

Influences of Xeno-Free Media on Mesenchymal Stem Cell Expansion for Clinical Application

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Abstract Mesenchymal stem/stromal cells (MSCs) are multipotent somatic stem/progenitor cells that can be isolated from various tissues and have attracted increasing attention from the scientific community. This is due to MSCs showing great potential for incurable disease treatment, and most applications of MSCs involve tissue degeneration and treatment of immune- and inflammation-mediated diseases. Conventional MSC cultures contain fetal bovine serum (FBS), which is a common supplement for cell development but is also a risk factor for exposure to animal-derived pathogens. To avoid the risks resulting from the xenogeneic origin and animal-derived pathogens of FBS, xeno-free media have been developed and commercialized to satisfy MSC expansion demands for human clinical applications. This review summarized and provided an overview of xeno-free media that are currently used for MSC expansion. Additionally, we discussed the influences of different xeno-free media on MSC biology with particular regard to cell morphology, surface marker expression, proliferation, differentiation and immunomodulation. The xeno-free media can be serum-free and xeno-free media or media supplemented with some human-originating substances, such as human serum, human platelet lysates, human umbilical cord serum/plasma, or human plasma-derived supplements for cell culture medium. These media have capacity to maintain a spindle-shaped morphology, the expression of typical surface markers, and the capacity of multipotent differentiation and immunomodulation of MSCs. Xeno-free media showed potential for safe use for human clinical treatment. However, the influences of these xeno-free media on MSCs are various and any xeno-free medium should be examined prior to being used for MSC cultures.

Keywords Mesenchymal stem cells · Xeno-free media · Xeno-free and serum-free media · Clinical application

1 Introduction

Mesenchymal stem/stromal cells (MSCs) were first described by Friedenstein et al. in 1970 and are among the most broadly studied adult multipotent stem cells due to their tremendous potential for disease and regenerative treatment [1–3]. This cell type can be obtained from several tissues, such as bone marrow, adipose tissue, umbilical cord, dental pulp, and peripheral blood [4]. According to the characterization criteria published by the International Society for Cellular Therapy (ISCT, <https://isctglobal.org/>), MSCs are defined by three major properties: (1) plastic adherence ability; (2) expression of specific surface markers (CD73, CD90 and CD105) and the lack of

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expression of hematopoietic antigens (CD45, CD34, CD14 or CD11b, CD79 α or CD19 and HLA-DR); and (3) multipotent differentiation into osteocytes, chondrocytes, and adipocytes. MSCs have been widely studied for the treatment of many diseases because of their immunomodulatory and anti-inflammatory effects [3, 5–9]. MSCs can interact with many cell types to modulate both the innate and adaptive immune systems via cell-to-cell contact or soluble factor secretion [6]. For instance, MSCs suppress lymphocyte responses, especially in the T cell population, and inhibit the activation, maturation, migration, differentiation, and proliferation of antigen-presenting cells as well as the switching cytokine-secreting cells into an anti-inflammatory phenotype [5, 6, 10]. MSC therapies have attracted considerable interest from the scientific community, even though the physiological role of MSCs and their possible pathogenicity in the body are unclear [11, 12]. To date, there have been more than 800 clinical trials using MSCs for disease treatment registered on the website <https://clinicaltrials.gov/> (accessed March 17th, 2020). Additionally, data from published investigations indicated that MSCs show promise for application for many incurable diseases, such as graft-versus-host disease (GvHD) [13], bronchopulmonary dysplasia [14], spinal cord injury [15], cerebral palsy [16], hepatic cirrhosis [17], and diabetes type I [18].

After isolation from tissues, MSCs were expanded *in vitro* to obtain an adequate cell quantity for clinical use. However, the challenge to scientists is to determine the appropriate cell culture media for cell growth and proliferation and to maintain the stem cell characteristics during cell expansion. The conventional medium used to culture MSCs is FBS-supplemented basal media [9]. FBS provides growth factors, hormones, adhesive proteins and other factors that are essential for cell proliferation and maintenance [3, 8]. However, the use of FBS originating from animals is only appropriate for *in vitro* research and not for human clinical applications because it poses many risks of transferring animal factors to patients [19]. FBS carrying xenogeneic components can cause immunological responses when infused into patients [5]. Additionally, FBS may carry animal-originated pathogens such as bacteria or viruses that can be transmitted to MSCs during cell culture [2, 20]. Moreover, undefined ingredients and batch-to-batch variation can affect the accuracy of research results and therapeutic outcomes [20]. Therefore, it is necessary to replace conventional FBS-containing media with xeno-free media that are safer and that can maintain MSC characteristics effectively for clinical application. In this review, we will discuss MSC xeno-free media and summarize the characteristics of MSCs when they are cultured in these media. The data indicate that xeno-free media could be better candidates for MSC expansion for clinical

applications in terms of both promoting cell proliferation and preserving stem cell characteristics.

