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Review article

Secretome-based acellular therapy of bone marrow-derived mesenchymal stem cells in degenerative and immunological disorders: A narrative review

Fatema Tuz Zohora [a], Mansur Aliyu [b,c], Ali Akbar Saboor-Yaraghi [b,*]

[a] Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Malaysia
[b] Department of Immunology, School of Public Health, Tehran University of Medical Sciences, International Campus, TUMS-IC, Tehran, Iran
[c] Department of Medical Microbiology, Faculty of Clinical Science, College of Health Sciences, Bayero University, Kano, Nigeria

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ABSTRACT

The bone marrow (BM) plays a pivotal role in homeostasis by supporting hematopoiesis and immune cells' activation, maturation, interaction, and deployment. "BMSC-derived secretome" refers to the complete repertoire of secreted molecules, including nucleic acids, chemokines, growth factors, cytokines, and lipids from BM-derived mesenchymal stem cells (BMSCs). BMSC-derived secretomes are the current molecular platform for acellular therapy. Secretomes are highly manipulable and can be synthesised in vast quantities using commercially accessible cell lines in the laboratory. Secretomes are less likely to elicit an immunological response because they contain fewer surface proteins. Moreover, the delivery of BMSC-derived secretomes has been shown in numerous studies to be an effective, cell-free therapy method for alleviating the symptoms of inflammatory and degenerative diseases. As a result, secretome delivery from BMSCs has the same therapeutic effects as BMSCs transplantation but may have fewer adverse effects. Additionally, BMSCs' secretome has therapeutic promise for organoids and parabiosis studies. This review focuses on recent advances in secretome-based cell-free therapy, including its manipulation, isolation, characterisation, and delivery systems. The diverse bioactive molecules of secretomes that successfully treat inflammatory and degenerative diseases of the musculoskeletal, cardiovascular, nervous, respiratory, reproductive, gastrointestinal, and anti-ageing systems were also examined in this review. However, secretome-based therapy has some unfavourable side effects that may restrict its uses. Some of the adverse effects of this modal therapy were briefly mentioned in this review.

1. Introduction

Due to their distinctive proliferative, regenerative, and differentiating abilities, stem cell studies have demonstrated that BMSCs can treat immunological and degenerative diseases. By giving hematopoietic stem cells (HSCs) a controlled environment, the BMSCs also host hematopoiesis. BMSCs are thus one of the most incredible medicinal breakthrough [1]. Various bioactive molecules directly secreted by BMSCs as soluble factors or through vesicular fractions/extracellular vesicles (EVs) are known as "BMSC-derived secretomes (BMSCs-secretomes)". Depending on size, surface characteristics, and density, BMSC-derived EVs are further classified as

* Corresponding author.
E-mail addresses: fatema.zohora@monash.edu (F.T. Zohora), maliyu.med@buk.edu.ng (M. Aliyu), asaboor@tums.ac.ir (A.A. Saboor-Yaraghi).

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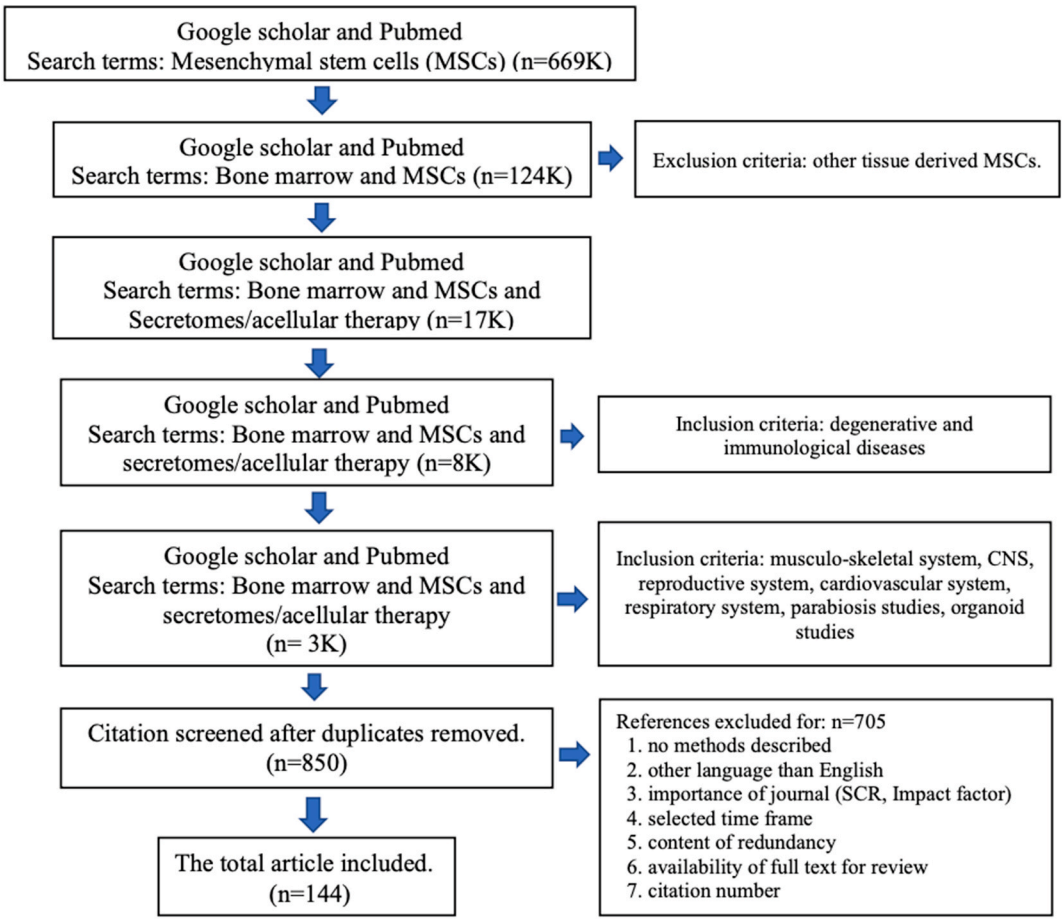

