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Original Article

Platelet-Rich Plasma as Xenofree-Growth Factor for Mesenchymal Stem Cells Culture

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Stem cell-based therapy is currently believed to be the future of medicine. Stem cells have considerable therapeutic, and biotechnological benefits in treating various progressive, and end-state diseases. The type of stem cell that is widely used in cell-based therapy is mesenchymal stem cells (MSCs). MSCs have some advantages, including being found in various body tissues, being relatively easy to isolate, having rapid proliferation ability, high differentiation capacity, and the ability to migrate to the injured areas. In stem cell-based therapy, the MSCs that will be used mostly need to be expanded first by culturing the cells. Both during the culture, and processing cells, often use supplements in the form of serum-containing various growth factors, and cytokines. The serum that is often used is a fetal bovine serum (FBS). However, regarding clinical applications, and Good Manufacturing Practices, the presence of animal elements should be avoided. FBS is not following the principles of Good Manufacturing Practice, because it can affect the safety, and efficacy of cell-based therapies. Therefore, alternative supplements are needed for cultures that are free from animal serum elements. 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.

Introduction

At present, stem cells are believed to be the future of medicine, because of their considerable therapeutic, and biotechnology benefits in treating progressive diseases such as cardiovascular disease, diabetes, and neurodegenerative diseases [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 unspecialized cells that can be isolated from various tissues in the body, including adipose tissue, dermal tissue, muscle-skeletal fluid, amniotic fluid, synovial fluid, tissue, and blood in the umbilical cord [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].

In stem cell-based therapy, cells that have been isolated are generally expanded first through culture. The process of stem cell expansion aims, among other things, to improve the quality, and quantity of cells, so that stem cells can be used for cell-based therapy [7]. In addition, cell culture has an important role in the study of human cells, in vitro, to determine disease mechanism simulation, vaccine discovery, or studying the toxicity of new drug compounds. Historically, cell culture methods have relied heavily on animal serum as the main supplement for a cell's growth medium [8]. The most widely used animal serum supplement in cell culture is a fetal bovine serum (FBS), which is known to stimulate the proliferation, differentiation, and survival of various cell types [8-9]. The advantages of FBS over other animal serums include, among others, that FBS is rich in adhesion molecules, growth factors, micronutrients, and hormones that can support attachment, growth, and proliferation of mammalian cells. FBS can also promote cell expansion, and support the differentiation potential of mesenchymal stem cells. The use of FBS is also relatively

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inexpensive [2].

Along with the development of regenerative medicine, and stem cell-based therapy, the use of animal elements in the cell preparation process has become a concern. The use of FBS as a supplement in cell culture carries the risk of transmission of viruses, mycoplasmas, prions, and other unknown zoonotic agents. In addition, the use of FBS in cell culture showed an accumulation of bovine protein in the cells, which potentially acts as an antigenic substrate when transplanted into the host. This condition could trigger an immunological reaction thereby increasing its immunogenicity potential [2-8]. For this reason FBS is ruled out from the principles of Good Manufacturing Practice, as it may affect the safety, and efficacy of cell-based therapies [8-10]. To avoid this problem, alternative supplements are currently being sought to replace FBS [2-8]. Human autologous, and allogeneic products, including platelet lysate, platelet-rich plasma (PRP), and poor platelet plasma (PPP) is an alternative being investigated as a supplement to FBS [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.

Mesenchymal Stem Cells

Stem cells are unspecialized cells of the human body. They can differentiate into various cells. Stem cells are present in both embryonic, and adult cells [14]. Embryonic stem cells (ESCs) originate from the inner cell mass of the blastocyst, which is the initial phase of pre-implantation of the embryo. ESCs are pluripotent cells, which are potentially immortal, and can maintain a normal karyotype. Meanwhile, stem cells from adult cells, are found throughout the body, mostly in the bone marrow. Adult stem cells can be unipotent, producing only one specific cell type, or multipotent capable of differentiation into a limited number of cell types such as chondrocytes, osteoblasts, and adipose cells. Adult stem cells are hoped for future cell therapy because they have the potential for differentiation, and the ability to divide in cell culture [1-14].

