Advances and Challenges in Serum-Free Culture Systems for Mesenchymal Stem Cells: Toward Clinical-Grade Expansion

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Abstract

Mesenchymal stem cells (MSCs) are advancing rapidly towards clinical translation based on their immunomodulatory and regenerative potential. However, fetal bovine serum (FBS) dependence is still compromising safety, reproducibility, and regulatory clearance. To overcome this, serum-free and xeno-free platforms such as human platelet lysate, recombinant proteins, and chemically defined media (CDM) are strong contenders as alternatives. These media are a step up in safety, minimizing variability, and in compliance with good manufacturing practice (GMP); there are still some issues though. Costliness of recombinant supplements, donor variation in human-source additives, and lack of standardized potency assays are roadblocks in large-scale adoption. New advancements in bioreactors, omics-directed quality control, and artificial intelligence–regulated optimization hold promise for crossing roadblocks. This review is a compilation of state-of-the-art developments, critical analysis of translational roadblocks ahead, and a blueprint for making MSC expansion in serum-free settings scalable, economical, and clinically compliant.

Keywords: Mesenchymal stem cells, Serum-free media, Xeno-free, Clinical-grade MSCs, GMP production, MSC-derived exosomes, Stem cell therapy

Introduction

Mesenchymal stem cells have emerged as a central focus in the past decade of regenerative medicine due to their immunoregulatory property and potential towards clinical use. Besides differentiation potential and self-renewal, MSCs possess high immunomodulatory potential with their ability to orchestrate proliferation and effector function of immune cells such as T cells, B cells, and NK cells [1,2]. Such immunoregulatory roles are carried out through release of numerous soluble factors. Besides that, cell-contact mechanism has also been shown to play a significant role in immunomodulation [2-4]. However, despite their promising therapeutic properties, the stability and reproducibility of MSC functions remain a major challenge, particularly under in vitro culture conditions.

MSC therapeutic function and phenotype are functionally dependent on culture media environmental conditions and can be influenced by culture medium composition [5].

Among the various components influencing MSC culture conditions, FBS remains one of the most critical yet controversial factors [6]. There are chief challenges to FBS use, including batch-to-batch variability, potential contamination, and ethical concerns [7]. These have generated interest in FBS alternatives and serum-free media production [8]. Efforts toward FBS reduction or replacement are science, biosafety, and ethics reasons-motivated [9]. Human platelet lysates (hPL), CDM, and other animal product constituents are alternatives being considered [8]. Animal cell culture

media replacement by animal component-free media increasingly becomes more compelling toward maximizing reproducibility and managing potential protection issue controls in biopharmaceutical production and clinical application [9]. Regulated compliance and product protection issue are driving the world trend toward serum-free, xeno-free, and chemically defined cell culture media production [10].

Given the increasing demand for standardized, xeno-free MSC culture systems, this review sets off a complete description of approaches, latest findings, and challenges towards constructing serum-free culture systems of MSCs. By this, the review also mirrors regulatory, technical, and translational challenges that are required towards developing clinical-grade products of MSCs.

By recently 2025, more than 1,600 MSC-related clinical trials are listed around the globe, covering autoimmune, orthopedic, and cardiovascular applications (ClinicalTrials.gov, keyword: mesenchymal stem cell). MSC biomanufacturing is estimated to exceed a value of over USD 30 billion in 2030 [11], reflecting a pressing need for scalable and standardized culture platforms. Despite developments with serum-free and xeno-free formulations, no standardized, universally applicable platform is currently available. Such a platform would be essential to ensure reproducibility, safety, and cost-efficiency across diverse tissue sources and clinical applications. Differing significantly from past reviews, this paper incorporates recent advances involving omics-based profiling, media optimization using artificial intelligence support, and novel bioreactors within a translational and regulatory framework for an attempt towards an MSC manufacturing roadmap for the next decade.

Foundations of MSC culture

MSCs produce energy mainly through glycolysis, so glucose is consumed rapidly and its depletion has a stronger impact on cells than other nutrients and serum [12]. However, high glucose concentrations accumulate ROS free radicals, causing oxidative stress and promoting cell aging by increasing p16, p21, and p52 gene expression; and activating the mTOR pathway [13]. In the study of Barzelay Aya *et al.* (2020), it was demonstrated that high glucose concentrations (4.5 g/L)

in the culture medium can reduce proliferation, potential differentiation, and increase the rate of aging in adipose-derived MSCs compared to low glucose concentrations (1g/L) [14]. In addition, MSCs cultured in glucose concentrations showed that they can maintain stemness, differentiation potential, reduce cell senescence, and activate PI3K/Akt signaling pathways to increase proliferation and migration [15]. Among 20 naturally occurring amino acids, Higuera *et al.* [16] showed that proliferating MSCs consume 8 essential amino acids and 6 non-essential amino acids, of which glutamine is the most consumed amino acid [16]. Glutamine provides energy for the TCA cycle, protein and nucleotide synthesis. In addition, branched-chain amino acids (valine, leucine, and isoleucine) upregulate the number of cells in S, G2, and M phases, thereby promoting cell proliferation [17]. In addition, fatty acids play an important role in stem cell physiology, involving quiescence and self-renewal states, division ability, and lineage determination [18]. MSCs require exogenously supplied essential fatty acids (such as linoleic and linolenic acids) to enhance growth and differentiation. Typically, essential fatty acids are provided by FBS, however for media that exclude animal products, serum albumin (human, natural or recombinant) can be substituted using diffusion techniques such as liposomes and emulsions [19].