2 Classification of xeno-free media

Along with the increasing demand for the use of MSCs for clinical application, innovative cell medium technologies for MSC cultures have been developed. Currently, there are two strategies for establishing xeno-free media for MSC cultures. The first is the substitution of FBS with “humanized” supplements derived from human blood, such as human serum (HS), human platelet lysates (HPL), human umbilical cord serum/plasma (hUCS/hUCP), or human plasma-derived supplement for cell culture medium (SCC). The second strategy is formulating media by using only synthetic, recombinant or human-derived purified substances without the addition of serum as a supplement [3, 8, 20]. This type of medium formulation is classified as serum-free media/xeno-free (SFM/XF) media. One of the advantages of SFM/XF media is that the media are defined with known compositions and concentrations.

Regarding supplements derived from human blood, a supplement type that can be used to support the growth of MSCs is HS. The benefit of replacing FBS with HS is to remove animal factors and thus to minimize infection risks due to nonhuman factors [19]. However, due to the limitation of using autologous HS because of its low availability and the variability among different HS donations, pooled allogeneic HS has been preferred for MSC cultures [21–23]. In addition to in-house human sera produced by several research teams, there are various commercial products with HS supplementation that have been approved for use in cell therapies from different manufacturers, such as Sigma-Aldrich, PAN-Biotech, and Life Science [19, 21]. Therefore, the use of HS-supplemented media for MSC expansion varies among groups and different MSC types.

Several research teams have replaced FBS with hUCS/hUCP for MSC expansion. The hUCS/hUCP could be harvested from whole umbilical cord blood [24, 25] or as a byproduct of the hematopoietic stem cell (HSC) separation procedure used for cryopreservation [26, 27]. Therefore, this approach can take advantage of the available raw material sources for hUCS/hUCP production, especially in cooperation with cord blood banks, to produce hUCS/hUCP supplements on a large scale. Additionally, the results of growth factor profiles indicated that hUCS/hUCP has significantly higher levels of vascular endothelial growth factor, granulocyte colony-stimulating factor, epidermal growth factor and basic fibroblast growth factor than human adult blood plasma [27]. These results suggested that hUCS/hUCP is a potential supplement for xeno-free media for cell growth.

Human platelets (HPLs) are another supplement type derived from blood used for MSC cultures. HPLs contain a wide range of growth factors, cytokines, adhesive proteins and other regulatory molecules to support MSC proliferation [8, 20, 28]. To release bioactive molecules, human platelets must be activated by freezing–thawing cycles to lyse platelets, by thrombin, or by sonication [28, 29]. The HPLs could be collected from blood banks, where there are expired platelet units that are not appropriate for patient transfusion. These HPLs could be stored and used for making cell culture medium despite the disadvantage of using platelet sources.

Additionally, SCC is a freeze-dried supplement for cell culture manufactured by the pharmaceutical company Grifols (Barcelona, Spain). SCC derived from human serum was produced through cold ethanol fractionation under good manufacturing practice (GMP) conditions [30–32]. Each plasma unit in a pool is tested for transmissible virus and pathogenic agents [31, 33], and a viral inactivation step with gamma irradiation is carried out during the manufacturing process to remove all residual viruses [31]. Due to the large number of blood units from approximately 1000 healthy donors used to produce each SCC batch, the components of SCC are consistent from batch-to-batch [31, 33]. Due to the safety and consistency of the quality of the SCC product, SCC is expected to be a good supplement to support MSC proliferation and maintain cell characteristics [32].

To address problems related to the use of serum for cell expansion, such as undefined compositions, variation among serum units or batches and risks of pathogen transmission, XF/SFM media have been developed to be more suitable for clinical applications. Currently, there are many commercial XF/SFM media available from different manufacturers for MSC expansion (Table 1). Most XF/SFM media require a suitable coating solution to support the attachment and spread of MSCs due to the lack of adhesive proteins added into the media. In this current review, we further focused on the two XF/SFM media that are used the most at present, which are StemPro™ MSC SFM XenoFree (Gibco™) and MesenCult™-XF Medium (STEMCELL Technologies).

3 MSCs cultured in xeno-free media maintain a spindle-shaped morphology

A spindle-shaped morphology or fibroblast-like morphology is not a requirement to define MSCs according to ISCT criteria; however, it has been described as one of the typical characteristics of MSCs. MSCs cultured in xeno-free media preserve their spindle-shaped morphology, which is similar to that of cells cultured in conventional medium supplemented with FBS [19, 24, 28, 34–36]. Intriguingly, many studies illustrated that MSCs cultured in XF/SFM media are more homogeneous, smaller in size and have a more defined spindle-shaped morphology compared to those cultured in FBS-supplemented medium [34, 36, 37]. The homogeneity of MSC morphology during the culture process in XF/SFM media may suggest that these media can maintain the morphological characteristics of MSCs even better than conventional media.

4 MSCs cultured in xeno-free media express typical surface markers

To classify MSCs, surface marker expression is one of critical requirements according to the ISCT. Over 95% of MSC populations must express CD73, CD90, and CD105, and less than 2% must express CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II [38]. The abnormality of surface markers might indicate a heterogeneous population or the poor quality of the cells due to low cell viability, poor differentiation, and a stressed or immune-stimulated status.