Human mesenchymal stem cells (hMSC) are one of the adult stem cells. MSCs are progenitors of non-hematopoietic cells, with the ability to differentiate along mesenchymal, and non-mesenchymal lines. MSCs can differentiate into osteocytes, adipocytes, myocytes, as well as lineages of chondrogenic cells [2,4,15]. MSCs can be isolated from various tissues, including adipose tissue, dermal tissue, skeletal muscle, amniotic fluid, synovial fluid, tissue, and blood in the umbilical cord. [3-4]. Bone marrow, cord blood, dental pulp, and adipose tissue are potential sources for autologous hMSCs in stem cell-based therapy [2, 4]. Bone marrow-derived mesenchymal cells (BM-hMSCs), and adipose cells (ADSCs) are more commonly used in stem cell-based therapy [2, 15].

MSCs can be isolated through various procedures, and can be propagated (expansion) using different media, seeding densities, and oxygen levels [16-17]. To minimize the diversity, *The* International Society for Cellular Therapy (ISCT) in 2006 established minimal criteria for defining human MSCs. First, the MSCs must adhere to the plastic when maintained under standard culture conditions. Second, MSCs should express CD105, CD73, and CD90 in a proportion of 95%, and not/only slightly with a proportion of 2% expressing the surface molecules CD45, CD34, CD14, or CD11b, CD79 alpha or CD19, and HLA-DR. Third, MSCs must be able to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro (figure 1) [6, 16, 18]. This criterion is useful for describing the purity of MSCs in culture growth. In addition, MSCs potential examination is also needed to predict clinically important factors such as differentiation ability, immunomodulating ability, and growth factor production [16-17].

When compared with other adult stem cell types, MSCs have many advantages. MSCs are known to have proliferative abilities rapid growth, high differentiation capacity, and the ability to migrate to damaged areas [5-6]. MSCs can play an important role in wound healing because they have the ability of cellular differentiation, immune modulation, and production of growth factors that can stimulate neovascularization, and re-epithelialization [15].

MSCs have a high in vitro proliferative capacity, and a low risk of tumor formation in vivo in transplanted cells, MSCs have been used in cell-based therapy for various diseases including graft-versus-host, multiple sclerosis, myocardial infarction, liver failure, and rejection after transplantation heart [16]. In addition, cultured MSCs have been used extensively in tissue engineering applications to repair large articular cartilage, and bone defects. MSCs play a role in stimulating osteogenesis because they have the potential for osteogenic differentiation [16,18].

The application of MSCs as cell-based therapy needs to pay attention to the principles of Good Manufacturing Practice, which is a system to ensure the quality of products used in the pharmaceutical, and biological industries [19]. MSCs that will be used for clinical applications need to be expanded first through cell culture. In MSCs cell culture, medium containing animal serum such as FBS is still commonly used. The components of the medium containing animal serum, have a risk of transmitting pathogens, and triggering immunological reactions. Since it can affect the safety, and efficacy of cell-based therapies, therefore any FBS content is not allowed according to the principles of Good Manufacturing Practice [2, 8, 10]. An alternative medium that is free from animal serum is needed to avoid this problem.

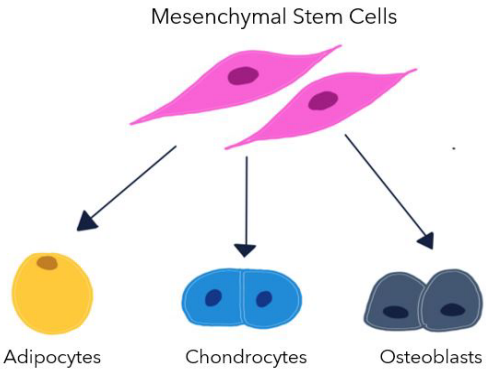

Figure 1: Trilineage differentiation capacity of mesenchymal stem cells

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.[20] 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, 20]. This growth factor is known to play crucial role in a series of molecular events, such as collagen synthesis, angiogenesis, mitogenesis, and cell growth, and differentiation that lead to tissue regeneration processes [12].

