, Cui J, Yu M, Zhang Y. Platelet-rich plasma enhanced umbilical cord mesenchymal stem cells-based bone tissue regeneration. Arch Oral Biol. 2014;59(11):1146–54.
5. Musiał-wysocka A, Kot M, Majka M. The Pros and Cons of Mesenchymal Stem Cell-Based Therapies. Cell Transplant. 2019;28(7):801–12.
6. Andrzejewska A, Lukomska B, Janowski M. Concise Review : Mesenchymal Stem Cells : From Roots to Boost. Stem Cells [Internet]. 2019;37:855–64.
7. Mckee C, Chaudhry GR. Advances and challenges in stem cell culture. Colloids Surf B Biointerfaces [Internet]. 2017;159:62–77.
8. Silva-carvalho AÉ, Assis F, Neves R, Saldanha-araujo F. The immunosuppressive mechanisms of mesenchymal stem cells are di ff erentially regulated by platelet poor plasma and fetal bovine serum supplemented media. Int Immunopharmacol. 2020;79(January):1–6.
9. Acebes-huerta A, Arias-fernández T, Bernardo Á, Muñoz-turrillas MC, Fernández-fuertes J, Seghatchian J. Platelet-derived bio-products : Classification update , applications, concerns and new perspectives. Transfusion and Apheresis Science. 2020;59(1):1–9.
10. Mcgrath M, Tam E, Sladkova M, Almanaie A, Zimmer M, Peppo GM De. GMP-compatible and xeno-free cultivation of mesenchymal progenitors derived from human-induced pluripotent stem cells. Stem Cell Res Ther. 2019;10(11):1–13.
11. Huang H, Sun X, Zhao Y. Platelet-rich plasma for the treatment of burn wounds : A meta-analysis of randomized controlled trials. Transfusion and Apheresis Science. 2021;60(1):1–7.
12. Chen Y chun, Hsu Y ming, Poay K, Fang H wei, Chang C hung. Intraarticular injection for rabbit knee osteoarthritis : effectiveness among hyaluronic acid , platelet-rich plasma , and mesenchymal stem cells. J Taiwan Inst Chem Eng. 2018;91:138–45.
13. Zhang J, Liu Z, Tang J, Li Y, You Q, Yang J, et al. Fibroblast growth factor 2 – induced human amniotic mesenchymal stem cells combined with autologous platelet rich plasma augmented tendon-to-bone healing. J Orthop Translat. 2020;24(January):155–65.
14. Bos-mikich A, de Oliveira R, Frantz N. Platelet-rich plasma therapy and reproductive medicine. J Assist Reprod Genet. 2018;35:753–6.
15. Kia C, Baldino J, Bell R, Ramji A, Uyeki C, Mazzocca A. Platelet-Rich Plasma: Review of Current Literature on its Use for

Tendon and Ligament Pathology. Curr Rev Musculoskelet Med. 2018;11:566–72.
16. Jain NK, Gulati M. Platelet-rich plasma: a healing virtuoso. Blood Res. 2016;51(1):3–5.
17. Amin I, Gellhorn AC. Platelet-Rich Plasma Use in Musculoskeletal Disorders: Are the Factors Important in Standardization Well Understood? Phys Med Rehabil Clin N Am. 2019;30(2):439–49.
18. Alves R, Grimalt R. A Review of Platelet-Rich Plasma : History , Biology , Mechanism of Action , and Classification. Skin Appendage Disord. 2018;4:18–24.
19. Aizawa H, Kawabata H, Sato A, Masuki H, Watanabe T. biomedicines A Comparative Study of the E ff ects of Anticoagulants on Pure Platelet-Rich Plasma Quality and Potency. :1–14.
20. Dhurat R, Sukesh M. Principles and Methods of Preparation of Platelet-Rich Plasma: A Review and Author's Perspective. J Cutan Aesthet Surg. 2014;7(4):189–97.
21. Yin W, Xu H, Sheng J, Zhu Z, Jin D, Hsu P, et al. Optimization of pure platelet-rich plasma preparation: A comparative study of pure platelet-rich plasma obtained using different centrifugal conditions in a single-donor model. Exp Ther Med. 2017;14:2060–70.
22. Gonzalez R, Silva G, Alkass N, Liana T, Ohana G, Furtado L, et al. The regenerative mechanisms of platelet-rich plasma : A review. Cytokine. 2021;144:1–8.
23. Fukaya M, Ito A. A new economic method for preparing platelet-rich plasma. Plast Reconstr Surg Glob Open. 2014;2(6):1–7.
24. Du L, Miao Y, Li X, Shi P, Hu Z. A Novel and Convenient Method for the Preparation and Activation of PRP without Any Additives : Temperature Controlled PRP. Biomed Res Int. 2018;2018:1–11.