Fig. 1. Flow chart of the literature selection process for this review.

exosomes, microvesicles, or apoptotic bodies. BMSCs-secretomes are made up of proteins (growth factors, cytokines, chemokines, transcription factors, ferments), lipids (eicosanoids), and free nucleic acids (DNA, RNA, siRNA, mRNA, miRNA) [2]. By focusing on multiple genes through post-transcriptional repression or degradation and on targeting different effector proteins of multiple signalling pathways, BMSC-secretomes have exhibited exceptional regenerative, antiapoptotic, antifibrotic, immunosuppressive and immunoregulatory properties in the domains of cell therapy, regenerative medicine, and the management of immunological dysregulations.

Therefore, BMSC-secretome, as a golden cell-free therapy, is being studied in the treatment of dermatological diseases (hair loss/alopecia, skin wounds, antimicrobial effects on skin wounds) [3], skeletal muscle degeneration (atrophic muscle disorders and muscle injuries) [4], intervertebral disc changes caused by trauma and degeneration [5], sepsis-induced cardiomyopathy [6], chemobrain/chemofog [7], spinal cord injury [8], neurodegenerative diseases [9], premature ovarian failure (POF) [10], liver diseases [11], cardiovascular diseases [12,13], and bone diseases [14].

Furthermore, given the unique capabilities of BMSC-secretomes for supporting the BM long-lived plasma cells (LLPCs), researchers further hypothesised that BMSC-secretome might be a significant source of long-lasting immunity against life-threatening infections, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). Recent studies further indicated that BMSC-exosome-derived miRNAs might suppress the inflammatory PANoptosis process by targeting specific PANoptosis-related genes, thereby minimising tissue damage and anomalies in the systemic inflammation [15]. Furthermore, due to their critical immune-regulatory capacity in preserving the integrity and homeostasis of the mucosal barrier, BMSCs-secretome-supported innate lymphoid cells (ILCs) types 2 and 3 have demonstrated new potential in allergen immunotherapy and intestinal implantation [16,17].

Despite their potential for treating a wide range of degenerative and immunological diseases, little is known about the biological types and morphological traits of BMSC- secretomes, their *in vitro* cultural properties with manipulating agents, isolation techniques, and delivery platforms to enhance their functional efficacy in different tissues and organs. Moreover, recently reported therapeutic components of BMSC-secretomes and their potentialities in various degenerative and immunological diseases of the musculoskeletal, CNS, cardiovascular, respiratory, reproductive, and digestive systems are understudied. This review will summarise the biological, structural and functional strategies of different types of BMSC-based secretomes and their components, their laboratory-based production with functional modifications, and their standard isolation, characterisation and delivery techniques for optimal effect. This review will also look at the advantages and disadvantages of BMSC-secretome-based acellular therapy compared to stem cell therapy

Table 1
Secretomes with their cellular sources and biological functions within the BM microenvironment.