Mechanically, in cartilage, and tendons, PRP enhances the injured tissues healing not only by inhibiting cell apoptosis, and reducing the inflammatory response but also by promoting cell proliferation, and collagen production [13, 20, 21]. Dense granules in platelets also contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), calcium ions, histamine, serotonin, and dopamine, which are important factors for tissue homeostasis [20]. There are several types of platelet concentrates based on the manufacturing process or production, namely [11, 20, 22] :

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).

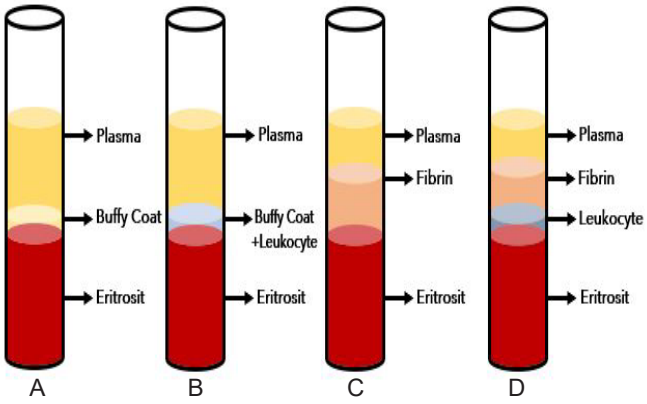

Figure 2: Type of Platelet Concentrates, (A) Pure platelet-rich plasma (P-PRP), (B) Leukocyte, and platelet-rich plasma (L-PRP), (C) Pure platelet-rich fibrin (P-PRF), (D) Leukocyte, and platelet-rich fibrin (L-PRF)

Isolation Methods of Platelet-Rich Plasma

Generally, the preparation of PRP isolation involves taking autologous whole blood from the patient followed by a 1-2 step centrifugation process to separate plasma from 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 [23]. The blood used is obtained from a vein puncture in an anticoagulant tube which contained citric acid dextrose, or sodium citrate solution [21, 24-25]. 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 [26]. 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 [24, 27]. 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) [20,28-29]. Several PRP isolation methods have been developed to obtain optimal PRP product results (table 1).

The changes in pH, temperature, and time after being taken from the vein puncture (within hours) can also affect PRP isolation result. The appropriate temperature, and pH will maintain the stability of the growth factor between plasma, and PRP. Soon after activation PRP will become a natural fiber scaffold that can trap more platelets, and reduce the rate of growth factors degradation growth factors [30]. 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 [24].

Growth Factor within Platelet-Rich Plasma

In order to gain the growth factors effect, the platelets within PRP must be activated before the application of PRP. Thrombin, and calcium chloride are known to induce platelet aggregation, which aims to activate platelets, and stimulate degranulation, causing the release of growth factors [24, 31]. In the use of PRP for cell culture, heparin is used to prevent blood clotting before being added to the medium [32]. If PRP is to be applied to a wound, activating factors such as thrombin can be applied to the final platelet concentrate to stimulate platelet degranulation, and exocytosis of factors that are stored in cytoplasmic granules.

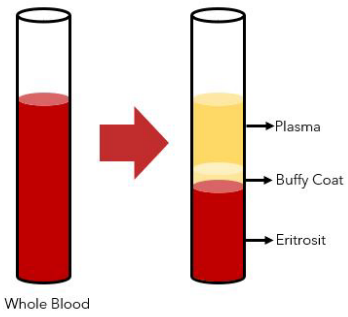

Figure 3: Platelet-Rich Plasma separation from the whole blood

Tabel 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	-	After second centrifuge, 1 ml plasma at the bottom, and precipitated platelets were used. It was transferred to a new glass tube, and then incubated for 15 minutes at 37° C	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

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 [16, 33].