In general, there is no significant difference in surface markers between MSCs cultured in xeno-free medium and those cultured in FBS-supplemented medium [26, 34, 37, 39, 40]. In addition to the markers that were suggested by the ISCT, other markers, such as CD29, CD44, CD166, CD146, Stro-1, CD16, CD54, HLA-ABC, CD31, CD80, and CD133, were also tested for cells cultured in both xeno-free medium and FBS-supplemented medium [19, 24, 31, 37]. Data from studies showed the similar expression of tested markers in all MSCs cultured

Table 1 Several types of commercial XF/SFM media

No.	XF/SFM media	Manufacturer
1	StemPro™ MSC SFM XenoFree	Gibco™
2	MesenCult™-XF Medium	STEMCELL Technologies
3	MSC NutriStem® XF Medium	Biological Industries
4	StemMACS MSC Expansion Media Kit XF, human	Miltenyi Biotec
5	MSCGM™ Mesenchymal Stem Cell Growth Medium BulletKit™	Lonza
6	PowerStem MSC1	PAN Biotech

both in xeno-free medium and FBS-supplemented medium [19, 31, 34, 37].

To examine whether the marker phenotypes of MSCs undergo any changes during a long period of culture in xeno-free medium, the expression of some MSC markers at late passages was investigated. Data from Al-Saqi et al. [35] showed that MSCs cultured in Mesencult-XF medium (a commercial XF/SFM medium) showed a significant decrease in the CD90 expression level at late passages compared to that in MSCs at early passages ($54.8\% \pm 16.5$ at passage 5 and $87.8\% \pm 7.9$ at passages 1 and 2) [35]. In addition, Brohlin et al. [41] noticed that the expression level of the CD105 marker was significantly decreased at passage 10 when MSCs were cultured in Mesencult-XF medium [41]. The decrease in CD105 expression level at late passages was also observed in other studies where MSCs were cultured in StemPro® MSC SFM Xeno-free [33, 42]. However, there has been no report of a reduction in MSC marker expression at late passages cultured in xeno-free media supplemented with HS, HPL, hUCS/hUCP or SCC. These results indicated that xeno-free and serum-free conditions might affect MSC marker expression at late passages specifically via a decrease in the expression level of some surface markers. More importantly, these data raise the issue of the quality of MSCs cultured in XF/SFM media, and doctors need to consider the period of the use of MSCs for transplantation.

5 MSCs cultured in xeno-free media preserve their multipotent differentiation capability

According to the literature, MSCs are able to differentiate into mesodermal lineages such as osteocytes, chondrocytes, and adipocytes [38]. The use of either xeno-free medium or conventional medium supplemented with FBS can maintain this differentiation capacity. There have been no studies reported on differences in MSC differentiation between HS or SCC-supplemented media and FBS-supplemented media [19, 32, 43]. Additionally, there were no obvious differences in the differentiation tendency of MSCs cultured in HPL-supplemented media or XF/SFM media compared to FBS-supplemented medium (Table 2 and Table 3). MSCs cultured in these media maintained their capacity to differentiate into osteocytes, chondrocytes, and adipocytes [28, 35, 42–44]. However, Caseiro et al. [26] reported that UC-MSCs could not differentiate into osteocytes in hUCBP-supplemented medium but could do so in FBS-supplemented medium [26]. Moreover, other studies also indicated an increase or decrease in differentiation level toward a specific lineage, for example,

osteogenic or adipogenic lineage; however, it varied among different studies [39, 40, 45]. It is noted that the dissimilarity of the comparison and quantification methods could cause differences among the reported data.

6 Xeno-free media significantly promote the proliferation of MSCs compared to FBS-containing medium

Apart from maintaining the characteristics of MSCs, media for MSC expansion must ensure that MSCs can proliferate well to obtain sufficient cell numbers in a certain culture period for clinical applications. Population doubling time (PDT) is an index of MSC growth kinetics, where the lower the PDT is, the faster the growth rate of cells is. Therefore, we compared the proliferation of MSCs cultured in xeno-free medium and FBS-supplemented media using the PDT. A summary of the PDT of MSCs cultured in different xeno-free media is reported in Fig. 1.

It is noticeable that the SCC-supplemented medium is the only type of media that exhibited a decreased ability to support MSC proliferation compared to FBS-supplemented medium (Table 1) [30–32]. The PDT of MSCs cultured in SCC-supplemented media was 1.5–2.5-fold higher than that of cells cultured in FBS-supplemented media [30–32]. However, this problem could be addressed by adding growth factors such as basic fibroblast growth factor, transforming growth factor β 1 (TGF β 1) or TGF β 3 into SCC-supplemented medium [33]. Blázquez-Prunera et al. (2017) showed that MSCs proliferated faster in SCC-supplemented medium supplemented with growth factors originating from platelet lysates than in SCC-supplemented medium; for example, the PDT of BM-derived MSCs cultured in these media was 5.5 and 8.3 days [31].

