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REVIEW: ANIMAL SERUM REPLACEMENT IN MESENCHYMAL STEM CELLS CULTURE

Kajian: Alternatif Pengganti Serum Hewan pada Kultur Sel Punca Mesenkim

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ABSTRACT
Mesenchymal stem cells (MSCs) are being used in clinical applications and must comply with Good Manufacturing Practices (GMP) standards and The National Agency of Drug and Food Control (NA-DFC)) regulations. MSCs cultured using a culture medium and added with several supplements like animal serums. However, animal serums can be a source of virus transmission. Therefore, it is necessary to substitute supplements for the animal serum that are safe to use in cell therapy using MSCs. The paper discusses substituting animal serum MSCs culture. This paper is a literature review through a literature search in scientific journals and research reports that explains the various studies on free serum in the culture of MSCs. It can be concluded that human platelet lysate (hPL), human platelet-rich plasma (hPRP), human serum (hS), human umbilical cord serum/plasma (hUCS/P), or human plasma-derived supplement for cell culture medium (SCC) can be used as substitutes for animal serum in MSCs culture.

Keywords: *animal, bovine, cell culture techniques, mesenchymal stem cells, serum albumin.*

ABSTRAK
Terapi menggunakan sel punca mesenkim (SPM) mulai digunakan dalam aplikasi medis dan harus memenuhi standar Good Manufacturing Practice (GMP) dan aturan serta regulasi dari Badan Pengawas Obat dan Makanan (BPOM). SPM sebaiknya dikultur menggunakan medium kultur ditambahkan suplemen tertentu, misalnya serum hewan. Namun, bahan ini masih mengandung materi hewan yang dapat menjadi sumber transmisi virus. Oleh karena itu diperlukan adanya suplemen pengganti serum hewan yang aman digunakan dalam kultur SPM untuk keperluan terapi sel. Tulisan ini menguraikan tentang bahan pengganti serum dalam kultur SPM. Tulisan ini berupa review literatur yang didapatkan melalui penelusuran pustaka yang didapatkan dari internet. Human platelet lysate (hPL), human platelet-rich plasma (hPRP), human serum (hS), human umbilical cord serum/plasma (hUCS/hUCP), atau human plasma-derived supplement for cell culture medium (SCC) dapat digunakan sebagai pengganti serum pada kultur SPM.

Kata Kunci: albumin serum, *bovine,* hewan, sel punca mesenkim, teknik kultur sel.

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INTRODUCTION

Currently, stem cell therapy against degenerative diseases is starting to be considered as an alternative in terms of availability, multiplication potential, and differentiation ability. The use of stem cells as a cell-based therapeutic strategy has shown promising results on a variety of health-related problems. In stem cell transplantation to patients, stem cells must comply with Good Manufacturing Practice (GMP) standards and regulations of the Food and Drug Administration (FDA) to produce quality products (Van Pham and Vu 2016).

Stem cells are cells that can multiply (self-renew). They do not have a specific form and function (undifferentiated) but can differentiate into other cells (Amin et al. 2019). One type of stem cell is mesenchymal stem cells (MSCs). MSCs can be isolated from bone marrow (BM), fat tissue, peripheral blood, cord blood, Wharton Jelly, etc. MSCs are multipotent and can proliferate and differentiate into several body cells such as osteoblasts, chondrocytes, adipocytes, and neurons (Halim 2010). The multipotential of MSCs which can differentiate into various cell types is an opportunity to utilize these cells as both diagnostic and therapeutic cells (Teixeira et al. 2013).

MSCs used for cell therapy are obtained from cell expansion. In MSCs expansion, materials in the culture medium, supplements, and other necessary supporting materials are needed, such as enzymes, saline solution, anti-fungal, anti-coagulant, and separation medium. The culture medium is used as a nutrient for growth and cell differentiation, supplement medium is used to optimize culture conditions while other supporting materials play an important role in the subculture of MSCs. In the MSCs expansion, a supplement that is often used to optimize MSCs culture is Fetal Bovine Serum (FBS) (Salzig et al. 2013, Tavakolinejad et al. 2014, Díez et al. 2015, Oikonomopoulos et al. 2015, Fong et al. 2017, Sagaradze et al. 2019). FBS supports the proliferation of MSCs, plays a role in the expansion of MSCs, and helps cell adhesion as well as a growth factor and essential nutrients for cell growth (Cardoso et al. 2012, Tavakolinejad et al. 2014).