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 (table 2) [32].

PDGF is a glycoprotein, which arises from platelet degranulation at the site of injury. PDGF activates cell membrane receptors on target cells which then activate signaling proteins to initiate target cell-specific activities. These specific activities include mitogenesis, angiogenesis, and macrophage activation. Meanwhile, TGF-â is secreted by platelets, and macrophages, which act as antiproliferative factors in normal epithelial cells. PRP also known to promote the migration, proliferation, and activation of fibroblasts. VEGF is a signaling protein produced by cells that stimulate vasculogenesis, and angiogenesis. Also known as vascular permeability factor. The content of VEGF in PRP is known to play a role in the revascularization of damaged tissue by stimulating migration, proliferation, differentiation, and stabilization of endothelial cells in new blood vessels. Neovascularization or the formation of new blood vessels is an important phase in the healing process, and or regeneration of injured tissue. While epidermal growth factor (EGF) is a growth factor that stimulates the growth, proliferation, and differentiation of epithelial cells by binding to their receptors [22, 28].

Tabel 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

Fetal bovine serum (FBS), is the most common serum for use in vitro in cell culture. FBS acts as a supporter of the process of cell adhesion, growth, and proliferation with a broad spectrum [2, 9]. FBS is generally extracted from fetal bovine blood, collected after slaughtering pregnant cows under sterile conditions. After freezing, centrifugation was carried out to separate the cellular components. Then it is filtered to remove potential bacterial, and viral contamination.

Until now, FBS is still the supplementary medium of choice in various cell culture protocols [2, 34]. 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 [35].

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 [36-37]. 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 [36-37]. 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 [23, 38–41]. 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+.[42] Moreover, studies also shown the immunomodulatory properties of PRP which potentially facilitate the less immunogenic of PRP [43]. In addition, studies have revealed the benefits of PRP compared to FBS, especially in MSCs culture (table 3). 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 [36,37,44].

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 [32-45]. Along with the advancement of stem cell-based therapies, and regenerative medicine, MSC is one of the most widely used types of stem cells. For cell expansion, MSCs can be cultured in vitro using a dish, or flask. However, generally, FBS is still widely used for cell expansion, and, or during the processing phase. This will greatly limit the clinical application of MSCs regarding the animal constituents of FBS. Therefore, some studies have been carried out to utilize platelet derivatives as growth factors in vitro culture of MSCs, as an alternative to FBS [46].

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 [35]. 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 [32].

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-

Tabel 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

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, 24]. Based on the above studies, PRP is a promising supplement for in vitro, and ex vivo expansion of MSCs [4,18].

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 [47].

Study by Lai et al, showed that the addition of PRP to human adipose stem cells (hASCs) can activate the JNK, ERK1/2, and Akt pathways. PRP can also significantly stimulate proliferative effects on chondrocytes via the ERK (Extracellular regulated-signal kinase) signaling pathway, and platelet-derived adenosine diphosphate in PRP is a key mediator of cell proliferation. In addition, growth factors in PRP stimulate DNA synthesis, and cell proliferation in hASCs which mediated by JNK activation, or through Akt activation. Growth factors such as FGF-2, EGF, or VEGF induces hASC proliferation through ERK1/2 activation. Since PRP contains large amounts of PDGF-BB, FGF-2, EGF, and VEGF, interactions among these growth factors can stimulate cell proliferation through various signaling pathways. In other words, PRP can induce human adipose stem cell (hASC) proliferation by activating the ERK1/2, Akt, and JNK signaling pathways [47].

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-â3 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 [37]. 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 [47]. 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.

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