Systematic review article

Human platelet-rich plasma as a biological stimulant for proliferation and differentiation of mesenchymal stem cellsDewi Sukmawati^{1,2}, Helsy Junaidi³, Rahimi Syaidah^{1,2}¹Department of Histology, ³Postgraduate Student, ²Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jln. Salemba Raya No.4 Jakarta 10430, Indonesia

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Corresponding author: **Dewi Sukmawati**. Email: ds_histoui@outlook.com; dewi.sukmawati@ui.ac.id**ABSTRACT**

Platelet is one of the cells within the blood that have potential in regenerative therapy. Recently platelet-related products got special attention due to the abundance of growth factors and easy availability and processing. With the robust in cell-based therapy, platelet-rich plasma (PRP) has been intensively studied for its potential to substitute the fetal bovine serum (FBS) as the supplement in cell culture. Our study aimed to investigate the use of human PRP in mesenchymal stem cells (MSC) culture and its related effect on stem cell biology. We searched in vitro studies that used human PRP as a supplementing factor on human MSCs culture. From the initial 172 studies, 14 studies fulfilled the selection criteria and were analysed. The results showed that the sources of MSCs were varied, including adipose tissue, bone marrow, and dental tissue. The PRP concentration showing the best effects was ranged from 10 to 20%. In addition, most of the studies demonstrated the superiority of PRP to FBS in promoting the proliferation and differentiation of MSCs in vitro. Therefore, PRP could be an alternative to FBS in supporting a xeno-free culture system. Studies are needed to reveal the mechanism of PRP in maintaining the physiology of MSCs.

Keywords: Platelet-rich plasma; mesenchymal stem cells; cell culture; proliferation; differentiation; xeno-free.

INTRODUCTION

To date, successes in regenerative medicine have included regenerating non-functional tissue or organ damage caused by trauma, congenital (acquired) abnormalities, and some end-stage diseases (1,2). Mesenchymal stem cells (MSCs) are among the promising stem cells for cell-based therapy. MSCs are known to have the pluripotent ability to differentiate into several cell types, including adipocytes, osteoblasts, and chondrocytes (3). To define the population of MSCs, there are minimal criteria according to The International Society for Cellular Therapy (ISCT). First, the cells have the ability to adhere on the plastic culture dish when cultured in vitro. Second, the cells positively express ($\geq 95\%$) the surface antigen of CD73, CD90 and CD105; but negatively ($\leq 2\%$) for the surface antigen of CD45, CD34, CD14 or CD11b, CD79 α or CD19, and HLA-DR. Third, the cells are able to differentiate into mesodermal cell types (i.e., chondrocytes, osteoblasts, and adipocytes) when cultured under specific conditions (4).

The origin of MSCs was revealed to be derived from many tissues and organs, including bone marrow, brain, lymphoid organs, kidney, muscle, etc (5). Due to its plasticity and multipotency, MSCs have been applied in various diseases and showed the advantages to promote tissue regeneration in neurological disorders, cardiac ischemia, diabetes, and musculoskeletal tissue (6-9).

Until recently, most of the procedures in stem cell expansion and propagation were still using animal sera, such as fetal bovine serum (FBS). FBS is widely

used for a broad spectrum of cell types, including cell lines (10). Therefore, with FBS content, it may hamper additional applications of the cells in clinical settings. Good manufacturing practices have required that the expansion of stem cells for therapeutic purposes in patients should avoid any xeno materials and must be safe from zoonotic disease transmission (11,12).

In the last decade, platelet-rich plasma (PRP) has attracted the attention of researchers as it has the potential to replace the use of animal sera in stem cell expansion. PRP is a concentrate of platelet that is rich in plasma protein, and obtained from the centrifuged-whole blood (13-16). PRP contains fibrinogen and abundant growth factors released by the platelets. These growth factors play important roles in promoting tissue regeneration and repair. PRP must be activated to release various growth factors and regulatory proteins, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-beta (TGF- β), insulin-like growth factors (IGFs), and vascular endothelial growth factor (VEGF) (14,17,18). These growth factors are known to play crucial roles in stem cell physiology, such as proliferation, migration, and differentiation. This study aims to review and identify the use of PRP as a potential biological growth factor for MSC culture that provides an alternative to animal sera, as well as its potential mechanism in maintaining MSC physiology *in vitro*.