In GMP, the use of FBS does not meet standards and most people do not approve of their use in stem cell transplantation because these materials come from animal materials (Krishnan and Woodard 2014). FBS was isolated from the pre-partus fetal blood of cows (Cheever et al. 2017). The use of animal material in cell cultures to be used for transplantation may trigger an immunological response due to the contamination of foreign compounds in the form of viruses, bacteria, fungi, and endotoxins. FBS is reported to contain xenogenic protein and a variety of other foreign components, which may transmit viral and prion diseases (Cardoso et al. 2012). There are two significant limitations to using FBS. First, the risk of exposure to residual bovine proteins that could be immunogenic and the potential for contamination with infectious agents (like the prion that causes mad cow disease or bovine spongiform encephalopathy) and second, FBS have variability in concentration from lot to lot (Mojica-Henshaw et al. 2013). So, we need a material that is safe to use in MSCs culture for cell therapy purposes. This review briefly discussed recent research that provides information regarding ingredients that can replace serum as supplements in MSCs cultures that are free of animal materials.

STEM CELL DEVELOPMENT

The characteristic of stem cells has the potential to be used as a source of transplantation in cell-based therapy for the treatment of degenerative diseases, for example, heart disease, brain ischemic, systemic lupus erythematosus (SLE), diabetes mellitus, graft versus host disease (GVHD), patients with COVID-19-related lung diseases, and other diseases (Halim 2010, Batsali et al. 2013, Laskowitz et al. 2018, Li et al. 2020).

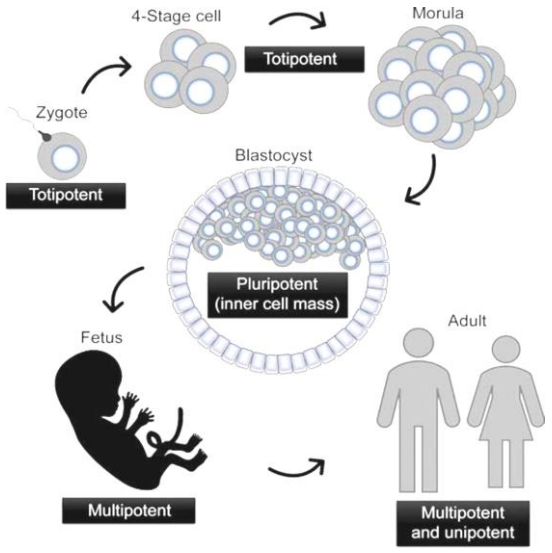

Figure 1. Characteristics of stem cell capabilities (Łos et al. 2019)

There are two kinds of stem cells, Embryonic Stem Cells (ESC) and Adult Stem Cells (ASC). Meanwhile, based on the potential or differentiation ability, stem cells can be divided into 4 types (Figure 1) (Zakrzewski et al. 2019):

1. Totipotent: can differentiate into all types of cells, for example, zygote,
2. Pluripotent: can differentiate into 3 germ layers: ectoderm, mesoderm, and endoderm, but cannot become extra-embryonic tissue such as placenta and umbilical cord, for example, embryonic stem cells (ESCs),
3. Multipotent: can differentiate into many cell types but is limited to only a group of cells, for example, MSCs, hematopoietic stem cells, and neural stem cells (NSCs),
4. Unipotent: can only produce 1 type of cell but still has properties that can renew or regenerate itself, for example, renal stem/progenitor cells (RSPCs) (Rantam et al. 2009, Liu et al. 2020).

Adult Stem Cells (ASCs) consist of hematopoietic stem cells (HSCs) and MSCs. When cultured, stem cells will produce a cell line that looks like a fibroblast (fibroblast-like cell). In research, stem cells can act as diagnostic cells where stem cells can be applied to test new drugs and test for cell damage due to viral infection (Rantam et al. 2009).