In a serum-free environment, exogenous growth factors supplemented to replace serum signals must adhere to the principles of concentration, timing, frequency, and mode of presentation. In terms of concentration, each growth factor has a distinct response curve. Low doses of TGF-β promote chondrogenic differentiation via Smad2/3, but prolonged administration induces EMT and fibrosis [20]. Timing and frequency are crucial because many factors are only effective within certain "signaling windows"; early PDGF-BB supports migration and angiogenesis, but long-term maintenance can lead to abnormal proliferation; meanwhile, cyclic FGF or Wnt have been shown to be much more effective in maintaining stemness than continuous supplementation [21]. The combination of three factors, TGF-β, PDGF, and FGF-2, has been shown to maintain MSCs proliferation for up to 5 passages without the need for serum [22]. The method of growth factor presentation maintains stable cell signaling. Soluble factors are often degraded by

proteases and are easily dispersed, leading to uncontrolled signaling. In contrast, immobilized growth factors on ECM proteins combined with controlled-release hydrogels help prolong survival time and biological activity, creating a spatial signal gradient that closely resembles the natural microenvironment [23]. Therefore, optimizing a serum-free medium is not only a suitable choice, but also a simultaneous fine-tuning of the dosage, timing, and presentation method in interaction with the signal background, to accurately simulate the natural microenvironment of cells [24].

In addition to their cytoskeletal functions, major components of the ECM, such as fibronectin and laminin, create a microenvironment that directly and indirectly regulates the maintenance, proliferation, self-renewal, and differentiation of MSCs [25]. Fibronectin and laminin are multidomain glycoproteins that support cell adhesion and mechanical signaling, with fibronectin binding to integrins including $\alpha5\beta1$, $\alpha4\beta1$, and $\alpha v\beta3$, and laminin binding to integrins such as $\alpha3\beta1$, $\alpha6\beta1$, and $\alpha7\beta1$ [26]. For example, fibronectin interacts with integrin $\alpha5\beta1$, activates PDGFR-β, the FAK and MAPK/ERK pathways, thereby promoting cell adhesion, proliferation, and facilitating cell migration [27]. Additionally, substrate stiffness influences the ability to direct MSC differentiation via mechanotransduction pathways [28]. ECM stiffness dictates lineage fate-soft matrices (~0.1 - 1 kPa) toward neurogenesis and stiffer surfaces (~10 - 40 kPa) toward myogenesis or osteogenesis via the YAP/TAZ signaling pathway [29].

Microenvironmental conditions also play an important role, with oxygen concentration being one of the determining factors. At hypoxia levels of 1% - 5% O_2, many studies have shown that cells tend to maintain their proliferative capacity and pluripotency, while shifting to a glycolysis metabolic pathway due to the stabilization of HIF-1α. In contrast, when cultured under normoxic conditions, cells are more likely to differentiate and are at risk of senescence [30]. In addition, there is intermittent hypoxia, where the cycle of deprivation and reoxygenation generates bursts of ROS. These ROS pulses act as signals to activate MAPK or NF-κB, supporting proliferation. If they exceed intracellular control, they become a source of oxidative stress, damaging DNA and activating senescence [31]. In addition to oxygen concentration, extracellular pH is also an important factor. Acidified environments affect the way growth factors bind to substrates and receptors, altering extracellular enzyme activity [32]. These findings suggest that culture systems are not only nutrient providers but also effective "programming" environments capable of shaping both the transcriptome and epigenome of stem cells.
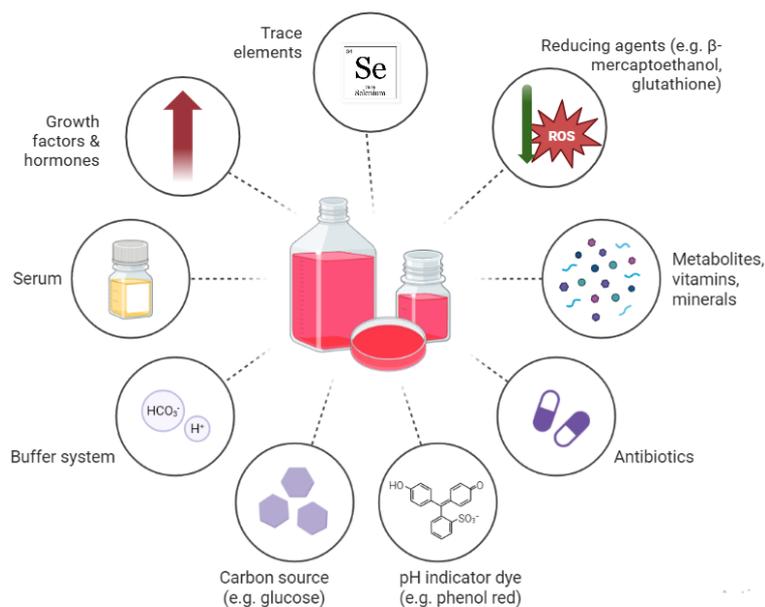
Figure 1 Basic components of animal cell culture medium. Taken together, MSC culture is governed by an interplay of metabolic, biochemical, mechanical, and environmental signals. These parameters not only sustain proliferation but also reprogram the epigenetic and functional landscape of MSCs, underscoring that medium design is effectively a form of biological programming. (Drawn with BioRender).

From FBS to defined alternatives

For more than a half-century of *in vitro* cell culture, including MSC expansion, FBS is the golden standard supplement. It is chosen for its unprecedented capability for cell proliferation support, cell viability support, and cell attachment [33]. Despite that being the case, there are staggering biological, ethical, and translational issues drowning its benefits by a substantial margin. As a field of standardized and clinically significant directions of production continues to improve in regenerative medicine, FBS is no longer an assistant but an obstacle [33].