MATERIALS AND METHODS

The literature search strategy was carried out through PubMed/MEDLINE by following the PRISMA-P protocol for systematic reviews and meta-analyses.

The following keywords were used for the search: “platelet-rich plasma” AND “mesenchymal stem cell culture”, “platelet-rich plasma” AND “cell-based therapy and platelet-rich plasma” AND “xeno-free”. In addition, relevant existing articles were added from our library. The selection process can be seen in Figure 1. The collected articles were then sorted based on the inclusion and exclusion criteria.

The inclusion criteria consisted of the following: literature needed to be available in English, published within the last 10 years (2010-2020), and freely accessible through PubMed; furthermore, the research had to have been conducted using in vitro experimental methods and with a focus on the use of platelet-rich plasma as a growth factor supplement in human mesenchymal stem cell culture. Literature published before 2010, non-English articles, articles not available in full-text form, review articles, research that did not document an in vitro study, experiments that did not use human mesenchymal stem cells, and those that did not use PRP or other forms of platelet lysate as a growth factor during cell culture were excluded.

Risk of bias and quality assessment

The methodological quality of the literature search, including determining which studies to include, was independently conducted and assessed by two reviewers.

RESULTS

Based on the selection methods, 172 articles were initially found (Fig. 1). Although most of these articles reflect the use of PRP in stem cell experiments, it is evident that the use of PRP as an alternative to animal sera is still not widely implemented. The articles showed PRP application in related to MSCs in various methods; these included in vivo study designs using animals, which were used conjointly to scaffold or by directly applying (injecting) the PRP alone or with the stem cell for tissue repair or regeneration without involving prior cell culture. Only several studies have used PRP for human MSC culture, as demonstrated by 14 eligible articles that met the inclusion criteria and were then analyzed. Their characteristics are summarized in Table 1.

Fig. 1: The selection process of the articles included in this study

The mesenchymal stem cells used in these studies were derived from various tissues ranging from adipose tissue, bone marrow, umbilical cords, endometrial stroma, deciduous teeth, synovium tissue, and dental pulp. The first two were the most widely used for PRP applications. This was in accordance with other studies that have shown that adipose tissue and bone marrow are rich sources of human MSCs (5,19,20).

The final result articles show that the application of PRP in human MSC culture will enhance the proliferation and differentiation capacity of the cells. Cell proliferation is a crucial component of tissue regeneration. During the healing process, cells have to enter and progress through the cell cycle, which results in an increase in cell count due to mitosis or cell dividing. These newly formed cells replace the old or damaged cells, thus regenerating the injured tissue crucial component of tissue regeneration (21).

Cell migration and differentiation are also substantial during tissue regeneration. According to the findings

in the result articles, PRP application in MSC culture stimulates migration and cell differentiation. This result shows the importance of PRP in stimulating undifferentiated stem cells to migrate according to the concentration of the PRP growth factor and in triggering differentiation of these cells once they are at the site of the injured tissue. This process is essential during tissue regeneration, such as the healing process of the wound or bone tissues (22,23). The concentration of PRP used in the included articles varied, ranging from 2% to 20%. The studies also reported the dose-related effect of the PRP when applied to MSC culture.

These findings provide evidence that when applied to MSC culture, PRP can stimulate major cell functions such as proliferation, migration, and differentiation, which are important for tissue regeneration. This could partly explain the underlying mechanism of the regenerative capacity of PRP in the wound and musculoskeletal tissues (24,25).