MSCs are adult stem cells that must have at least 3 criteria according to the

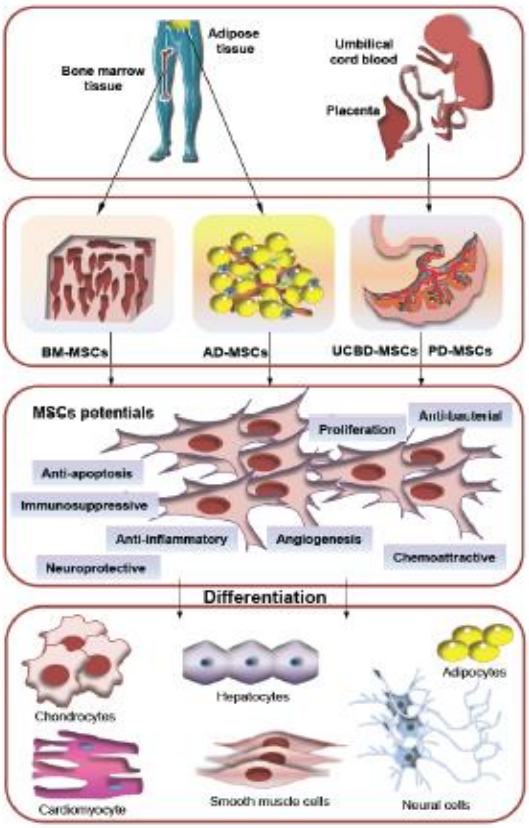

Figure 2. Source and differentiation of MSCs (Alishah et al. 2020)

Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, namely: adhering to a plastic Petri dish base (plastic-adherent) with a standard culture medium, expressing marker CD105, CD73, CD90, and do not express CD45, CD34, CD14 or CD11b, CD79alpha or CD19, and HLA-DR on the cell surface. In humans, the most specific marker of MSCs from bone marrow is CD271. The third criterion is that MSCs can differentiate into osteoblasts, adipocytes, and chondroblasts in vitro (Dominici et al. 2006).

Friedenstein et al. (1974) successfully isolated and described MSCs from mouse bone marrow that is similar to fibroblasts and can be attached to the bottom of a culture plate (Friedenstein et al. 1974). After that, several researchers reported that MSCs are not only present in bone marrow but can be isolated from fat tissue, peripheral blood, umbilical cord blood, amniotic fluid, placenta, Wharton jelly, dental pulp, dermis, etc. (Figure 2) (Macrin et al. 2017).

This is due to the isolation of mesenchymal stem cells originating from the

bone marrow, an invasive procedure for the patient and often accompanied by a risk of infection. Before being used for testing, confluent MSCs (grows evenly monolayer) need to be subcultured (passages). So, the cells can grow optimally in the culture medium (Rantam et al. 2009).

In MSCs culture in vitro, several reagents are needed, such as basal medium, supplements, salt or saline solution, and other additives in isolation and stem cell culture. The culture of MSCs needs nutrients like organic nutrients, inorganic nutrients, and non-nutrient factors. Carbohydrates, lipids, amino acids, and vitamins in the basal medium are organic nutrients; inorganic nutrients like salts, antibiotics, antifungals, trace minerals, anticoagulants, separation medium; and non-nutrient factors like serum that contains growth factors and hormones or other supplements, and a conducive environment like oxygen, humidity, and temperature (Devireddy et al. 2019).

A basal medium is a medium used to culture several kinds of cells and the composition of the constituent materials to support cell productivity and growth. Basal mediums commonly used for stem cell isolation are Dulbecco's modified essential medium (DMEM), alpha-modified essential medium (α-MEM), Iscove's medium, TC199, Ham F12, and others. The components of the basal medium are as follows: carbohydrates (glucose, galactose, and others), amino acids, vitamins, organic salts, and trace elements (zinc, copper, iron, and others) (Rantam et al. 2009). Currently, there are special media for stem cell cultures such as StemPro, MethoCult for hematopoietic stem cell medium, MesenCult for MSCs medium, StemMACS, and others (Bui et al. 2021).

Salt or saline solutions are useful as a base medium for short-term culture, adjusting pH, and osmotic pressure such as balanced salt solution (BSS) or phosphate buffer saline (PBS). The addition of antibiotics and antifungals is intended so that contamination from bacteria and fungi is reduced. Antibiotics or antifungals that are often used for stem cell culture are Penicillin, Streptomycin, Gentamycin, Fungizone, and others. Anticoagulants are used to isolate stem cells from blood specimens to prevent blood clots, for example, sodium or lithium heparin (Rantam et al. 2009).