Traditionally, FBS was the gold standard for MSC culture due to its wide range of growth factors, adhesion proteins, and buffering proteins for proliferation support, cell survival support, and medium stability. However, its use is now marred by significant limitations: Immunogenic xenoproteins, risk for transmission of pathogens, severe batch-to-batch variation, and animal welfare issues in its sourcing [33]. Regulators (FDA, EMA, GMP) increasingly recommend against animal-derived reagents since FBS is not compatible with the needs for standardized, safe, reproducible clinical applications. As a result, serum-free, xeno-free, and CDM alternatives are now available [34]. Serum-free media circumvent animal serum use but retain animal proteins; xeno-free platforms substitute with human-source supplements or recombinant proteins; while CDM offers fully defined media for maximum reproducibility and regulatory acceptability. These platforms improve safety, decrease variation, and maintain MSC potency preservation and migration capability such that FBS elimination is both a technical imperative for clinical-grade MSC production and an ethical imperative due to animal welfare issues [34].
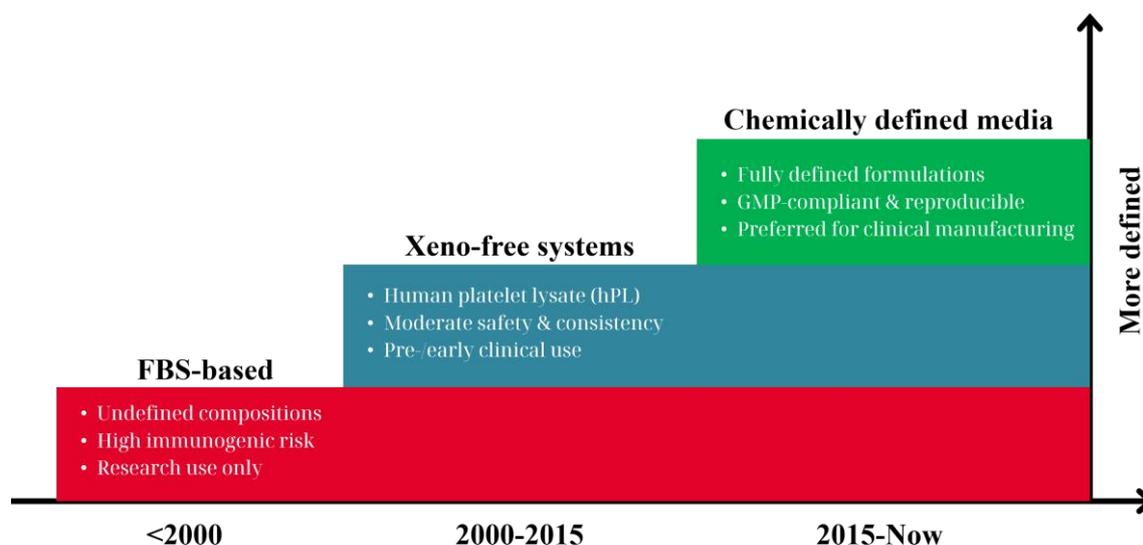
Figure 2 Timeline of MSC culture system development: From FBS to CDM.

Serum-Free Medium (SFM)

SFM definition refers to the medium that non absence of animal serum, such as FBS, calf or horse serum [35]. As its definition, serum-free just can avoid the using of animal serum, but not all the animal-derived components such as bovine insulin, porcine trypsin [36], making high risk of heterologous immune response of clinical application on human.

Xeno-free medium

The term "xeno" originates from the Greek word "xénos", meaning "foreign" or "strange". In the context of cell culture system, xeno-free refers to the absence of components originating from a different species. In the case of human MSCs culture systems, xeno-free media are defined as culture environments that contain no components of animal origin, including proteins, enzymes, or biological additives. These media are not required to be chemically defined [4]. Animal-derived components are replaced by human-derived supplements (such as serum, plasma, or platelet lysate) or by recombinant proteins produced in non-animal expression systems (including bacteria, yeast, or immortalized human cell lines) [5]. The main objective of xeno-free systems is to eliminate the risk of transmitting animal-derived pathogens (e.g., viruses, prions), reduce the potential for xenogeneic immune responses, and enhance the safety profile of cell culture systems intended for clinical applications. Yet, donor-to-donor heterogeneity remains the Achilles' heel of

xeno-free systems, as proteomic and metabolomic analyses consistently reveal batch-dependent shifts in the MSC secretome that complicate comparability across trials

Chemically defined

Chemically defined medium (CDM) refers to a culture system in which all components are precisely known in identity and concentration, with no undefined biological extracts or animal-derived supplements. It excludes serum, hydrolysates, and lysates, aiming to enhance reproducibility, batch-to-batch consistency, and compliance with clinical-grade standards [5] Typically, CDM consists of a defined basal medium (e.g., DMEM/F12), supplemented with recombinant growth factors, hormones (e.g., insulin, transferrin), essential amino acids, trace elements, vitamins, and synthetic lipids (**Table 1**). These components are often produced in non-animal systems to reduce immunological and contamination risks [37] CDM offers tight control over MSC behavior, promoting consistent expansion, phenotype maintenance, and lineage-specific differentiation. However, developing CDM requires extensive optimization, as stem cells may rely on subtle cues from serum or xeno-free additives [37]. Despite this, several studies support its clinical potential. For instance, Li *et al.* [38] formulated a fully CDM that enabled long-term expansion of human MSCs, showing better safety and uniformity than serum- or xeno-free systems. As regulatory demands increase,

CDM is widely recognized as the gold standard for stem cell culture and clinical applications [39].

To compare with conventional media that contain serum, these systems serve more defined conditions so that improving the reproducibility and reducing batch-to-batch variability - which are big concerns in conventional media [40] (**Table 2**). In a preclinical study of Wu *et al.* [41], UCMSCs cultured in serum-free, xeno-free, CDM were less likely to get trapped in the lungs after injection and showed better migration to the kidneys and colon. In contrast, serum-cultured UCMSCs tended to stay in the lungs, limiting their reach to other organs [38]. In terms of feasibility, these systems enable scalable, reproducible, and GMP-compliant manufacturing processes meeting critical requirements for clinical applications and regulatory approval.

In summary, the transition from serum to xeno-free and chemically defined systems represents not only a technical improvement but also an ethical and regulatory imperative. The critical question now is whether these alternatives can reproducibly preserve MSC potency, genomic stability, and therapeutic function - criteria that will determine their ultimate clinical adoption.

Emerging strategies and components in serum-free culture

Human-derived supplements

Human platelet lysates is a blood-derived product obtained through the lysis of human platelets by various methods, including freeze-thaw cycles, activation by Ca^{2+}, thrombin, or ultrasound [42,43]. hPL is particularly rich in growth factors such as PDGF, IGF-1, TGF-β, VEGF, EGF, and bFGF, which collectively enhance cell proliferation, migration, differentiation, and immunomodulation [44,45]. In addition, hPL contains chemokines such as RANTES, CXCL1, CXCL2, and CXCL3; soluble adhesion molecules including sCD40L, VCAM-1, and ICAM-1 [46], and cytokines such as IL-1, IL-6 [44], all of which support cell adhesion, migration, and signaling regulation. Moreover, hPL comprises a substantial proportion of proteins such as albumin and immunoglobulins, which contribute to the stabilization of the culture environment and provide essential nutrients for cell maintenance [44].