In contrast, MSCs cultured in hUCS/hUCP-supplemented media exhibited stronger growth kinetics with a greater cell yield compared to those cultured in FBS-supplemented media [46]. The PDT of MSCs cultured in hUCS/hUCP-supplemented medium was 1.1 ± 0.3 days, whereas it was 2.1 ± 1.0 days in FBS-supplemented medium [24]. hUCS/hUCP not only supported the outgrowth of MSC colonies in primary cultures but also exerted a strong expansion effect on MSC proliferation in later passages [24]. That is, hUCS/hUCP displayed superior *in vitro* cell prolongation when it could maintain MSCs in culture for at least 20 passages [24]. Although there has been limited literature on culturing MSCs in media supplemented with hUCS/hUCP, the results from these studies suggest that hUCS/hUCP might be a potential xeno-free supplement that supports MSC proliferation.

In addition, HS seems to be a good candidate xeno-free supplement for MSC expansion. Paula et al. [19] showed that HS exerted a significant proliferative effect on MSC proliferation [19]. MSCs cultured in medium supplemented with 10% HS expanded much faster (PDT: 24.3 ± 1.9 h) than those cultured with medium supplemented with 10% FBS (PTD: 122.1 ± 1.8 h) [19]. However, Venugopal et al. [21] reported that there was no statistically significant difference between the proliferation of MSCs cultivated in HS-supplemented medium and those cultured in in FBS-supplemented medium [21]. This may be due to a lower concentration of HS (5%) and the addition of 2 ng/ml basic fibroblast growth factor in the xeno-free medium compared to the control medium supplemented with 10% FBS, which was designed by Venugopal's team [21]. Thus, further studies need to be performed to reveal the appropriate concentration of HS and may be additional elements to effectively support MSC proliferation.

In addition, HPL is also a potent supplement to support MSC proliferation. Hemeda et al. (2018) showed that MSCs cultured in HPL-supplemented medium grew faster and possessed a lower PDT than those cultured in FBS-supplemented medium [28]. This might be initiated by higher levels of mitogens, such as platelet-derived growth factor, epidermal growth factor, basic fibroblast growth factor, hepatocyte growth factor, transforming growth factor β 1 and vascular endothelial growth factor, present in HPL compared to serum [47]. The outstanding proliferation of MSCs cultured in HPL-supplemented medium has been confirmed by other studies using different measurement methods, such as BrdU incorporation assays or MTT cell proliferation assays [39, 40]. The yield of MSC

proliferation under HPL-supplemented conditions was even five times higher than that under FBS-supplemented conditions [40]. On the other hand, Xia et al. [45] reported that the growth capacity of MSCs cultured with HPL was not different compared to MSCs cultured with FBS [45]. Additionally, there are still some drawbacks for the use of HPL, such as the significant variation between donations and the inconsistency of culture conditions, leading to inaccurate results [40, 44]. Despite these drawbacks, it is undeniable that HPL is a very good potential supplement for xeno-free medium used to expand MSCs because of its superior capacity to promote MSC proliferation compared to FBS.

XF/SFM media are preferable choices for MSC expansion due to their safety, consistency, and capacity to support MSC proliferation. However, different media from different manufacturers may be appropriate for each MSC source. Data collected in the current study indicated that MSCs cultured in MesenCultTM-XF medium could grow faster than those cultured in FBS-supplemented medium, with average PDTs of 37.082 h and 54.964 h, respectively [34, 35, 41, 43]. However, Brohlin et al. [41] also noted that the growth kinetics of BM-derived MSCs cultured in MesenCultTM-XF medium declined after five passages [41]. The decrease in the cell expansion rate might be due to cellular senescence during culture [35, 41]. However, this problem was only observed in BM-derived MSCs but not in MSCs derived from other sources, such as AD [35, 41]. Therefore, MesenCultTM-XF medium may be more appropriate for AD-derived MSCs than BM-derived MSCs.

Table 2 Effect of HPL-supplemented medium on the MSC differentiation tendency compared to FBS-supplemented medium

Studies	Osteogenic differentiation	Chondrogenic differentiation	Adipogenic differentiation
Oikonomopoulos et al. [39]			
BM-MSCs	↑	NA	↑
AD-MSCs	↑	NA	↑
Riordan et al. [44]			
UC-MSCs	–	–	–
Wuchter et al. [52]			
BM-MSCs	–	↑	–
Mojica-Henshaw et al. [40]			
Commercial hMSCs	–	–	↑
Kandai et al. [28]			
UC-MSCs	–	–	–
Xia et al. [45]			
BM-MSCs	↑	NA	↓

↑ denotes an increased effect, ↓ denotes a decreased effect, – denotes no difference, NA denotes not available

Table 3 Effect of XF/SFM media on the MSC differentiation tendency compared to FBS-supplemented medium