The separation medium is used during the isolation of stem cells from peripheral blood, cord blood, and bone marrow. Blood contains various types of cells, namely blood cells, blood progenitor cells, and stem cells. Separating these cells requires a separation medium, namely Ficoll-hypaque. Ficoll-hypaque is a dextran polymer that induces erythrocyte aggregation to increase osmolarity and fluid density. After the blood is mixed and centrifuged with Ficoll, several layers are formed. The lowest layer is erythrocytes and neutrophils, then the clear layer, namely Ficoll, the uppermost layer is the plasma layer, and the layer between Ficoll and plasma is a buffy coat that is rich in mononuclear cells (MNCs) as a source of stem cells. Then the buffy coat is cultured in a flask or petri dish (Rantam et al. 2009).

Enzymes like trypsin, papain, collagenase, and dispase can be used in stem cell isolation. Trypsin and papain enzymes can be used for subculture, while collagenase and dispase can be used for the isolation of stem cells by enzymatic methods, for example, the isolation of MSCs from Wharton Jelly or fat tissue. The role of collagenase and dispase is to remove the extracellular matrix that binds the cells. So, the stem cells can separate from the extracellular matrix. Dispase can also be used to separate stem cells from their tissues and can prevent the formation of cell groups in a cell suspension (Lee et al. 2010, Chang et al. 2014).

Stem cell culture requires more complex nutrition, so it is necessary to add supplements to support cell proliferation, for example, the buffer system, serum, glutamine, and growth factors. The buffer system is used to adjust the pH so that stem cell growth can be optimal, namely by adding sodium bicarbonate or Hepes solution. Glutamine is an important precursor in the metabolism of nucleic acid in protein synthesis and as a fuel for cellular respiration as an energy source. Glutamine consists of amino acids that are unstable and can undergo change to ammonia which can be toxic when accumulated. Therefore, glutamine must be added just before using the medium. GlutaMax (L-alanyl-L-

glutamine) is a form of L-glutamine that is more stable in aqueous solutions (Jagušić et al. 2016, Chen et al. 2018, Devireddy et al. 2019).

Table 1. Some research about the substitute of animal serum in MSCs culture

No	Medium Composition	Serum Substitute	Source of MSCs	Result	Reference
1	Serum-Free Medium (SFM; StemPro®)	Growth factor: Recombinant Human PDGF-BB, bFGF, TGF-β1	BM	Cell proliferation in SFM medium is higher than medium with growth factor containing serum	(Chase et al. 2010)
2	α-MEM containing 10% FBS or 5% PL + 0.5% *Cifloxacin* + 1% *glutamine* + *Heparin* (2 IU/mL)	PL	BM	Human PL allogenic can help the proliferation of MSCs	(Chevallier et al. 2010)
3	α-MEM containing 1% penicillin/streptomycin	Human serum	Skeletal MSCs	Human serum can help hMSC proliferate and differentiate in vitro and bone-forming ability in vivo	(Aldahmash et al. 2011)
4	Low glucose DMEM-LG + 100 U/mL penicillin + 100 µg/mL streptomycin + 10% FBS/hPL	*hPL*	AT	ASCs with PL-cultured had a 3-fold higher proliferation rate, larger amounts of CD73, CD90, and CD166 than FBS-cultured ASCs, and have a similar strong capacity to differentiate into cardiomyocytes.	(Naaijkens et al. 2012)
5	DMEM/F12 medium + 1% penicillin/streptomycin, 4 IU/ml heparin + 10% hPLF-fresh + 10% hPLO-outdated/expired or 10% FBS	*hPL*	Human BM	hPLO can be a substitute for FBS in MSCs culture same as hPLF	(Jonsdottir-Buch et al. 2013)
6	*Serum-free medium* (ultra-culture), 100 U penicillin–streptomycin, 1% V SITE, 1 µM/L *non-essential amino acids*, 1.5 mg/L L-glutathione, 0.1 mM/L β-mercaptoethanol, 1 mM/L pyruvate, suplementasi : 1, 10, or 100 nM/L EGF	10 nM/L EGF	BM	Optimal doses of EGF: 10 nM/L in the *serum-free medium* can replace serum	(Wang et al. 2013)
7	*high glucose* DMEM + *human AB type PRP* (*Indonesian Red Cross*) 5%, and 10% + 100 unit (U) *penicillin*/100 µg *streptomycin*/ mL + *amphotericin* B 2.5 µg/mL	PRP	Lipoaspirate MSCs	DMEM/10% PRP can replace FBS	(Suryani et al. 2013)
8	*Ultra Culture Media* + 2% *Ultroser G*	2% *Ultroser G*	AT	MSC cultured well as FBS	(Ahrari et al. 2013)
9	*Mesencult-XF culture kit*	*Mesencult*-SF *attachment substate* dan *Mesencult-ACF Dissociation Kit*	BM and AT	MSCs have stable morphology, differentiation potential marker is higher than medium supplemented with FBS. It is preferable to use it to support adipose culture, not for bone marrow culture.	(Al-Saqi et al. 2014)
10	αMEM (Gibco, Invitrogen Carlsbad, CA)	hPRP	*human adipose-derived stem cells* (hADSCs)	Cell proliferation was significantly higher in the 15% hPRP group compared to FBS. hPRP not only increases proliferation but may also be useful in osteogenic differentiation for clinical purposes	(Tavakolinejad et al. 2014)
11	*DMEM + 1 µg amphotericin β + 10 µg gentamicin + human AB serum / stemulate human platelet lysate (HPL)*	XerumFree (XF), HPL	Stroma kornea	Cell proliferation in cultures using HPL was higher than in cultures with FBS	(Matthyssen et al. 2017)
12	*DMEM F12 supplemented with the supplement for cell culture (SCC contain HP) and other growth factors from platelet lysate*	HP	hMSC lines obtained from BM, umbilical cord (UC), and AT	The use of a xeno-free medium with SCC to expand hMSCs for advanced therapies could be viable.	(Blázquez-Prunera et al. 2017)
13	*Stromal Media*	HPL	Adipose tissue	The HPL concentration of 0.75% is equivalent to 10% FBS which is used to support cell proliferation, immunophenotype, and determination of colony-forming units-fibroblasts.	(Cowper et al. 2019)