Human platelet lysates has emerged as a promising xeno-free supplement to replace FBS in cell culture media, particularly in the large-scale expansion of MSCs intended for clinical applications (**Table 2**). Culture media supplemented with hPL has been shown to significantly promote MSC proliferation, exhibiting shorter doubling times and higher population doublings compared to FBS-based cultures [47]. In blood banks, platelet concentrates typically have a short shelf life of 5 to 7 days, despite retaining their biological functionality beyond this period [48]. Utilizing expired platelet units to produce hPL not only addresses concerns associated with FBS such as the risk of zoonotic pathogen transmission, immune responses to xenogeneic antigens, and animal welfare issues - but also contributes to waste reduction and aligns with circular bioeconomy principles [44]. However, like FBS, hPL is challenged by batch-to-batch variability in composition. Two main factors affect the quality and performance of hPL: Donor-related characteristics (such as age, sex, blood type) and manufacturing parameters (like lysis technique, storage conditions). These variables can affect platelet concentration, residual plasma content, and levels of growth factors and fibrinogen in the resulting culture medium [49,50].

To address the challenges above, standardizing hPL manufacturing processes is essential to ensure safety and consistency in clinical-grade cell culture applications. Delabie *et al.* [51] demonstrated that pooling multiple individual hPL samples into a single batch can reduce inter-donor variability in composition. However, pooled preparations may increase the risk of transmitting blood-borne pathogens from different donors, necessitating stringent screening protocols, robust donor management, and traceability systems. Activated hPL preparations often retain residual fibrinogen, which can induce coagulation in the culture medium. To prevent this, heparin is commonly added; however, as pharmaceutical-grade heparin is typically derived from porcine sources, it may not only suppress cell proliferation but also elicit immunogenic responses upon clinical application [52]. An alternative approach involving platelet activation with low concentrations of Ca^{2+} and glass beads has been shown to efficiently deplete fibrinogen while circumventing the need for heparin. This method also offers broader applicability across different platelet sources [53].

In addition, umbilical cord plasma (UCP) and autologous serum (AS) are human-derived supplements considered safe alternatives to FBS for the culture of MSCs. UCP contains platelet-derived growth factors and exhibits higher glucose levels, alongside lower concentrations of lactate, glutamate, alanine, and branched-chain amino acids compared to FBS [54]. These characteristics contribute to enhanced cell proliferation, adhesion, morphological maintenance, and the preservation of multilineage differentiation potential [55,56]. However, UCP is exclusively derived from neonatal umbilical cord blood, and its composition varies considerably among donors. In contrast, AS is obtained from the individual's peripheral blood, thereby minimizing the risks of immune incompatibility and cross-contamination. Studies have shown that supplementing culture media with 10% AS supports MSC isolation and expansion with comparable efficacy to FBS [57]. Thaweesapphithak *et al.* [58] further reported that MSCs proliferate more rapidly in AS-supplemented media compared to those cultured with FBS.

Human-derived supplements such as hPL, UCP, and autologous serum have emerged as practical alternatives to FBS, offering improved safety and compatibility with clinical translation. However, donor heterogeneity, batch-to-batch variability, and residual risks of pathogen transmission remain unresolved, limiting their acceptance as universal GMP-grade solutions.

Recombinant and synthetic components

This transition from serum-containing to xeno-free and serum-free systems necessitates the substitution of undefined serum components with synthetic and recombinant proteins. Besides offering reproducibility and safety, such components are GMP-compliant to generate clinical-grade MSCs.

Growth factor supplements are the cornerstone of recombinant protocols. Recombinant FGF-2 is a powerful mitogen, delaying senescence and initiating proliferation through ERK/PI3K-Akt pathways[59]; PDGF-BB initiates migration and angiogenesis through PDGFR chemotaxis [60]; IGF-1 extends lifespan and maintains stemness through IGF1R–MAPK signaling [61]; EGF activates mesenchymal and neural progenitor division [62]; and ascorbic acid diminishes oxidative

stress and enhances ECM alignment [63]. Their activities are listed in **Table 1**, where each growth factor is paired with its application concentration, MSC effect, and dominant signaling pathway. Nutrient and synthetic additions like ITS (Insulin-Transferrin-Selenium), B27, and N2 deliver standardized micronutrients, trace components, and antioxidants to support MSC homeostasis [64]. Their well-characterized formulation circumvents batch-to-batch variability engineered into animal-derived sera. Analogously, vitamins (e.g., ascorbic acid, vitamin E, selenium) and polyamines maintain proliferation, genomic integrity, and collagen biosynthesis [63] (**Table 1**). Small molecules and epigenetic modulators carry out fine-tuning of MSC performance. Valuable examples include valproic acid (VPA), to initiate proliferation by histone deacetylase (HDAC) inhibition, lithium chloride, to initiate Wnt/β-catenin activation and osteogenesis, and rapamycin to maintain autophagy and initiate senescence delay [65,66]. These drugs, when mixed in defined low concentrations, permit fine tuning of MSC phenotype and therapeutic potential (**Table 1**, "Small Molecules/Epigenetic Modulators").

Together, recombinant and synthetic components offer not only direct control of MSC activity but also scalability and approbability to regulatory bodies. Their worth has been validated experimentally in the clinic: Jia *et al.* [67] demonstrated that combined bFGF and EGF at 10 ng/mL optimally enhanced fibroblast proliferation and collagen expression for pelvic floor tissue regeneration; Hanley *et al.* [68] described that GMP-compliant xeno-free media with recombinant factor supplementation reached therapeutic MSC doses by 14 days' culture [68]. Liu *et al.* [69]'s study showed that the characteristics of the medium, including DMEM/F12, 1xITS, 0.5% human serum albumin, 10 ng/mL bFGF and 100 ug/mL L-ascorbic acid, also showed similar results to the medium containing FBS, but the expression of MMP2, VEGF, KGF, TGF-β, IGF-I and PDGF genes was enhanced, showing the potential of SFM to be replicated in clinical practice. Another study by Leonardo Solmesky et al. showed that: The medium supplemented with EGF and bFGF alone combined with retinoic acid was sufficient for bone marrow MSC cell growth compared to the traditional medium [70].