Studies	Osteogenic differentiation	Chondrogenic differentiation	Adipogenic differentiation
StemPro® MSC SFM Xenofree			
Oikonomopoulos et al. [39]			
BM-MSCs	↑	NA	–
AD-MSCs	↓	NA	↓
Bobis-Wozowicz et al. [42]			
UC-MSCs	–	–	–
Simoes et al. [36]			
UC-MSCs	–	–	–
BM-MSCs	–	–	–
AD-MSCs	–	–	–
Wuchter et al. [52]			
BM-MSCs	–	–	–
Hartmann et al. [51]			
UC-MSCs	–	NA	–
MesenCult XF/SF medium			
Swamynathan et al. [34]			
UC-MSCs	↑	–	–
Al-Saqi et al. [35]			
BM-MSCs	–	NA	↓
AD-MSCs	↑	NA	–
Gottipamula et al. [43]			
BM-MSCs	–	↓	–
Hartmann et al. [51]			
UC-MSC	–	NA	–

↑ denotes an increased effect, ↓ denotes a decreased effect, – denotes no difference, NA denotes not available

However, BM-derived MSCs were reported to be inappropriate for culturing in StemPro™ MSC SFM XenoFree even with supplementation with 2% HS (n = 3) [43]. In the case of UC-derived MSCs cultured in StemPro™ MSC SFM XenoFree, even if the cell can grow, the cell lifespan is short, and two of three UC-derived MSC samples could not grow to passage five [34]. In contrast, Simoes et al. [36] isolated MSCs successfully from all UC tissues in their study (n = 8) [36]. The number of isolated MSCs in StemPro™ MSC SFM XenoFree at passage zero was nearly eight times higher than the number of MSCs isolated from FBS-supplemented medium. Additionally, both BM-derived MSCs and AD-derived MSCs cultured in StemPro™ MSC SFM XenoFree medium exhibited higher proliferation capacity with a significantly higher number of cells during S-phase compared to those cultured in FBS-supplemented medium [36, 39]. The proliferation capacity of MSCs under StemPro™ MSC SFM XenoFree is controversial; this may be due to inconsistent data generated from different studies. Thus, more studies are required to confirm these results and the use of commercial media.

7 MSCs cultured in xeno-free media retain their immunomodulatory capacity

MSCs exert their immunomodulatory effects by secreting many regulatory molecules, such as cytokines, growth factors, and other anti-inflammatory mediators, which may or may not envelop secreted exosomes [48–50]. These functional molecules can trigger many subsequent reactions by inhibiting T helper cells, NK cells, B cells, and neutrophils, and stimulating regulatory T cells and B cells [4]. This is one of the reasons why an increasing number of MSC-based therapies have been developed, especially for immune-mediated diseases [48, 49].

The use of xeno-free medium to expand MSCs for clinical application can not only be safer for patients but also retain MSC immunomodulatory potency. Many studies indicated that XF/SFM medium could preserve MSC immunomodulatory properties exhibited by the capacity of MSCs to inhibit the proliferation of PBMCs compared to FBS-containing medium [34, 37, 43, 51]. Additionally, the immunomodulation property of MSCs cultured in xeno-

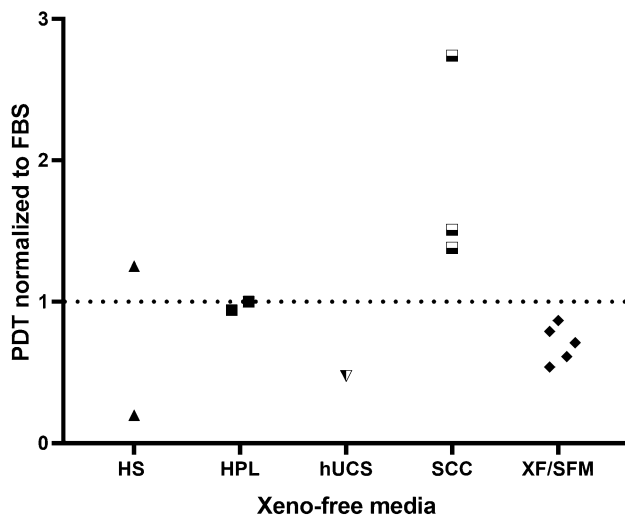


Fig. 1 Population doubling times (PDT) of MSCs cultured in xeno-free media normalized to FBS-supplemented media. Xeno-free media including hUCS-supplemented media and XF/SFM media expressed a higher capacity to support MSC proliferation than FBS-supplemented media (lower than 1), whereas SCC-supplemented media expressed a lower capacity to support MSC proliferation (higher than 1). The HS-supplemented media are various where different studies expressed different capacity to enhance MSC proliferation. Data was analyzed by GraphPad Prism 8 software. MesenCult™-XF Medium is represented for XF/SFM media. Black triangle (▲) indicates PDT of MSCs cultured in HS; black square (■) indicates PDT of MSCs cultured in HPL; black and white triangle (▼) indicates PDT of MSCs cultured in hUCS; black and white square (◻) indicates PDT of MSCs cultured in SCC; black diamond (◆) indicates PDT of MSCs cultured in XF/SFM