25. Fukuda S, Hagiwara S, Fukuda S, Yakabe R, Suzuki H. Safety assessment of bone marrow derived MSC grown in platelet-rich plasma. Regen Ther. 2015;1(2015):72–9.
26. Laura C. Burzynski, Nicholas Pugh MCHC. Platelet Isolation and Activation Assays. Bio Protoc. 2019;9(20):1–14.
27. Subiran C, Kristensen SG, Andersen CY. Umbilical cord blood – derived platelet-rich plasma : a clinically acceptable substitute for fetal bovine serum ? Fertil Steril. 2021;115(2):336–7.
28. Zhai W, Yong D, El-jawhari JJ, Cuthbert R, Mcgonagle D, Naing MAYWIN, et al. Identification of senescent cells in multipotent mesenchymal stromal cell cultures : Current methods and future directions. Cytotherapy [Internet]. 2019;21:803–19. Available from: https://doi.org/10.1016/j.jcyt.2019.05.001
29. Cimino M, Gonçalves RM, Barrias CC, Martins MCL. Xeno-Free Strategies for Safe Human Mesenchymal Stem / Stromal Cell Expansion : Supplements and Coatings. Stem Cells Int. 2017;2017:1–13.
30. Menezes K, Takamori ER, Teixeira MVT, Carias RBV, Borojevic R. Integrative review for replacement of fetal bovine serum by platelet-rich human plasma for culture and ex vivo expansion of human cells for advanced therapies. Vigilância Sanitária em Debate. 2018;6(1):1–9.
31. Yao T, Asayama Y. Animal cell culture media: History, characteristics, and current issues. Reprod Med Biol. 2017;16:99–117.
32. Qian Y, Han Q, Chen W, Song J, Zhao X. Platelet-Rich Plasma Derived Growth Factors Contribute to Stem Cell Differentiation in Musculoskeletal Regeneration. Front Chem. 2017;5(89):1–8.
33. Shala R. Platelet-rich plasma for tendinopathy and osteoarthritis: a narrative review. Bulletin of Faculty of Physical Therapy. 2021;26(1):1–9.
34. McLarnon M, Heron N. Intra-articular platelet-rich plasma injections versus intra-articular corticosteroid injections for symptomatic management of knee osteoarthritis: systematic review and meta-analysis. BMC Musculoskelet Disord. 2021;22(1):1–13.
35. Karina K, Rosliana I, Rosadi I, Sobariah S, Christoffel LM, Novariani R, et al. Phase I/II Clinical Trial of Autologous Activated Platelet-Rich Plasma (aaPRP) in the Treatment of Severe Coronavirus Disease 2019 (COVID-19) Patients. Int J Inflam. 2021;2021(March 2020):1–10.
36. Akbarzadeh S, McKenzie MB, Rahman MM, Cleland H. Allogeneic Platelet-Rich Plasma: Is It Safe and Effective for Wound

Repair? European Surgical Research. 2021;62(1):1–9.
37. Zhang ZY, Huang AW, Fan JJ, Wei K, Jin D, Chen B, et al. The potential use of allogeneic platelet-rich plasma for large bone defect treatment: Immunogenicity and defect healing efficacy. Cell Transplant. 2013;22(1):175–87.
38. Everts P, Onishi K, Jayaram P, Mautner K. Platelet-Rich Plasma : New Performance Understandings and Therapeutic Considerations in 2020. Int J Mol Sci. 2020;21:1–36.
39. Sukmawati D, Junaidi H, Syaidah R. Human platelet-rich plasma as a biological stimulant for proliferation and differentiation of mesenchymal stem cells. Biomedicine (India). 2021;41(2):168–73.
40. Markmee R, Aungsuchawan S, Tancharoen W. Differentiation of cardiomyocyte-like cells from human amniotic fluid mesenchymal stem cells by combined induction with human platelet lysate and 5-azacytidine. Heliyon. 2020;6:1–10.
41. Atashi F, Jaconi MEE, Pittet-Cuénod B, Modarressi A. Autologous platelet-rich plasma: A biological supplement to enhance adipose-derived mesenchymal stem cell expansion. Tissue Engineering - Part C. 2015;21(3):253–62.
42. Patel P, Ghosh K, Mishra KK. Efficacy of Mesenchymal Stem Cells (MSCs) enhanced with Platelet Rich Plasma PRP in Osteoarthritis (OA) Treatment. 2017;3(1):1–7.
43. Lai F, Kakudo N, Morimoto N, Taketani S, Hara T, Ogawa T. Platelet-rich plasma enhances the proliferation of human adipose stem cells through multiple signaling pathways. Stem Cell Res Ther. 2018;9(107):1–10.