BM cells	Secretomes	Biological functions	References
Osteoblast	Granulocyte-macrophage colony stimulating factor (GM-CSF), Granulocyte-colony stimulating factor (G-CSF), Leukaemia inhibitory factors (LIFs), Tumor necrosis factor-α (TNF-α), Transforming growth factor-β (TGF-β), Interlekine-6 (IL-6), C-X-C Motif Chemokine Ligand 12 (CXCL12), Wnt, A*lkaline phosphatase* (ALP), Receptor activator of nuclear factor kappa-B ligand (RANKL), Collagen alpha-1(I) chain Col1a, Osteocalcin, MicroRNAs (miR), Lipocalin2	1. Communication within cells of the BM 2. Skeletal homeostasis and mineralisation 3. Apoptotic regulation and endocytosis, 4. Immune cells activation, maturation, and immune regulation	[19–21]
Osteoclast	miR-324, TGFβ, *Fibroblast growth factors* (*FGFs*), Proteases	1. Regulation of osteogenic differentiation 2. Bridge between osteoclasts and MSCs.	[22]
Megakaryocytes	S100 calcium-binding protein P	1. Osteoclast differentiation and bone resorption activity	[23]
Myoblast	miR-196a-5p	Suppress osteoclast formation	[24]
Osteocytes	Sclerostin	Anti-anabolic and inhibitory effects on bone formation	[25]
Fibroblast	Peirostin, *Plasminogen activator inhibitor* 1 (PAI-1), Laminin, Collagen, Proteoglycans, Fibronectin	Maintain ECM structure and functional homeostasis	[20]
Endothelial cells	Lipoprotein receptor-related protein-1 (LRP1), CXCL12, Stem cell factors (SCFs), Angiopoietin	1. Osteoclastin induction 2. Regulate insulin sensitivity 3. HSCs-maintenance	[26]
Adipocytes	Angioprotein4, TEK receptor tyrosine kinase, Kruppel like factor 5 (KLF5), Insulin-like growth factor 2 (IGF2), Insulin receptor substrate 2 (IRS2), Activin A receptor type 2A (ACVR2A), TGF-β, Dipeptidyl-peptidase 4 (DPP4), Bone morphogenic protein (BMP), RUNT-related transcription factor 2 (RUNX2), Nuclear factor-κβ (NF-κβ), Lipocalin2, SCFs, Chemerin	1. Transdifferentiation of osteoblasts and bone remodelling 2. Cholesterol metabolism and lipolysis, 3. Immune cell regulations, maturation, and chemotaxis 4. Apoptotic regulation 5. Functional homeostasis of hormones and chemokines 6. Degradation of incretins (gut hormones) 7. Blood cell formation 8. Maturation and self-maintenance of HSCs	[25,27]
MSCs	**miRNAs:** miR-15, miR-16, miR-17-92, miR-19/19a, miR-21, miR-21a-5p, miR-22, miR-23a/b, miR-30b, miR-30d-5p, miR-31, miR-92a-3p, miR-122, miR-124a, miR-125a/b, miR-126/126a, miR-128, miR-133, miR-133b/133b-3p, miR-135b, miR-140, miR-141, miR-142-3p, miR-145, miR-146a/b, miR-147, miR-148a/148b-3p, miR-155, miR-181/181c/181-5p, miR-199a/b, miR-210, miR-221, miR-223, miR-224, miR-295, miR-320c, miR-340, miR-344a, miR-375, miR-378, miR-379, miR-423-3p, miR-451, miR-485-3p, miR-495, miR-532-5p, miR-564, miR-872-3p,miR-1587, miR-let-7b, miR-hCO1/2 **Immunomodulation:** *Sphingosine-1-phosphate* (*S1P*), Thrombospondin-1 (*TSP1*),TGF-β, IL-1a, IL-1b, IL-6, IL-8, IL-10, IL-12, Forkhead box P3 (FOXP3), *Human leukocyte antigen G* (*HLA-G*), Galectin 1/9, Nitric oxide (NO), Vascular endothelial growth factor (VEGF), Prostaglandin E2 (*PGE2*), Krüppel-like factor 3 antisense RNA 1 (KLF3-*AS1*), Annexin, Indoleamine 2,3-dioxygenase (*IDO*), IGF-1R **Angiogenesis:** CXCR4, *Hepatocyte growth factor* (HGF), VEGF, Hypoxia-inducible factor 1-alpha (HIF-1a), Transcription factor 4 (TCF4), Hairy and enhancer of split-1 (HES1), Protein kinase B (PKB), Cluster differentiation (CD)105, Angiopoietin 1 (*Ang1*) **Chemokines:** CXCL1, CXCL2, CXCL6, CXCL8, CXCL9, CXCL10 CXCL16, CXCL20, C–C motif chemokine ligand 2 (CCL2), CCL5, C–C motif chemokine receptor 2 (CCR2), Defensin alpha 1 (DEFA1), Interferon-induced transmembrane (IFITM) **Cell adhesion and binding:** Calmodulin-like protein 5 (CALML5), Dolichyl-diphosphooligosaccharide–protein glycosyltransferase non-catalytic subunit (*DDOST*), G protein subunit alpha I3 (GNAI3), Transmembrane protein 119 (TMEM119), HLA-A, Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), Integrin subunit beta 3 (ITGB3), Mammalian class I myosin (MYO1), Gap junction protein alpha 1 (GJA1), Matrix metalloproteinase-14 (MMP14), GLI pathogenesis related 2 (GLIPR2), Ras-associated binding protein 23 (RAB23), Staphylococcal nuclease and tudor domain containing 1 (SND1), Plexin-B2 (PLXNB2), Plastin 3 (PLS3), Extended synaptotagmin-1 (ESYT1), PDZ and LIM Domain 5 (PDLIM5), CD29,CD44,CD49 A/C, CD59, CD166 **Degradation:** 20s proteasome member, CD10, CD13	1. Proliferation and differentiation osteoblast, inhibit osteoclast formation and bone loss 2. Regulation of BMSC's functions 4. Regulation of stem cell fate and differentiation, lineage regulation, and angiogenesis 5. Immunoregulation, tissue repair, and wound healing	[25, 28–32]

(*continued on next page*)

Table 1 (*continued*)

BM cells	Secretomes	Biological functions	References
	Adipogenesis: KLF Transcription Factor 7 (KLF7), CCAAT enhancer-binding protein alpha (CEBPA) **Extracellular matrix and protein-protein interaction:** Aldolase, Fructose-Bisphosphate C (ALDOC), Fibronectin, Galectin-1, Actinin Alpha 1 (ACTN1), Collagen Type VI Alpha 1 Chain (COL6A1/3), Aminopeptidase N (ANPEP), Filamin C (FLNC), Vesicle Amine Transport 1 (VAT1), MMP14, Hemoglobin Subunit Beta (HBB), Low-density lipoprotein receptor–related protein-1 (*LRP1*), FLNB, Vimentin (VIM), CD95 **Apoptotic regulation:** POU Class 3 Homeobox 1 (POU3F1), SP1, GATA-4, Wnt4, CD73 **Growth factors:** FGF, Keratinocyte growth factor (KGF), Platelet-derived growth factor-D (PDGF-D), Glial Cell Line-Derived Neurotrophic Factor (GDNF), HGF, Ciliary neurotrophic factor (CNTF) **RNA binding:** MyoD family inhibitor domain containing (MDFIC), Argonaute RISC Catalytic Component 2 (AGO-2), *Nuclear receptor interacting protein 1* (*NRIP1*)		

and the most recent developments in the therapeutic applicability of various components of BMSC-secretomes in treating a wide range of regenerative and immunological disorders in humans.