free medium proved that MSCs cultured in XF/SFM medium secreted pro-inflammatory cytokines, including IL-1 β , IL-8, IFN- γ , and TNF- α , at lower or at least the same levels as those cultured in FBS-supplemented medium [42]. It is noted that various XF/SFM media could affect the MSC immunomodulatory molecule profile with different levels of pro-inflammatory or anti-inflammatory cytokines secreted by MSCs [42]. Meanwhile, the immunomodulatory capacity of MSCs cultured in HPL-supplemented medium is controversial. For example, Kandoi et al. (2018) showed that the immunosuppressive ability of UC-derived MSCs expanded in HPL-supplemented medium was preserved and even better than those in FBS-supplemented medium [28]. These data are inconsistent with the data reported by Oikonomopoulos et al. [39] that BM-derived MSCs and AD-derived MSCs cultured in HPL-supplemented medium did not suppress PBMCs [39]. Thus, further studies are essential to understand the influences of xeno-free media on MSC cultures

for human clinical application, especially in the field of immunomodulation.

8 Recommendation for choosing appropriate xeno-free media for MSC expansion

In addition to *in-house* media developed by research teams, there are several commercial xeno-free media used to expand MSCs to produce a safe and well-qualified cell product for clinical transplantation. This review indicates that almost xeno-free media are appropriate for expanding clinical grade MSCs. However, each type of xeno-free medium subclass has its own advantages and disadvantages. While SFM/XF media are quite consistent and made up of defined ingredients, media made up of serum and blood-derived products such as HS, HPL, hUCS/hUCP and SCC are less consistent from batch-to-batch and contain undefined ingredients [20]. Moreover, it seems that one type of xeno-free medium is not appropriate for all MSCs, as different types of MSCs showed dissimilar biological characteristics under different mediums [31, 35, 41]. It is noteworthy that different studies reported inconsistent data for MSC cultures [34, 36]; thus, care should be taken when expanding MSCs for clinical application. Medium testing is needed to ensure that MSCs can grow well and maintain their stem cell characteristics.

9 Conclusion

An increasing demand for the clinical application of MSCs has promoted the development of xeno-free MSC culture media. All these xeno-free media showed promise for safe use for human clinical treatment by producing equivalent or even increased proliferation of MSCs and maintained MSC characteristics in terms of morphology, surface marker expression, multipotent differentiation capacity and immunomodulation capacity. However, the influences of these xeno-free media on MSCs vary and are inconsistent among different studies. In addition, xeno-free and serum-free media are more consistent in their components but seem to be appropriate for a particular MSC type. Additionally, other xeno-free media comprising human blood-derived components are affected by limitations in accessing serum sources that are inconsistent in their components from batch-to-batch and that might cause immune responses. Thus, we suggest that any xeno-free medium should be tested prior to being used for MSC cultures.

Compliance with ethical standards

Conflict of interest The authors have no conflicts to declare.

Ethical statement There are no animal experiments carried out for this article.