PPRF-msc6 was cultured in DMEM:Ham's F-12 (1:1) and supplemented with key biological components including L-glutamine (4.0 mM), lipid concentrate (0.1% v/v), sodium bicarbonate (20.5 mM), HEPES (4.9 mM), insulin (4.01 μM), transferrin (0.318 μM), putrescine (55.9 μM), progesterone (17.8 nM), fetuin (1.0 mg/mL), hydrocortisone (100 nM), L-ascorbic acid-2-phosphate (197.6 μM), human serum albumin (4.0 mg/ml), along with growth factors such as bFGF (2.0 ng/mL) and TGF-β1 (1.0 ng/mL). The results showed that hMSCs cultured in PPRF-msc6 had significantly shorter PDT during the period from P1 to P10, with mean values of 25.7 ± 3.2 h (BM1), 20.6 ± 1.9 h (BM2), and 20.8 ± 1.8 h (BM3), respectively. Meanwhile, cells cultured in FBS–DMEM medium had longer PDTs, 38.2 ± 7.2, 35.4 ± 2.6, and 36.3 ± 4.8 h,

respectively, indicating that PPRF-msc6 medium had superior efficacy in promoting hMSCs proliferation [71].

Recombinant growth factors, synthetic nutrients, and small molecules offer the greatest reproducibility and regulatory acceptability, directly addressing the variability inherent in serum or hPL. Yet, the high cost of recombinant proteins and the incomplete replacement of serum-derived complexity continue to restrict their widespread use, underscoring the need for hybrid systems and cost-reduction strategies.

Table 1 is not offered here as a catalogue but rather as a blueprint for a rational medium design-combining recombinant growth factors, synthetic nutrients, and small-molecule modulators into serum-free systems of improved safety, consistency, and clinical translatability.

Table 1 Comprehensive list of biochemical, nutritional, and physical modulators influencing MSC culture in serum-free/xeno-free conditions (Source: Authors).

Category	Factor/ Compound	Application conditions	Effects on MSCs	Main mechanism	References
Growth Factors	FGF-2 (bFGF)	10 - 20 ng/mL	Increases Proliferation, Decreases Senescence	ERK/PI3K-Akt activation	[59]
	PDGF-BB	Synergistic with FGF/TGF	Increases Migration, Increases Proliferation, Angiogenesis	PDGFR-mediated chemotaxis	[60]
	TGF-β1	~0.1 ng/mL	Increases ECM, Osteo-/Chondrogenesis, Decreases Adipogenesis	SMAD3 phosphorylation, RhoA activation	[72]
	IGF-1	10 - 100 ng/mL	Increases Proliferation, Survival	IGF1R-mediated PI3K/Akt, MAPK	[61]
	VEGF	10 - 50 ng/mL	Increases Angiogenesis, Vascular Support	VEGFR2-mediated PI3K/Akt, ERK	[73]
	HGF	10 - 50 ng/mL	Increases Migration, Anti-fibrotic	c-Met activation, Anti-fibrotic pathways	[74]
Cytokines / Immunomodulators	IL-6	Low levels / paracrine	Increases Immunomodulation, Increases Angiogenesis	STAT3/MAPK activation	[75]
	IL-10	Therapeutic conditioning levels	Increases Immunosuppression, Decreases Inflammation	STAT3 activation, Anti-inflammatory	[76]
	PGE2	Physiologic levels	Increases Immunosuppression, Increases Migration	EP2/EP4 → cAMP/PKA	[77]

Category	Factor/ Compound	Application conditions	Effects on MSCs	Main mechanism	References
ECM Components	Fibronectin	Surface coating	Increases Adhesion, Spreading, Cytoskeletal Organization	α5β1 integrins →FAK signaling	[78]
	Laminin	Coating (basement membrane mimic)	Increases Neural/Myogenic Differentiation	α6β1integrins →YAP/TAZ modulation	[79]
	Fibrin / Fibrinogen	Scaffold / coating	Increases Adhesion, Osteo- /Chondrogenesis	Integrin-mediated FAK/YAP activation	[80]
Nutrients & Vitamins	Vitamin C (AA, AA-2G)	50 - 200 μM	Increases Proliferation, DecreasesROS, Increases Collagen	Cofactor for hydroxylases, Antioxidant	[63]
	Vitamin E	Antioxidant supplement	Antioxidant protection	Direct ROS scavenging	[81]
	Selenium	With bFGF or as trace element	Increases Proliferation, Maintains Potency	Antioxidant enzyme support	[64]
Small Molecules / Epigenetic Modulators	Valproic Acid (VPA)	Low μM	Increases Proliferation, Epigenetic Modulation	HDAC inhibition	[65]
	Lithium Chloride (LiCl)	Low mM	Activates Wnt/β-catenin, IncreasesOsteogenesis	G5K3β inhibition	[66]
	5-aza-dC (Decitabine)	Low μM	DNA demethylation, Modulates Differentiation	DNMT inhibition	[82]
	Rapamycin	Low nM-μM	mTOR inhibition, Increases Autophagy, Decreases Senescence	mTOR inhibition → Autophagy	[83]
Bioactive Molecules	Keratinocyte Growth Factor (KGF)	10 - 20 ng/mL	Increases Epithelial Interaction, Wound Healing	FGFR2b activation	[84]
	Polyamines (Putrescine, Spermidine)	Physiologic levels	Increases Proliferation, DNA/RNA Stability	DNA/RNA stabilization	[85]
	Amino Acids (Arginine, Lysine)	Balanced in media	Supports ECM, Nitric Oxide Balance	Collagen synthesis, NO modulation	
Physical / Environmental Factors	Hypoxia (1% - 5% O_2)	1% - 5% O_2	Maintains Stemness, Decreases Senescence	HIF-α stabilization	[86]

Category	Factor/ Compound	Application conditions	Effects on MSCs	Main mechanism	References
	Mechanical Cues (Stiffness, Stretch)	0.1 - 40 kPa stiffness / cyclic stretch	Guides Differentiation	Integrin tension, YAP/TAZ	[87]
	Electrical Stimulation	Low voltage / patterned	Increases Neural Differentiation	Voltage-gated modulation	[88]
	PEMF (Pulsed Electromagnetic Field)	~75 Hz, 2 mT	Increases Osteogenesis, Increases ALP/BMP2 Expression	Ca^{2+}-dependent signaling	[89]

Table 2 Comparative summary of MSC culture supplements (Source: Authors).