2. Literature search for the present review

A literature search was performed for the present study. It included two databases, PubMed and Google Scholar, using several search terms: 'mesenchymal stem cells', 'bone marrow', 'secretomes', 'acellular therapy', 'degenerative diseases', 'immunological diseases', 'parabiosis' and 'organoids'. (Fig. 1).

3. Secretomes within the BM microenvironment: a molecular platform for acellular therapy

BM-HSCs and endosteal niches are rich in osteoblasts, osteoclasts and supporting cells such as endothelial cells, adipocytes, macrophages, fibroblasts, and MSCs, which form a complex communication network with BM-secretomes to maintain functional crosstalk and homeostasis within the BM microenvironment [18]. The cellular sources and biological roles of BM-secretomes that contributed to the BM microenvironment and functional homeostasis are shown in Table 1. The secretome-derived bioactive compounds control gene expression and mediate cellular communication, cell adhesion, migration, proliferation, and differentiation.

3.1. Manipulation of MSC-secretomes for enhanced biological effects: recent advancements

MSCs-secretomes are manipulable. Several priming methods have been proposed to increase MSCs' activity, survival, and therapeutic efficacy [33] (Fig. 2), here highlights several approaches.

3.1.1. MSCs priming with hypoxia increase angiogenesis and reduce apoptosis

Oxygen concentrations ranging from 0 to 5% promotes MSCs-secreted angiogenic factors like VEGF-A, and IL-8, rwhich are regulated upon activation of normal T cells expressed and presumably secreted (RANTES), IGF-1, and monocyte chemoattractant protein-1 (MCP-1), and significantly downregulates immune mediators, thereby increasing the regenerative potentiality of MSCs in various degenerative diseases [34]. Moreover, under hypoxia, MSCs priming with IFN-γ also upregulated pro-angiogenic genes, including TIMP metallopeptidase inhibitor 1 (TIMP-1), vascular cell adhesion protein-1 (VCAM-1), mesenchymal-epithelial transition factors (MET), HIF-1α, and IL-8. It also downregulates the anti-angiogenic genes IGFBP-1 and TIMP-4, resulting in a valuable cell source with super angiogenic properties [35]. In addition, hypoxic-MSCs-derived secretomes highly expressed activating transcription factor 4 (ATF4), which further upregulates autophagy-related 5 (ATG5) and autophagy-associated microtubule-associated protein 1 light chain 3 beta (MAP1LC3B) genes, resulting in autophagosome formation and phagophore expansion to enhance autophagy, thereby reducing apoptosis [36].

3.1.2. MSC-priming with cytokines or mediators for immune regulation

This is a valuable strategy for harvesting MSC-exosomes for immune therapy. For instance, cytokine-primed MSC-exosomes showed increased expression of TNF-α-induced protein 3 (TNFAIP3) and TNF-stimulated gene 6 (TSG-6) -anti-inflammatory molecules, thereby negatively regulating T cell proliferation [37]. It is reported that the IFNγ-primed-MSCs-secretome significantly secreted more than 60 cytokines and 240 miRNAs, which were involved in chemotaxis, immune cell migration, extracellular matrix remodelling, M2 macrophage polarising, chondroprotective, tissue regeneration, and anti-inflammation [38]. Moreover, IFN-γ and TNF-α priming was associated with adhesion, immunogenicity and migration features regulated by MSC-derived immunomodulatory factors, including