References

- Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet.* 1970;3:393–403.
- Han Y, Li X, Zhang Y, Han Y, Chang F, Ding J. Mesenchymal stem cells for regenerative medicine. *Cells.* 2019;8:886.
- Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. *NPJ Regen Med.* 2019;4:22.
- Hass R, Kasper C, Bohm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. *Cell Commun Signal.* 2011;9:12.
- Weiss ARR, Dahlke MH. Immunomodulation by mesenchymal stem cells (MSCs): Mechanisms of action of living, apoptotic, and dead MSCs. *Front Immunol.* 2019;10:1191.
- Qin Y, Guan J, Zhang C. Mesenchymal stem cells: mechanisms and role in bone regeneration. *Postgrad Med J.* 2014;90:643–7.
- Spees JL, Lee RH, Gregory CA. Mechanisms of mesenchymal stem/stromal cell function. *Stem Cell Res Ther.* 2016;7:125.
- Shin TH, Kim HS, Choi SW, Kang KS. Mesenchymal stem cell therapy for inflammatory skin diseases: clinical potential and mode of action. *Int J Mol Sci.* 2017;18:244.
- Wang LT, Ting CH, Yen ML, Liu KJ, Sytwu HK, Wu KK, et al. Human mesenchymal stem cells (MSCs) for treatment towards immune- and inflammation-mediated diseases: review of current clinical trials. *J Biomed Sci.* 2016;23:76.
- Wang M, Yuan Q, Xie L. Mesenchymal stem cell-based immunomodulation: properties and clinical application. *Stem Cells Int.* 2018;2018:3057624.
- Feng Y, Zhan F, Zhong Y, Tan B. Effects of human umbilical cord mesenchymal stem cells derived from exosomes on migration ability of endometrial glandular epithelial cells. *Mol Med Rep.* 2020;22:715–22.
- Laganà AS, Vitale SG, Salmeri FM, Triolo O, Ban Frangež H, Vrtačnik-Bokal E, et al. Unus pro omnibus, omnes pro uno: a novel, evidence-based, unifying theory for the pathogenesis of endometriosis. *Med Hypotheses.* 2017;103:10–20.
- Amorin B, Alegretti AP, Valim V, Pezzi A, Laureano AM, da Silva MA, et al. Mesenchymal stem cell therapy and acute graft-versus-host disease: a review. *Hum Cell.* 2014;27:137–50.
- Augustine S, Avey MT, Harrison B, Locke T, Ghannad M, Moher D, et al. Mesenchymal stromal cell therapy in bronchopulmonary dysplasia: Systematic review and meta-analysis of preclinical studies. *Stem Cells Transl Med.* 2017;6:2079–93.
- Cofano F, Boido M, Monticelli M, Zenga F, Ducati A, Vercelli A, et al. Mesenchymal stem cells for spinal cord injury: current options, limitations, and future of cell therapy. *Int J Mol Sci.* 2019;20:2698.
- McDonald CA, Fahey MC, Jenkin G, Miller SL. Umbilical cord blood cells for treatment of cerebral palsy; timing and treatment options. *Pediatr Res.* 2018;83:333–44.
- Zhang Y, Li Y, Zhang L, Li J, Zhu C. Mesenchymal stem cells: potential application for the treatment of hepatic cirrhosis. *Stem Cell Res Ther.* 2018;9:59.
- Li L, Li F, Gao F, Yang Y, Liu Y, Guo P, et al. Transplantation of mesenchymal stem cells improves type 1 diabetes mellitus. *Cell Tissue Res.* 2016;364:345–55.
- Paula AC, Martins TM, Zonari A, Frade SP, Angelo PC, Gomes DA, et al. Human adipose tissue-derived stem cells cultured in xeno-free culture condition enhance c-MYC expression increasing proliferation but bypassing spontaneous cell transformation. *Stem Cell Res Ther.* 2015;6:76.
- 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;2017:6597815.
- Venugopal P, Balasubramanian S, Majumdar AS, Ta M. Isolation, characterization, and gene expression analysis of Wharton's jelly-derived mesenchymal stem cells under xeno-free culture conditions. *Stem Cells Cloning.* 2011;4:39–50.
- Tonti GA, Mannello F. From bone marrow to therapeutic applications: different behaviour and genetic/epigenetic stability during mesenchymal stem cell expansion in autologous and foetal bovine sera? *Int J Dev Biol.* 2008;52:1023–32.
- Jung S, Panchalingam KM, Rosenberg L, Behie LA. Ex vivo expansion of human mesenchymal stem cells in defined serum-free media. *Stem Cells Int.* 2012;2012:123030.
- Julavijitphong S, Wichitwiengrat S, Tirawanchai N, Ruangvutitert P, Vantanasiri C, Phermthai T. A xeno-free culture method that enhances Wharton's jelly mesenchymal stromal cell culture efficiency over traditional animal serum-supplemented cultures. *Cytotherapy.* 2014;16:683–91.
- Cooper K, SenMajumdar A, Viswanathan C. Derivation, expansion and characterization of clinical grade mesenchymal stem cells from umbilical cord matrix using cord blood serum. *Int J Stem Cells.* 2010;3:119–28.
- Caseiro AR, Ivanova G, Pedrosa SS, Branquinho MV, Georgieva P, Barbosa PP, et al. Human umbilical cord blood plasma as an alternative to animal sera for mesenchymal stromal cells in vitro expansion - A multicomponent metabolomic analysis. *PLoS One.* 2018;13:e0203936.
- Ehrhart J, Sanberg PR, Garbuzova-Davis S. Plasma derived from human umbilical cord blood: Potential cell-additive or cell-substitute therapeutic for neurodegenerative diseases. *J Cell Mol Med.* 2018;22:6157–66.
- Kandoi S, Praveen Kumar L, Patra B, Vidyasekar P, Sivanesan D, Vijayalakshmi S, et al. Evaluation of platelet lysate as a substitute for FBS in explant and enzymatic isolation methods of human umbilical cord MSCs. *Sci Rep.* 2018;8:12439.
- Schallmoser K, Henschler R, Gabriel C, Koh MBC, Burnouf T. Production and quality requirements of human platelet lysate: a position statement from the working party on cellular therapies of the international society of blood transfusion. *Trends Biotechnol.* 2020;38:13–23.
- Blazquez-Prunera A, Almeida CR, Barbosa MA. Human bone marrow mesenchymal stem/stromal cells preserve their immunomodulatory and chemotactic properties when expanded in a human plasma derived xeno-free medium. *Stem Cells Int.* 2017;2017:2185351.
- Blazquez-Prunera A, Diez JM, Gajardo R, Grancha S. Human mesenchymal stem cells maintain their phenotype, multipotentiality, and genetic stability when cultured using a defined xeno-free human plasma fraction. *Stem Cell Res Ther.* 2017;8:103.
- Diez JM, Bauman E, Gajardo R, Jorquera JL. Culture of human mesenchymal stem cells using a candidate pharmaceutical grade xeno-free cell culture supplement derived from industrial human plasma pools. *Stem Cell Res Ther.* 2015;6:28.
- Cimino M, Gonçalves RM, Bauman E, Barroso-Vilares M, Logarinho E, Barrias CC, et al. Optimization of the use of a pharmaceutical grade xeno-free medium for in vitro expansion of