THE ROLE OF SERUM

Another supplement in MSCs culture is serum. The serum is an important factor influencing cell growth to be more optimal in MSCs culture because there are contain essential materials like growth factors, vitamins, hormones, trace elements, and transport proteins to support the environment for cell proliferation and maintenance in isolation and expansion of MSCs (van der Valk et al. 2018).

The serum plays a role in protein synthesis. The serum can contain albumin, amino acids, lipids, hormones, vitamins, or growth factors. Albumin is a key component of FBS that is normally present in quantities ranging from 20 to 50 mg/mL. Extracellular lipids are abundant in FBS. They are vital components. FBS also contains lactate dehydrogenase (LDH), alkaline phosphatase (ALP), prostaglandins, endotoxin, hemoglobin, bilirubin, urea, creatinine, prolactin, and other unidentified components. This component's function in cell growth and metabolism is unknown. Besides FBS, Fetal Calf Serum (FCS), Newborn Calf Serum (NCS), and others can be used in stem cell culture (Díez et al. 2015, Devireddy et al. 2019).

For some research purposes, FBS batches with low immunoglobulin (IgG) concentration (less than 5 mg/mL) are used. A high gamma globulin concentration in FBS have the potential to prevent cell lysis. (Burnouf et al. 2016).

The culture of MSCs with FBS is not for human clinical applications, but only appropriate for in vitro research because FBS is originating from animals and have many risks of transferring animal factors to patients. FBS contains xenogeneic components, so it can cause immunological responses when transplanted to patients. FBS is generally used in risk culture as a carrier of infectious agents such as bacteria or viruses from animals. This can lead to inflammation and fibrosis with MSCs therapy or even rejection when stem cells are transplanted (Al-Saqi et al. 2014, Yoshida et al. 2018, Bui et al. 2021).

FBS that is produced can cause heterogeneity in every batch. The concentrations of nutrients in FBS vary substantially between suppliers and can also change between batches. So it may contribute to variations in cell growth and yield between stem cell products (Devireddy et al. 2019).

Furthermore, unclear components and batch-to-batch heterogeneity of FBS can skew research findings and therapeutic outcomes. FBS also raises ethical difficulties because it is derived from bovine fetuses extracted from pregnant cows. Furthermore, FBS does not meet GMP standards because these materials come from animal materials. FBS is also the most expensive component of cell culture. As a result, MSCs medium that contains FBS must be replaced with others that are safer and can effectively retain MSC properties for clinical use (Bui et al. 2021).