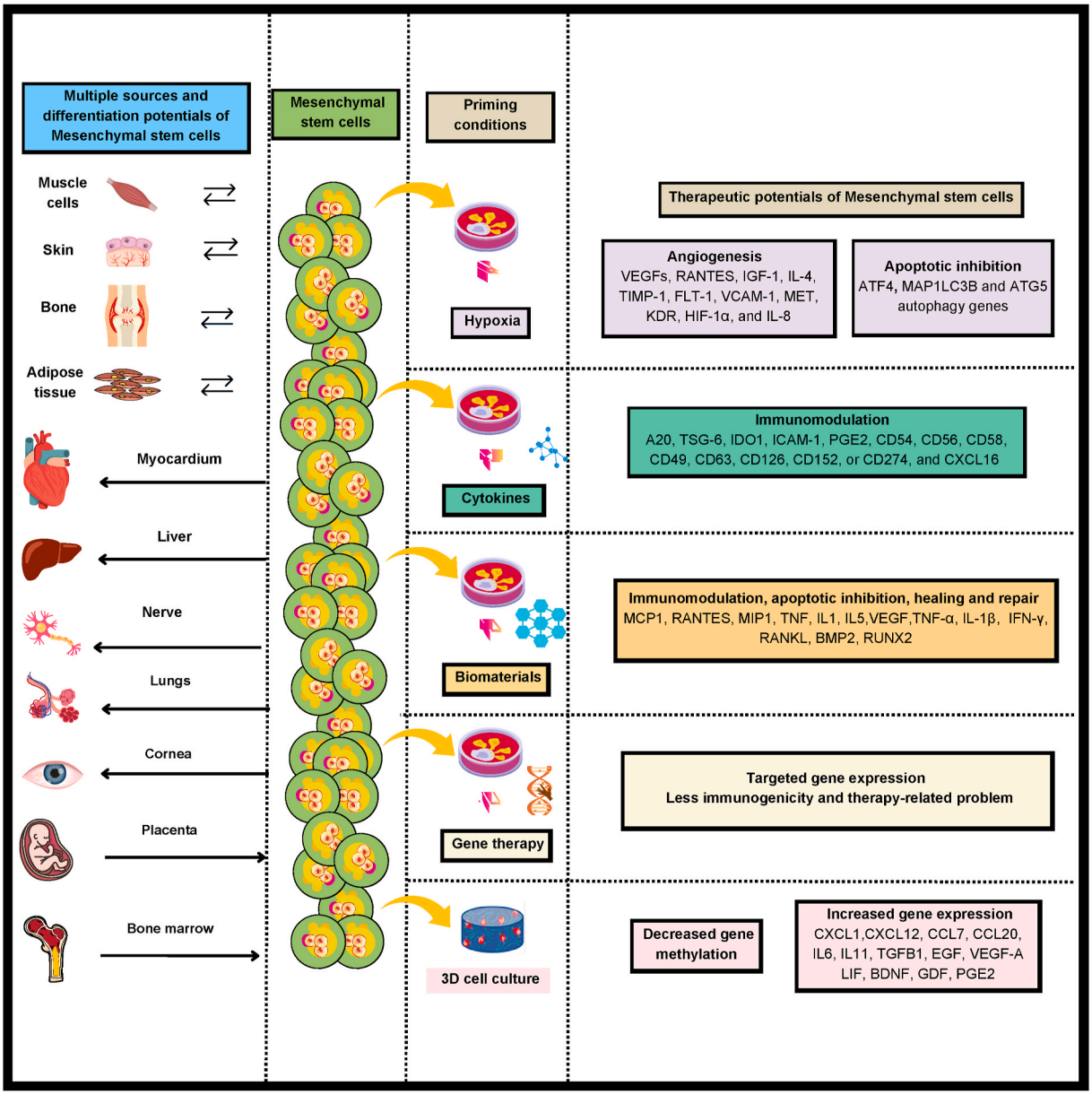

Fig. 2. Displays a visual presentation of the MSCs' biological characteristics. Tremendous potential and therapeutic usefulness lie in the fact that MSCs can be produced from and differentiated into diverse cellular lineages. In addition, MSCs are modifiable and can be primed with various laboratory-based cultural conditions such as hypoxia, immune modulators, biomaterials, gene delivery, and 3D culture to improve therapeutic efficacy and clinical feasibility. MSC-priming has thus become the standard of care in regenerative medicine, gene therapy, and immunotherapy.

IDO, intercellular adhesion molecule 1 (ICAM-1), PGE2, CD49, CD54, CD56, CD58, CD63, CD126, CD152, or CD274, and CXCL16 [39, 40]. In addition, TGF-stimulated MSCs-exosomes exhibited more extracellular matrix (ECM) genes, collagens, and elastins to enhance fibroblast proliferation and migration during wound healing [41].

3.1.3. MSCs priming with biomaterials

This has been used as an innovative therapy for treating complex diseases. For instance, EVs produced from MSCs loaded on biphasic calcium phosphate biomaterial have treated maxillofacial bone abnormalities. Since these EVs secrete MCP1, RANTES, MIP1, TNF, IL-1, IL-5, and VEGF cargo, they dramatically boost the transition from M1 to M2 polarised macrophages, which in turn modulate macrophage-induced inflammation and repair responses [42]. Moreover, MSC-exosomes loaded with polypeptide hydrogel scaffold provide sustained release of exosomes to inhibit the production of inflammatory TNF-α, IL-1β, and IFN-γ and to upregulate the expression of IL-6, arginase (Arg-1) and IL-10 *in vitro*; which induce anti-inflammation, cell migration, and tissue regeneration *in vitro*