Criteria	FBS	hPL	Recombinant/Synthetic
Origin	Animal-derived (fetal bovine)	Human-derived (platelet concentrate)	Recombinant proteins/ synthetic molecules
Composition	Undefined, variable mix of proteins, growth factors	Semi-defined, rich in growth factors and cytokines	Fully defined and quantifiable
Batch-to-batch consistency	Low	Moderate (dependent on donor and preparation method)	High
Immunogenicity risk	High (xenogeneic proteins)	Lower (but alloantigenic risk exists)	Minimal (no biological contaminants)
Pathogen transmission risk	Moderate to high	Low to moderate (requires blood screening)	Minimal (GMP-grade production with QA/QC control)
Ethical concerns	Significant (animal welfare, fetal extraction)	Moderate (human donor consent, blood usage)	None
GMP compliance potential	Poor	Moderate to high (if standardized)	High
Scalability and cost	Low cost, widely available	Moderate cost; source-limited	High cost (recombinant proteins), scalable with bioprocessing
Regulatory acceptability	Limited (discouraged in clinical-grade production)	Increasing (used in many clinical studies)	Preferred (aligned with FDA/EMA requirements)
Functional support for MSCs	Proliferation, attachment, differentiation support	Comparable or superior to FBS in proliferation and immunomodulation	Controlled support with tunable effects on MSC behavior
Genomic and epigenetic stability	Low	Moderate	High
Lineage-specific differentiation control	Non-specific	Moderate	High (customizable)

Criteria	FBS	hPL	Recombinant/Synthetic
Secretome/exosome purity	Low (contaminated vesicles)	Moderate	High
Compatibility with 3D/bioreactor culture	Limited	Moderate	High
In vivo biodistribution after injection	Pulmonary trapping	Improved	Best (targeted migration)
Industrial-scale supply reliability	Low	Medium	High

Commercial serum-free/xeno-free media

In the study of Carmelo *et al.* [90], despite the use of the same culture medium (StemPro® MSC SFM Xeno Free), there was a significant difference in population doubling time between the 2 culture methods. Additionally, the manufacturer of StemPro® MSC SFM Xeno Free reported that hBM-MSCs cultured long-term on CELLstart-coated flasks using this medium show a similar growth rate compared to those maintained in DMEM supplemented with 10% FBS [91], showing no significant improvement in proliferative capacity compared to the conventional medium.

Another factor making variation in PDT between studies is coating method. In the study of Nguyen *et al.* [92] conducted on several different coating methods (CELLstart™ CTS™ and Autologous Serum), and they also pointed that MSCs without coating showed highest PDT value, indicating the poorest proliferation [92]. Additionally, a study of Chen *et al.* [93] also indicated that different donors making different PDT values, even

when using the same culture medium. Consistently, PDT varied notably across age groups, with the most distinct differences observed in donors younger than 10 years, suggesting that donor age influences cell proliferation rates [94]. Yang *et al.* [95] showed that cells in MesenCult-XF medium were smaller in size, retained their originality, and had higher immunoregulatory characteristics on T lymphocyte cell lines than the control group using FBS. Lucas G Chase's results were similar, but also pointed out that the SFM components included PDGF-BB, bFGF, and TGF-β1 [96]. SFM offers improved batch consistency, safety, and GMP compliance compared to serum-supplemented media [97]. It enhances MSC proliferation, maintains differentiation potential, and preserves anti-inflammatory properties [97]. SFM cultivation results in lower cellular senescence, reduced immunogenicity, and higher genetic stability compared to FBS-containing media [98].

Table 3 Summary of commercial media for MSC culture and expansion (Source: Authors).

No.	Name of product	Manufacturer	Defined level	MSC source compatibility	PDT		Clinical using	Price/mL of complete medium (*)
1	StemPro® MSC SFM Xeno Free	Thermo Fisher Scientific	Serum-free, Xeno-free, Chemically defined	hBM	45 - 60 [91]	25.8 - 72.5 [90]	Research use only	~$1.06
				hUC	38.63 - 53.03 [99]	26.9 - 29.5 [100]		
				hAD	36 [90]	18.48 [90]		
2	Mesencult-XF	STEMCELL Technologies	Serum-free, Xeno-free, Chemically defined	hBM	38 [101]	24.8 - 40.5 [102]	Research use only	Not publicly available
				hUC	31.2 - 40.8 [38]	39 - 53 [93]		
				hAD	19.6 - 47 [102]	38.72 [103]		
3		Miltenyi Biotec	Serum-free, Xeno-free,	hBM	30 [104]	43 - 53 [105]	Research use only	~$1.03

	StemMACS MSC expansion media kit XF		Chemically defined	hUC	18.3 - 25.16 [106]	25 [92]		
				hAD	31.51 - 39.83 [106]	30 [107]		
4	StemFit For Mesenchymal Stem Cell	Ajinomoto Bio-Pharma Services	Serum-free, Xeno-free, Chemically defined	hBM	36 [108]	N/A	Clinical use	~$1.34
				hUC	24 [108]			
				hAD	40 [108]			
5	TheraPEAK™MS CGM™	Lonza	Serum-free, xeno-free, chemically defined	hBM	52.8 - 62 [109]	50 [101]	Research use only	$0.96
				hUC	60 - 72 [110]	N/A		
				hAD	N/A			
6	RoosterNourish MSC-XF	RoosterBio	Xeno-free, chemically defined	hBM	50.6 [111]	36 - 40 [112]		~$0.68
				hUC	N/A			
				hAD	30 [112]	44 - 48 [113]		
7	MSC NutriStem XF	Sartorius AG	Serum-free, xeno-free, chemically defined	hBM	30 - 34 [104]	25 [114]		~$0.80
				hUC	50 [115]	N/A		
				hAD	N/A			
8	MSCCult1 medium	Regenmedlab, Vietnam	Serum-free, xeno-free, chemically defined	hUC	15.32 ± 0.44 [116]		For research or production	~$0.65
9	ADSCCult1 medium	Regenmedlab, Vietnam	Serum-free, xeno-free, chemically defined	hAD	17.15 ± 0.56 [116]		For research or production	~$0.6

N/A: Not available; (*) This data not included coating materials.