SUBSTITUTE OF ANIMAL SERUM

Based on the consideration of the effects of using a serum with animal materials, a serum-free medium is currently being developed. Ideally, the culture medium should contain an animal-free serum with an efficient media formulation while maintaining the resulting therapeutic quality (Jung et al. 2012). Here are several supplements that can substitute FBS (Table 1) such as human plasma, human serum (HuS), human platelet lysates (hPL), human platelet-rich plasma (hPRP), human umbilical cord serum/plasma (hUCS/hUCP), or human plasma-derived supplement for cell culture medium (SCC) (Aldahmash et al. 2011, Bui et al. 2021).

Human serum

Human serum (HuS) can help hMSC proliferate and differentiate in vitro, as well as keep their bone-forming ability in vivo. It is better to use the human serum in hMSC cell cultures for cell-based treatment. In the presence of HuS, primary bone marrow-derived hMSC induced differentiation to osteoblast or adipose. It showed comparable levels of gene expression and protein production of osteoblast markers (CBFA1/Runx2, alkaline phosphatase, collagen type I, and osteocalcin) or adipose markers (PPAR-gamma2, lipoprotein lipase

(LPL), and aP2) with FBS (Aldahmash et al. 2011).

HuS is a good alternative to FBS in terms of supporting hMSC development and biological functions. However, there are several issues with using HuS to replace FBS because the transmission of human infections during cell transplantation is a major concern, hence autologous serum or comprehensive testing of allogenic serum is advised. serum effects on hMSCs growth and differentiation are age-dependent, with serum effects on hMSCs growth and differentiation decreasing as the donor gets older. Because cell-based protocols are used to treat degenerative disorders in the elderly, this has significant clinical implications. It is recommended to utilize allogeneic serum in these circumstances. Other human materials, such as human plasma and human platelet lysate, have been demonstrated to stimulate MSCs growth in addition to HuS (Aldahmash et al. 2011).

Human plasma

One of the sources MSCs is bone marrow. Furthermore, human bone marrow (hBM)-MSCs were found to be multilineage differentiated. Thus, employing human plasma-supplemented medium to expand and differentiate hBM-MSCs is acceptable for clinical use in autologous transplantation and tissue engineering. In an autologous serum/plasma medium, hBM-MSCs can be grown and proliferated (Lin et al. 2005). Human plasma can support the growth, function, and morphology of fibroblasts, human endothelial cells, and smooth muscle cells as well as FBS (Castells-Sala et al. 2017).

Human platelet lysate (hPL)

Human Platelet Lysate (hPL) can be used to replace FBS in MSCs cultures where FBS can transmit pathogens, be xenogenic, and stimulate immunogenic reactions. hPL is reported to contain several growth factors, chemokines, and cytokines, like epidermal growth factor (EGF), HGF, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-AA, PDGF-AB, PDGF-BB, basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), transforming growth factor (TGF-β), serotonin, fibronectin, platelet factor 4 (PF-4), and platelet-derived epidermal growth factor (PDEGF). This growth factor can increase MSCs growth in vitro. hPL is reported as effective as FBS in promoting proliferation, adhesion, and survival of the culture of MSCs. MSCs that culture in hPL have the same morphology, cluster of differentiation (CD) markers, higher proliferation, and no significant variation in differentiation potency to osteocyte, adipocyte, and chondrocyte. The use of hPL also eliminates the need for heparin, resulting in a xeno-free expansion culture medium. hPL should be a viable alternative to FBS in MSCs culture (Mirabet et al. 2008, Chevallier et al. 2010, Naaijkens et al. 2012, Mojica-Henshaw et al. 2013, Alberts et al. 2014, Guiotto et al. 2020).

PL has been suggested as an efficient alternative to FBS for cell and tissue expansion, reducing the risks of transmission of xenogeneic contaminants such as viruses, bacteria, and prions, as well as xenogeneic antigens. Increased safety for cell therapy protocols and cost reduction are the major benefits of human alternatives (Santos et al. 2018).

The hPLs must be activated by freezing and thawing cycles, thrombin, or sonication to release bioactive chemicals. The hPLs could come from blood banks with expired platelet units that aren't suitable for patient transfusion. Despite the disadvantage of employing platelet sources, these hPLs could be preserved and employed to produce cell culture media. Platelets have a short shelf life of about 5 to 7 days, after which they are discarded due to the increased risk of pathogen contamination. But the hPL-outdated unit or expired (hPLO) can be a substitute for FBS in MSCs culture same as hPL-fresh unit (hPLF) (Jonsdottir-Buch et al. 2013, Bui et al. 2021).