Table 1: Summary of the use of PRP as a biological stimulant in mesenchymal stem cell culture

Type of stem cells	Characteristic findings	Biological effects	Reference
Human adipose-derived stem cells (hADSCs)	PRP promotes cell proliferation and chondrogenesis through increases in glycosaminoglycan (GAG) and TGF- β 1 secretion and mRNA expression of collagen type-1	Cell proliferation and differentiation	(26)
Adipose-derived MSCs (h-AdMSCs)	The ratio of platelet/leukocyte during PRP preparation, drove cell behavior including AdMSC proliferation activity, through the balance between the anabolic/catabolic.	Cell proliferation	(27)
<ul style="list-style-type: none"> Cell of stromal fibroblasts MSCs Ishikawa endometrial adenocarcinoma cells (IC) 	PRP promotes endometrial regeneration through cell migration, proliferation, and expression of pro-inflammatory markers of cytokines, chemokines, and MMPs.	Cell migration and proliferation	(28)
MSCs from adipose tissue	PRP enhances chondrogenesis through increased gene expression of chondrogenic markers (Sox9 and collagen II) and also enhances chondrogenic differentiation	Cell differentiation	(9)
MSCs from human exfoliated deciduous teeth (SHED)	PRP showed its superiority to FBS in promoting proliferation and differentiation of MSCs-SHED cells, in a lower concentration.	Cell proliferation and differentiation	(29)
MSCs from human exfoliated deciduous teeth (SHED)	PRP can substitute the fetal calf serum (FCS) for SHED cultivation, maintaining the proliferation and differentiation capacity	Cell proliferation and differentiation	(30)
Human adipose-derived stem cell (hADSC)	The culture of hADSC in the presence of platelet rich growth factors shows an increase in proliferation rate and promotes osteogenic differentiation	Cell proliferation and differentiation	(31)
Adipose-derived MSCs (AT-MSC)	PRP in 20% concentration is effective in promoting higher cell proliferation compared with FBS in AT-MSC, without changing its differentiation capacity	Cell proliferation	(32)
Umbilical cord-derived MSCs	The 10% concentration of PRP in primary cultures exhibits the highest success in promoting a faster cell adherent and proliferation rate compared with FBS, while maintaining their immunophenotypes, multilineage differentiation potential, and does not form tumors when injected at a high dose into athymic nude mice.	Cell adherent, proliferation, and differentiation	(12)

Synovium-derived mesenchymal stem cells	In the presence of PRP, the cell proliferation was significantly higher in the cultured cell, in all three-differentiation lineages. However, PRP alone (without medium differentiation), did not show the evidence to promote SDSC differentiation	Cell proliferation	(33)
Mesenchymal dental pulp stem cell	PRP in 10% concentration shows highly increased proliferation activity by shortening the doubling time	Cell proliferation	(16)
Human mesenchymal stromal cells from bone marrow, adipose tissue, and Wharton's Jelly	10% PRP increases cell proliferation as compared with FBS, however MSCs isolated from different tissues have distinct properties regarding differentiation, angiogenic, and inflammatory potential when cultured in PRP supplemented media	Cell proliferation	(34)
Human adipose-derived stem cells (hADSCs)	PRP significantly increase cell proliferation, with less osteogenic differentiation compared with FBS	Cell proliferation	(35)
Bone marrow-derived MSCs	PRP stimulates cell proliferation in a dose-dependent manner. MSC proliferation was positively correlated with TGF- β 1 concentration, and ALP activity was negatively correlated with PDGF-BB concentration in PRP	Cell proliferation	(36)

Table 2: The major growth factors in PRP

Growth Factor Proteins of PRP	Major biological activities
Platelet-derived growth factor (PDGF)	Promotes cell proliferation
Vascular endothelial growth factor (VEGF)	Promotes neovascularization, cell survival
TGF- β (transforming growth factor)	Promotes cell proliferation, differentiation
Hepatocyte growth factor (HGF)	Stimulates mitogenesis, cell motility, and matrix invasion
Epidermal growth factor (EGF)	Stimulates cell growth/proliferation and differentiation
Insulin-like growth factor (IGF)-1	Stimulates cell proliferation and migration
Fibroblast growth factor (bFGF)	Stimulates angiogenesis, cell differentiation

DISCUSSION

Platelets are nucleated fragments of megakaryocytes that originate from bone marrow and migrate into the bloodstream, circulating for 7-10 days. As cellular elements, platelets contain a wide protein content in the form of secretory granules (25). Platelet-rich plasma (PRP) is a human platelet concentrated in small volumes of plasma; it is known to be rich in major proteins of growth factors that are actively secreted for wound healing initiation. Moreover, PRP contains major blood proteins such as fibrin, fibronectin, and vitronectin. These proteins are essential for cell adhesion molecules and as a matrix for bone, connective tissue, and epithelial cell migration (37).