- human mesenchymal stem/stromal cells. *J Tissue Eng Regen Med*. 2018;12:e1785–95.
34. Swamynathan P, Venugopal P, Kannan S, Thej C, Kolkundar U, Bhagwat S, et al. Are serum-free and xeno-free culture conditions ideal for large scale clinical grade expansion of Wharton's jelly derived mesenchymal stem cells? A comparative study. *Stem Cell Res Ther*. 2014;5:88.
 35. Al-Saqi SH, Saliem M, Asikainen S, Quezada HC, Ekblad A, Hovatta O, et al. Defined serum-free media for in vitro expansion of adipose-derived mesenchymal stem cells. *Cytotherapy*. 2014;16:915–26.
 36. Simões IN, Boura JS, dos Santos F, Andrade PZ, Cardoso CM, Gimble JM, et al. Human mesenchymal stem cells from the umbilical cord matrix: successful isolation and ex vivo expansion using serum-/xeno-free culture media. *Biotechnol J*. 2013;8:448–58.
 37. Wu X, Kang H, Liu X, Gao J, Zhao K, Ma Z. Serum and xeno-free, chemically defined, no-plate-coating-based culture system for mesenchymal stromal cells from the umbilical cord. *Cell Prolif*. 2016;49:579–88.
 38. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. *Int Soc Cell Therapy pos statement*. 2006;8:315–7.
 39. Oikonomopoulos A, van Deen WK, Manansala AR, Lacey PN, Tomakili TA, Ziman A, et al. Optimization of human mesenchymal stem cell manufacturing: the effects of animal/xeno-free media. *Sci Rep*. 2015;5:16570.
 40. Mojica-Henshaw MP, Jacobson P, Morris J, Kelley L, Pierce J, Boyer M, et al. Serum-converted platelet lysate can substitute for fetal bovine serum in human mesenchymal stromal cell cultures. *Cytotherapy*. 2013;15:1458–68.
 41. Brohlin M, Kelk P, Wiberg M, Kingham PJ. Effects of a defined xeno-free medium on the growth and neurotrophic and angiogenic properties of human adult stem cells. *Cytotherapy*. 2017;19:629–39.
 42. Bobis-Wozowicz S, Kmiolek K, Kania K, Karnas E, Labedz-Maslowska A, Sekula M, et al. Diverse impact of xeno-free conditions on biological and regenerative properties of hUC-MSCs and their extracellular vesicles. *J Mol Med (Berl)*. 2017;95:205–20.
 43. Gottipamula S, Ashwin KM, Muttigi MS, Kannan S, Kolkundkar U, Seetharam RN. Isolation, expansion and characterization of bone marrow-derived mesenchymal stromal cells in serum-free conditions. *Cell Tissue Res*. 2014;356:123–35.
 44. Riordan NH, Madrigal M, Reneau J, de Cupeiro K, Jiménez N, Ruiz S, et al. Scalable efficient expansion of mesenchymal stem cells in xeno free media using commercially available reagents. *J Transl Med*. 2015;13:232.
 45. Xia W, Li H, Wang Z, Xu R, Fu Y, Zhang X, et al. Human platelet lysate supports ex vivo expansion and enhances osteogenic differentiation of human bone marrow-derived mesenchymal stem cells. *Cell Biol Int*. 2011;35:639–43.
 46. Shetty P, Bharucha K, Tanavde V. Human umbilical cord blood serum can replace fetal bovine serum in the culture of mesenchymal stem cells. *Cell Biol Int*. 2007;31:293–8.
 47. Rauch C, Feifel E, Amann EM, Spötl HP, Schennach H, Pfaller W, et al. Alternatives to the use of fetal bovine serum: human platelet lysates as a serum substitute in cell culture media. *ALTEX*. 2011;28:305–16.
 48. Saeedi P, Halabian R, Imani Fooladi AA. A revealing review of mesenchymal stem cells therapy, clinical perspectives and modification strategies. *Stem Cell Investig*. 2019;6:34.
 49. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. *Nat Rev Nephrol*. 2018;14:493–507.
 50. Liu F, Li Y, Bai L, Yang Z, Liao G, Chen Y, et al. Immunomodulatory effects of rhesus monkey bone marrow-derived mesenchymal stem cells in serum-free conditions. *Int Immunopharmacol*. 2018;64:364–71.
 51. Hartmann I, Hollweck T, Haffner S, Krebs M, Meiser B, Reichart B, et al. Umbilical cord tissue-derived mesenchymal stem cells grow best under GMP-compliant culture conditions and maintain their phenotypic and functional properties. *J Immunol Methods*. 2010;363:80–9.
 52. Wuchter P, Vetter M, Saffrich R, Diehlmann A, Bieback K, Ho AD, et al. Evaluation of GMP-compliant culture media for in vitro expansion of human bone marrow mesenchymal stromal cells. *Exp Hematol*. 2016;44:508–18.

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