Human platelet-rich plasma (hPRP)

PRP is another supplement of stem cell culture medium to increase proliferation, differentiation, and migration because PRP contains high concentrations of growth factors and proteins (Santos et al. 2018). Autologous human platelet-rich plasma (hPRP) is a substitute for the serum that is easily produced at a relatively low cost.

Several in vitro studies reported that hPRP increased proliferation in several cell types including MSCs. Platelets can contain many cytokines, including platelet-derived growth factor, β-transforming growth factor, insulin-like growth factor-1, and vascular endothelial growth factor in α-granules (Tavakolinejad et al. 2014). hPRP also contains several other growth factors such as EGF, acidic FGF, PDGF keratocyte growth factor, and hepatocyte growth factor (Fekete et al. 2012).

PRP is easily obtained by differential centrifugation of a whole blood sample. Platelet activation is then imperative for the release of the soluble factors. Platelet activation is usually achieved by three different methods, namely the addition of calcium chloride and thrombin, contact with collagen, or freeze/thaw cycles. Compared to chemical activation of platelets which may cause side effects, mechanical lysis via freezing and thawing is easier, less time-consuming, and also cost-effective (Santos et al. 2018).

The major component of plasma is albumin and globulins that act as carriers for various biomolecules, other components include fibrinogen which plays an important role in the blood clotting cascade. GFs present in human PRP and PL include platelet-derived growth factor (PDGF), VEGF, fibroblast growth factor (FGF), insulin-like growth factor I and II (IGFI and II), TGF-β22, epidermal growth factor (EGF), epithelial cell growth factor (ECGF), connective tissue growth factor (CTGF), platelet factor 4 (PF4), interleukin 8 (IL-8), and keratinocytes growth factor (KGF). These GFs perform a function such as chemoattraction, cell proliferation, maturation, matrix synthesis, and angiogenesis. Also, clotting factors are present – factor V, factor XI, protein S, and antithrombin – and all of them are responsible for thrombin activation and fibrin clot formation (Santos et al. 2018).

In a study conducted by Fekete (2012), MSCs proliferation with 2-8% PRP has higher efficacy than FCS. The study results indicated that platelet-based (PL) or PRP media efficiently maintained the phenotype and genotype of cells in long-term culture. Cell regeneration, differentiation potential, and surface marker expression of MSCs were also retained in these cultures.

Human umbilical cord serum/plasma

The Human umbilical cord serum/plasma (hUCS/hUCP) could be extracted from the entire umbilical cord blood or as a byproduct of the cryopreservation method for HSCs. As a result, this strategy can take advantage of the readily available raw material sources for hUCS/hUCP production, particularly in collaboration with cord blood banks, to mass-produce hUCS/hUCP supplements. hUCS/hUCP also has much larger amounts of basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and granulocyte colony-stimulating factor than human adult blood plasma, according to growth factor profiles. These findings suggested that hUCS/hUCP could be used as a xeno-free medium additive for cell proliferation (Bui et al. 2021).

Human plasma-derived SCC medium

SCC is a freeze-dried cell culture supplement made by Grifols, a pharmaceutical firm (Barcelona, Spain). Cold ethanol fractionation under GMP conditions was used to create SCC obtained from human serum. Each plasma unit in a pool is screened for transmissible viruses and harmful agents, and during the manufacturing process, a viral inactivation step using gamma irradiation is performed to eradicate any remaining viruses. Because each SCC batch is made up of a large number of blood units from roughly 1000 healthy donors, the components of SCC are similar from batch to batch. SCC is expected to be an effective supplement to boost MSC proliferation and retain cell properties due to its safety and uniformity of quality (Bui et al. 2021).

CONCLUSION

Human platelet lysate (hPL), human platelet-rich plasma (hPRP), human serum (hS), human umbilical cord serum/plasma (hUCS/hUCP), or human plasma-derived supplement for cell culture medium (SCC) can be used as substitutes for FBS in MSCs culture because they can stimulate of MSCs in adhesion, proliferation, maintain cellular

morphology, enhance cell functionality, and survival similar with FBS. Some supplements above can be used in cell therapy with MSCs to replace animal serum.

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