As summarized in Table 1, studies reveal that PRP application in MSC culture could promote cell proliferation, migration, and differentiation—the main physiological functions of stem cells, which are required for tissue regeneration. The effect of PRP on MSC culture in part is due to its rich content of secretory granules of various growth factors, chemokines, cytokines, and other plasma proteins (37–41). The growth factor proteins of PRP are summarized in Table 2.

Our results suggest that in the near future it would be possible to replace animal sera for MSC culture with PRP. PRP is not only suitable for maintaining MSC

culture but also shows its superiority to FBS in stimulating cell proliferation, migration, and differentiation. The PRP concentration showing superior effects to FBS ranges from 10 to 20% (12,16,32,34). After activated, PRP releases its secretory granules enriched with growth factors, including PDGF, VEGF, TGF- β , HGF, EGF, IGF, and FGF. These growth factors are suggested as the active biological agents that promote cell proliferation, migration, and differentiation. When PRP is used as a supplement in stem cell culture, the growth factors will interact with the cells to influence their biological activities. After being secreted, the growth factors will bind to the outer surface of the cell membrane of the affected cells, such as mesenchymal stem cells, fibroblasts, osteoblasts, endothelial cells and epidermal cells which express the receptor of

PRP-derived growth factors (37). The important message is that the active secretion of these growth factors is initiated by the clotting process of the blood, therefore the PRP should be in anti-coagulated state before use. The underlying mechanism of the mitogenic effect of PRP in MSCs proliferation is suggested through multiple signalling. A previous study using BrdU incorporation assay demonstrated that PRP induced the increase of BrdU incorporation which showed the stimulation of DNA synthesis. In addition, PRP also promotes cell cycle transition from G0/G1 to S phase, therefore promoting cell to proliferate. Moreover, PRP also works through

activating the phosphorylation of ERK1/2, Akt and JNK which is known as crucial pathway for cell proliferation (42). All of these effects indicated the potential of PRP in promoting cell proliferation and growth.

The differentiation process promoted by PRP translated the MSCs into various cell types, such as chondrogenic, osteogenic, adipogenic, and neurogenic differentiation. Research conducted by Elgamal et al. (2019) used 10% PRP in culture medium for MSCs from adipose tissue, showing an increase in neurogenic proliferation and differentiation (43). Another study showed the effect of PRP in promoting the AD-MSCs differentiation into chondrogenesis (9). Based on our article results, most studies in MSCs differentiation using PRP supplement, showed that PRP can maintain the differentiation capacity of the MSCs, especially the multi-lineage differentiation potential into three lineages of differentiation: osteogenic, chondrogenic and adipogenic. It is suggested that the growth factors contained within PRP also contribute to cell differentiation. Studies have revealed that TGF- β and PDGF are the potent growth factors for cell differentiation. These growth factors are suggested to work by binding to the extracellular domain of the targeted receptor which leads to the activation of the intracellular signal transduction pathway and stimulates cell differentiation (44, 45).

Our review showed that PRP is prominently beneficial for human MSC culture in terms of enhancing the capacity of cell proliferation, migration, and differentiation. Thus, it is possible to use PRP to replace the animal sera in human MSC culture to support a xeno-free culture system and enhance the potential use of human MSC in clinical settings.

CONCLUSION

Few studies use PRP as a growth factor supplement/stimulant for human mesenchymal stem cell culture. This suggests that PRP is still less popular than FBS. Our results have revealed and showed the potential of human PRP as a biological supplement in human MSC culture, acting as an alternative to FBS or other animal sera. Our results indicated the potential benefit of PRP for providing a xeno-free culture method for human MSCs which supports the clinical applications of MSCs in regenerative medicine. Further studies are required to understand the detailed mechanism pathway explaining how PRP stimulates cell proliferation and differentiation.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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