For customers utilizing these media, cost is also a significant consideration. **Table 3** provides a comparison of the cost-effectiveness of different MSC culture media. MSCCult I and ADSCCult I are the most economical ($0.65/mL and $0.6/mL), while StemFit is the most expensive ($1.34/mL) due to its ready-to-use format, and all components were fully disclosed. TheraPEAK™ MSCGM™ offers good value among complete media ($0.96/mL). Mid-range options like StemPro® and StemMACS cost around $1.03 - 1.06/mL. Pricing varies based on formulation and supplement volume, emphasizing the need to balance cost with performance and clinical requirements (**Table 3**).

Although almost these commercial products were approved by many safety standards such as GMP, FDA, Lots of concerns over undisclosed ingredients still occur. Firstly, concerns originated from the misunderstanding of terms: chemically defined and fully disclosed. Companies refer their products as "chemically defined" in compositions but "proprietary formulation", so users couldn't verify about what's in it. Proteins or peptides that are not disclosed could affect cell behavior and may pose clinical safety concerns, such as triggering unintended immune responses [117]. Besides, some proprietary supplements such as plant hydrolysates may carry harmful by-product or allergenic contaminants [118,119]. In summary, regardless of whether the proprietary components have positive or negative effects, they may contribute to variability and instability in study outcomes. Collectively, emerging strategies highlight a trade-off: human-derived supplements provide biological relevance but remain variable, recombinant components ensure reproducibility but are costly, and commercial products promise GMP

alignment yet face barriers in transparency and adoption. Bridging these approaches through hybrid, cost-effective, and omics-guided systems will be key to future clinical translation.

Biological outcomes of serum-free cultivation

Although removal of animal-derived constituents of them FBS obviates central objections concerning immunogenicity, transfer of infection, as well as ethical fitness, it does at the same time pose essential issues with regards to replacement medium capacity of maintaining MSC biological functionalities. These inherent qualities include proliferative potential, multipotency, immunomodulatory capacity, as well as genomic and epigenetic fidelity - each one of these being central in definition of therapeutic effect as well as safety of interventions with MSCs.

Regarding standards, cultured MSC products are usually determined according to GMP standards before treatment on humans. Specifically, these factors include: endotoxin, sterility, MSC markers and differentiation ability based on ISCT guidelines, karyotype, DBT, and other methods to test MSC functions (such as immunoregulatory ability, aging and apoptosis rate). MSCs cultured in a serum-free environment must retain their good properties and capabilities, at least not statistically different from the standard serum environment [120]. Only then can the serum-free environment be considered for application in clinical production [121]. Throughout, serum-free/xeno-free culture systems have immense promise in maintaining the biological integrity of MSCs, with due care in fine-tuning their formulations and orientation towards functional output. Proliferation, differentiation, immunomodulation, and genomic integrity-being multifactorial parameters affected not only by basal composition of medium but also prime regimen, passage number, as well as donor variability-can only undergo standardization using omics-based profiling as well as with predictive biomarkers of therapeutic effect. Standardizing assays of potency, along with omics-based profiling, as well as establishing predictive biomarkers of therapeutic effect will be most fruitful in ensuing times. Only with such meticulous

characterization will serum-free systems realize their complete potential in enabling the safe, reproducible, as well as scalable manufacture of MSCs for regenerative medicine (**Table 4**).

Studies have shown that MSCs can accumulate genetic alterations, primarily telomeric deletions, during culture [122]. DNA methylation changes also occur, with genes associated with aging and tumorigenesis tending to become demethylated over time [123]. However, the overall genomic stability of MSCs appears to be maintained throughout culture, despite transient clonal aneuploidies [98]. Epigenetic modifications play a crucial role in MSC differentiation and fate determination [124]. Culture conditions can impact genomic stability, with autologous serum showing a stronger tendency to maintain unmethylated states compared to FBS [122]. These findings highlight the importance of monitoring genetic and epigenetic alterations in MSCs before clinical application [123].

Potency assays are still the greatest bottleneck for translating serum-free MSC products to the clinic. Traditional assays (that is, T-cell suppression, angiogenesis, and tri-lineage differentiation) generate functional readouts, but are laborious, not well standardized, and frequently lack in vitro-in vivo predictive capability [125]. That limitation is exacerbated under serum-free and xeno-free conditions, where fine variations in nutrient content or supplement source can re-define the MSC secretome and product therapeutics [125]. Next-generation assays involving senescence, apoptosis, and secretome profiling offer more immediate signals of cell fitness but still fail to reflect MSC heterogenicity. Here, omics-based profiling has become a game-changing method for establishing in vitro predictive biomarkers for potency [126]. These technologies not only allow early detection of drift in afferent culture but are also consistent with regulatory requirements for reproducibility, comparability, and functional relevance. Down the line, integration of omics-defined biomarkers with functional assays in a standardized, GMP-compatible pipeline is imperative. Such multi-layer potency testing will form the crux for confirming serum-free MSC products for safety-, efficacy-, and globally-regulatory-acceptance (**Table 4**).

Table 4 Comparative summary of potency assays for MSCs under serum-free culture (Source: Authors).