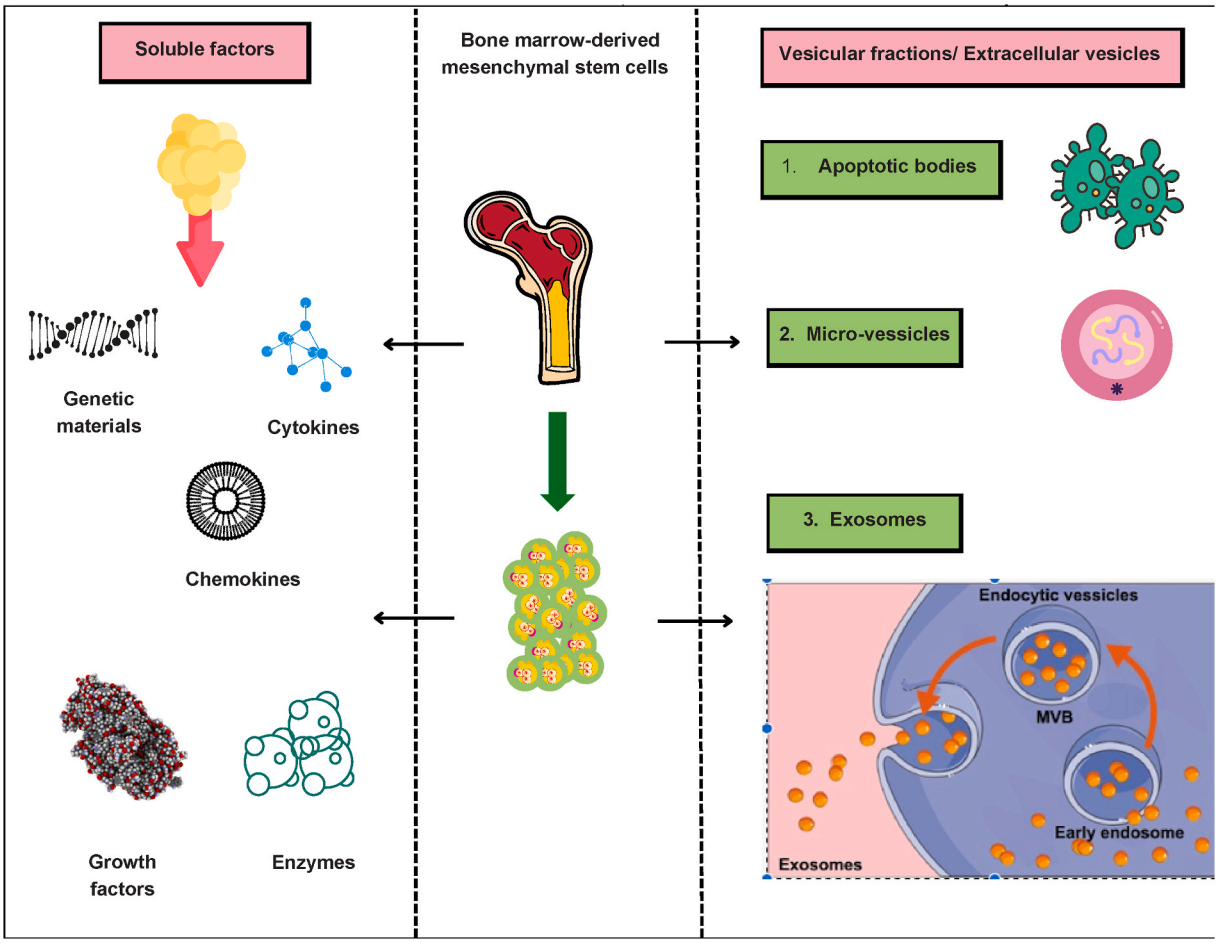

Fig. 3. The secretome of BMSCs contains a wide variety of bioactive molecules (growth factors, inflammatory mediators, enzymes, and genetic materials) and vesicular fractions consisting of apoptotic bodies, microvesicles and exosomes.

[43]. Additionally, the priming of BMSCs with biphasic calcium phosphate granules (BCP) was reported to enhance the expression of RANKL, BMP2, RUNX2, IL-10, and VEGF to suppress inflammatory IL-6 and IL-8 genes *in vitro*, showing enhanced effects of BCP on the pro-healing activities of MSCs [44]. Likewise, MSC-laden porous gelatin micro-carriers generated resilient and soft micro-tissues, which dynamically regulated the IL-17-CXCL1 axis and neurotrophin-3 (NT-3)-mediated phosphorylation of collapsing response mediator protein-2 (CRMP2); thereby reducing cell apoptosis, glial cell infiltration, and Nissl body degradation, and increasing the neurotrophic factors to promote neuronal regeneration in spinal cord injury [45].

3.1.4. MSCs primed with gene therapy

The unique qualities of MSCs and gene therapy are combined in MSCs primed with gene therapy to provide a simple cell supply with targeted gene expression and low immunological rejection, tumorigenesis, and therapy-related issues [46]. Recent research using a nonhuman primate model of Parkinson's disease found that MSCs engineered to express three critical genes guanosine triphosphate cyclohydrolase 1 (GCH1), tyrosine Hydroxylase (TH), and aromatic L-amino acid decarboxylase (AADC) for dopamine synthesis and provide a continuous striatal supply of dopamine, resulted in significant and safe improvements in motor and non-motor functions that lasted at least 51 months [47]. Moreover, functionally engineered BMSCs-EVs with miR-424 showed advanced cytoprotection, reduced microglial activation, suppressed IL-6, IL-1β, MCP, and reactive oxygen species (ROS) production, ameliorating ischemia and neuronal death in the rat retina [48].

3.1.5. MSCs in 3D culture

3D culture is a potent priming strategy to improve therapeutic efficacy and transcriptome profiles. 3D-culturing of highly expanded MSCs induces the expression of immunomodulatory, anti-inflammatory and therapeutic genes *in vitro* [49]. RNA-sequencing of these 3D MSC spheroids identified the top 3000 elevated genes linked to cellular growth, differentiation, angiogenesis, and immune modulation in 3D cultures compared to 2D [50]. 3D MSC-spheroids also showed decreased methylation status with more secretory patterns of 16 genes including CXCL1, CXCL12, CCL2, CCL7, CCL20, IL-6, IL-11, TGF-β1, EGF, VEGF-A, brain-derived neurotrophic

Table 2
Comparison between different types of MSCs-derived EVs [2,52,53,55,56].

Properties	Large EVs	MVs	Exosomes
Biogenesis	The appearance of protrusions and blebs in the plasmatic membrane, as well as the release of apoptotic bodies	Outward budding of extracellular membrane	Plasma membrane internally budding and developing into multivesicular bodies
Cell type	Dead cells	Live cells	Live cells
Production requirement	Rho-associated protein kinase and the plasma membrane channel Pannexin	Calcium-dependent proteins e.g., aminophospholipid translocases, scramblases, and calpain	ESCRT, ALIX, TSG101, vacuolar protein sorting-associated protein 4 and SNAREs, ceramides and tetraspanins
Fate and release	Determined by intracellular apoptotic factors	Determined by intracellular calcium levels	Determined by intracellular cholesterol levels and cellular homeostasis
Diameter	50–100 μm	200–1000 nm	30 to 200 nm
Surface markers	Express annexin V, phosphatidylserine, and thrombospondin bio-markers	ARF-6, CD40 ligand, vesicle-associated membrane protein 3, selectins, integrins, secondary metabolites, nucleic acids, such as mRNA, miRNA, and non-coding RNAs, lipids, and proteins	CD9, CD63, CD81, flotillin, tumor susceptibility 101, Alix, and the endosomal sorting complex necessary for transport-3

Table 3
Comparison between existing techniques for EVs isolation [59–64].