Category	Assay	Readout	Advantages	Limitations	Relevance to Serum-Free systems
Classical functional assays	T-cell proliferation inhibition	Suppression index (%)	Direct immunomodulatory measure; simple	Labor-intensive; donor-dependent	Critical for MSCs intended for immunotherapy
	Mixed lymphocyte reaction	IFN-γ / IL-2 secretion	Physiological mimic of allo-response	Variability across donor PBMCs	Useful for regulatory comparability
	Angiogenesis assays (tube formation with HUVECs)	Branch points, tube length	Models paracrine pro-angiogenic activity	In vitro model, limited in vivo correlation	Important for wound healing, ischemia indications
	Tri-lineage differentiation (osteo/chondro/adipo)	ALP, Oil Red O, Alcian blue staining	Widely accepted ISCT criterion	Not predictive of clinical efficacy	Baseline QC, but insufficient alone
Advanced assays	Senescence markers (SA-β-gal, p16^{INK4a}, p21)	% senescent cells	Links culture stress to therapeutic decline	May not capture functional capacity	Needed to compare FBS vs serum-free culture
	Apoptosis/resistance (Annexin V/PI)	% apoptotic cells	Rapid, standardized	Snapshot only, not predictive	Reflects culture stress from SFM
	Secretome profiling (ELISA, multiplex cytokine panels)	IL-6, PGE2, TGF-β, VEGF levels	Direct link to paracrine potency	Requires robust normalization	Reveals how SFM alters paracrine output
	Extracellular vesicle characterization	NTA, proteomics, miRNA content	Reflects regenerative "cargo"	Technical standardization lacking	Key for MSC-EV product pipelines
Omics-based & predictive biomarkers	Transcriptomics (bulk RNA-seq, scRNA-seq)	Pathway activation, subpopulation maps	Captures heterogeneity, mechanistic insight	High cost; data interpretation complex	Identifies serum-free media effects at single-cell level
	Epigenetic profiling (DNA methylation signatures)	Stemness vs aging-related methylation patterns	Predictive of potency decline	Not standardized yet	Aligns with regulatory push for predictive biomarkers
	Proteomics/metabolomics	Secretome signatures, energy metabolism	Global functional fingerprint	Needs GMP-compatible pipelines	Strong for QC & comparability studies

Challenges and future directions in serum-free MSC manufacturing

In spite of the potential of SFM for growing MSCs under clinical conditions, several inherent limitations prevent large-scale application in GMP-compliant production. Foremost among these is recombinant growth factor and chemically defined supplement cost, the major discouragement to commercial-scale production [127]. Even though SFM reduces the immunogenicity and risk of contamination of animal components, the introduction of a new set of sources of variability occurs, most notably when human-derived supplements like platelet lysate or umbilical cord plasma are employed - owing to donor heterogeneity and inter-lot and inter-operator variation in processing protocol [128]. Scaling-up from 2D optimized

monolayer cultures to 3D bioreactors still remains technically challenging and requires tuning of the shear stress, diffusion of nutrients and aggregation kinetics [129]. No commonly standardized measure of potency assay for MSCs is yet established other than primary phenotyping and tri-lineage differentiation, and cross-study reproducibility and regulatory clearance are therefore impaired. No universally standard, totally defined formulation to cover sources of MSC has been realized despite developments from improved recombinant technologies and DoE–based media optimization [129].

The upcoming developments will need to focus on generating xeno-free, chemically defined systems that are not only biologically efficient but economical, scalable, and compatible with regulatory and biosafety procedures. Bioreactor platforms involving microcarriers could perhaps provide solutions to high-density culture of MSCs with real-time process control and cellular identity and functionality maintenance [130,131]. In addition, artificial intelligence and machine learning are transforming cell manufacturing to facilitate predictive modeling of the best culture conditions from omics data, and to maximize speed of development and reproducibility [132]. Customized MSC culture systems for individual patient factors such as status of the disease, immunity, or age are another area of innovation in alignment with the vision of precision medicine. In tandem, these technological and conceptual breakthroughs predict a transformative leap in MSC production-i.e., of reproducibility, precision, and patient-specific fine-tuning [133]. Bioengineering, computational modeling, and vision of regulation each will be the passport to the complete translational potential of serum-free therapies of MSCs.

Three converging technologies in the future are poised to overcome current hurdles in serum-free MSC production. First, next-generation bioreactor systems consisting of vertical-wheel, hollow-fiber, and microcarrier-based technologies allow for high-density expansion within controlled shear stress, diffusion of nutrients, and real-time measurement [134]. Such systems hold promise for filling the gap between flasks used for lab-scale expansion and clinically meaningful batch production while maintaining cellular identity and potency. Second, quality control directed by omics promises a route towards standardization of potency assays. Single-cell transcriptomics, epigenomics for epigenetic profiling, proteomics, and metabolomics allow for predictive biomarkers reflecting heterogeneity and drift during culture while linking MSC product characterization with regulatory requirements [135]. Third, artificial intelligence and machine learning are poised for use in linking such large-scale datasets for describing complicated cell-environment behavior, predicting favorable culture conditions, and pushing towards "quality by design" adoption in GMP pipelines [120]. Through a combination of bioreactor scalability, omics-mediated functional readouts, and AI-mediated optimization, next-generation MSC production can take a step towards reproducible, economic, and personalized production. Such systems level integration is not only a technical upgrade but also a conceptual shift in how serum-free MSC products are developed, validated, and translated towards clinical therapies.

Conclusions

Conversion of MSC biomanufacturing from serum-dependent to xeno-free and chemically defined conditions is a paradigm shift for regenerative medicine. Despite continued issues-including high cost of recombinant factors, donor heterogeneity, scalability constraints, and standard potency assay deficiency-cross-disciplinary technologies are emerging rapidly in line to overcome such impediments. Out of these, advanced bioreactor platforms enable expanded high-density as well as GMP-compatible expansion; omics-mediated profiling enables standardization of quality control using predictive biomarkers; and machine learning enables optimisation of culture conditions using data-informed approaches. These three pillars in combination furnish a roadmap for reproducible, cost-effective, and personalized MSC products. As a next step, convergence of bioengineering, systems biology, and computational modelling shall become essential for clinically compatible, scalable, and patient-specific therapies. Through such technologies, serum-free MSC expansion is not only in a position to replace conventional FBS-dependent approaches but shall become a cornerstone technology for next-generation regenerative medicine.

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CRediT Author Statement

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