Techniques	Advantages	Disadvantages
Ultracentrifugation	1. Gold standard 2. Handle large volume	1. The low recovery rate (~25%) 2. Time-consuming and laborious 3. High g force, the low structural integrity of EVs and aggregation of EVs 4. Deposition of soluble factors of secretomes
Ultrafiltration	1. Size uniformity 2. Faster than ultracentrifugation 3. Need no special equipment	1. Low yield 2. Clogging and blockage of the membrane and trap EVs
Size exclusion and Antibody-based capture mechanisms	1. Useful techniques for purity 2. Have returned with good results	1. Low throughput 2. Requires final-concentration step 3. High costing
Microfluidic technique	1. Simultaneous isolation and molecular characterization 2. Less time consuming 3. Precise manipulation of fluids 4. Low consumption of sample and reagents 5. High level of integration, sensitivity and selectivity	1. Lack of purity, as exosome overlaps with other particles in size and surface markers 2. Single exosome heterogeneity cannot be addressed 3. Need multiple biomarkers panel for specificity and sensitivity 4. Complicated equipment, difficult to operate
Anion-exchange chromatography	1. High purity 2. One-step EV isolation, less time consuming (3 h/1 lit cell supernatant) 3. Suitable for larger cell culture volume 4. Intact EVs with minimal protein contamination	1. Elution dynamics may be affected by cell lines and the membrane composition of EVs 2. Need optimization and modification
Commercialised rapid isolation kits	1. Simple precipitation method 2. Widely employed 3. Can separate EVs easily from soluble factors of secretome	1. Poor purity 2. Non-exosome contaminants
Capture-based (Immunoaffinity and magnetic beads) methods	1. Useful for specifically marked EVs 2. High purity	1. Less employed 2. Only for exosome with targeted proteins 3. High reagent cost 4. Need cell-free sample
Polymer-based methods	1. Easy and convenient operation 2. Quick procedure	1. High expenditure 2. Unstable quality of kits

factor (BDNF), PGE2, HGF, growth differentiation factor 15 (GDF15), leukaemia inhibitory factor (LIF), and BMP2 [50]. In addition, EVs derived from 3D MSC-spheroids reported 6.7 times more secretion capacity and altered surface markers of MSCs than 2D cultures. Correspondingly, 3D MSCs-spheroids cultured with IFN-γ and TNF-α produced nine-fold more EVs than 2D culture without a priming [51]. Therefore, the secretomes produced from preconditioned MSCs play crucial roles in stimulating cell proliferation and wound healing, migration, angiogenesis, and suppressing inflammation, apoptosis, and fibrosis.

3.2. Organization, isolation and characterisation of the BMSCs-secretomes: recent updates

The BMSC-derived soluble factors, large EVs, ectosomes/microvesicles (MVs), and exosomes, all of which have distinct features that may make them valuable therapeutic tools. Imune-regulating chemicals, cytokines, chemokines, and growth factors are significant among the soluble fractions (Fig. 3).

3.2.1. BMSC-large EVs

They contain (i) apoptotic bodies (50–100 μm in diameter, liberated by dying cells, and expressing thrombospondin, annexin V, and phosphatidylserine bio-markers); (ii) oncosomes (1000 nm in size, originate from cancer cells, and express ARF2, annexin A1/A2, and cancer-specific biomarkers); (iii) DNA, coding and non-coding RNA, proteins, lipids, nuclear fractions, and organelles [2,52].

3.2.2. BMSC-MVs

They are medium-sized EVs with an average size of 200–1000 nm that express ARF 6, VAMP3, selectins, integrins, CD40L, mRNA, miRNA, non-coding RNAs, secondary metabolites, lipids, and proteins. MVs protrude from the plasma membrane and convey the parent cell's cytoplasm [52,53].

3.2.3. BMSC-exosomes/nanovesicles

Exosomes/nanovesicles originate from the endocytic pathway and have a 30–200 nm size range. They express CD9, CD63, CD81, flotillin, TSG101, Alix, soluble NSF attachment protein receptors (SNAREs) and ESCRT biomarkers. Exosomes are generated when the endosomal membrane invaginates into the multivesicular body (MVB), merges with the cytoplasmic membrane, and is liberated into the extracellular space [2,52,53].

In contrast, three distinct EVs can also be distinguished based on their densities: low-density (1.01–1.06 g/cm3), medium-density

Table 4
There are super therapeutic advantages of BMSCs-secretome-based acellular therapy over stem cell transplantation in clinical applications.

Properties of secretome	Therapeutic benefits of secretome	References
Cell-free soluble molecules and Nano- and micro-sized EVs	Break cellular boundaries and transduce signals, no emboli formation; aim to ensure cell survival	[65]
Expression of fewer cell surface proteins	Less immunogenicity, no graft rejection	[66]
Ready to use secretory therapies	Lowers the extremely high cell counts required for transplantation, time saving, cost effective, less phenotypic changes, and therapeutic potentials	[67]
Suitable for dynamically controlled laboratory conditions and can be modified easily	Higher production rates with great therapeutic efficacy and higher biosafety	[68,69]
No invasive cell collection procedures	Promising cell-free therapy that is more affordable and useful in clinical settings	[68]
No toxic cryo-protectant agents	Can be safely and securely stored in the operating room without losing any of its effectiveness.	[65,70]
Safety, dosage and potency can be easily calculated like conventional pharmaceutical agents	More reproducibility and scalability for clinical applications	[68,70]

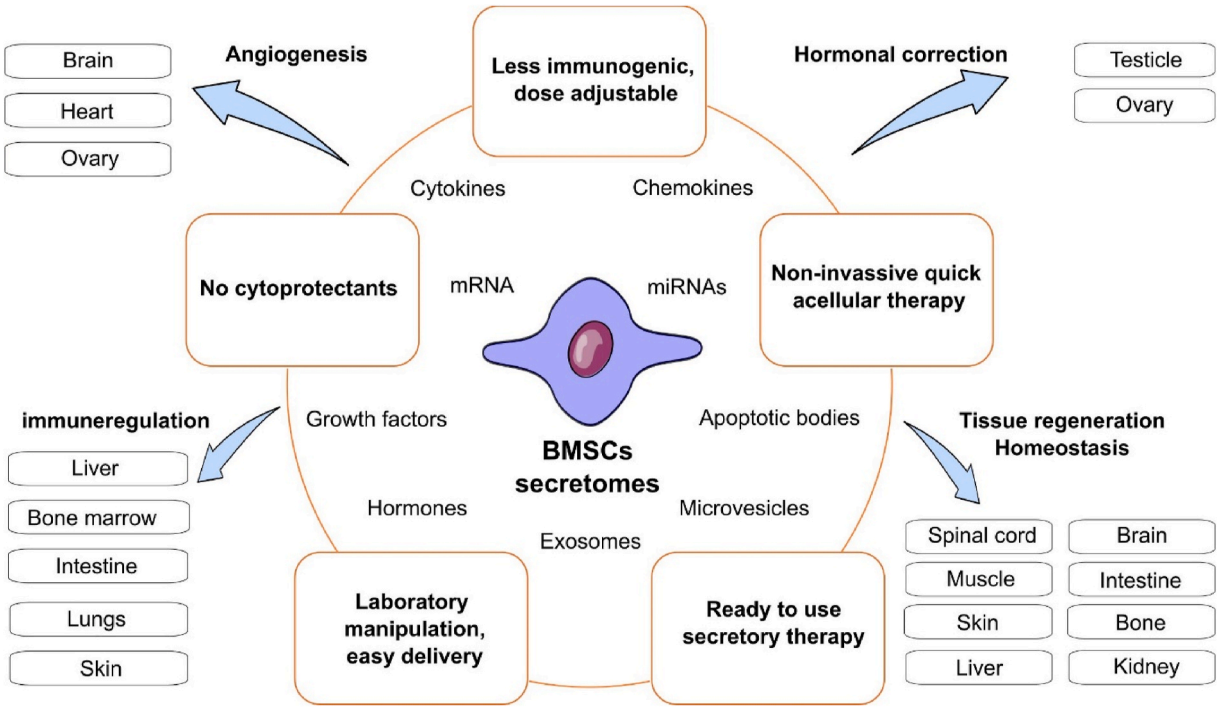

Fig. 4. Illustrations of BMSC-derived secretomes' therapeutic potential and clinical utility in various tissues and organs. The superiority of BMSC-derived secretomes as a non-invasive, ready-to-use, and less immunogenic-tumorigenic therapy indicates that they may treat multiple human disorders.

(1.08–1.14 g/cm3), and high-density (1.16–1.28 g/cm3). These discrepancies in density were also linked to their surface markers [54]. Table 2 represents a comparative analysis between apoptotic bodies, MVs and exosomes.

3.2.4. Isolation of BMSC-derived secretomes

BMSCs are obtained via a positive selection technique based on the cells' ability to adhere to tissue-culture plastic and survive in an FBS-supplemented medium. Therefore, BMSCs' "conditioned medium" (CM) contains both fractions of MSC-secretomes. A vastly complex secretome-isolation method results from the fact that the quantity and type of secretomes depend on the MSCs' culture period or growth phase [57,58]. Modern science has already established several approaches for secretome isolation, some of which are outlined in Table 3 below.

3.2.5. Characterisation and delivery

Flow cytometry, nanoparticle tracking analysis (NTA), resistive pulse sensing (RPS), and transmission electron microscopy (TEM) have been introduced for the analysis of the morphology, subpopulations, and bio-markers of MSC-exosomes [61]. BMSC-secretomes are most often given systemically. Intravenous administration generally causes excessive liver and spleen stem cell secretome build-up, requiring several injections with an increased dosage for therapeutic efficacy. Therefore, researchers are developing various

Table 5
Bioactive components of MSC-secretomes with their molecular roles and functional activities in treating different human diseases.

Bio-active compounds	Biological activities	Functional dynamics	Diseases	References
2-arachidonoylglycerol (2AG) and palmitoylethanolamide (PEA)	1. Initiation of inflammatory responses 2. Initiation of anti-inflammatory responses	1. TNFα-2AG treatment enhanced the secretion of inflammatory lipids, prostaglandin E2, cyclooxygenase-2 (COX2), and nitric oxide (NO) production 2. PEA down-modulated the TNFα-mediated secretion of prostaglandin E2, cyclooxygenase 2 (COX2), and NO	Osteoarthritis	[123]
miR-497-5p	1. Inhibition of myocardial damage and apoptosis and increase cell survival	1. miR-497-5p directly bound with CircRTN4 and upregulated MG53 repair protein in myocardiocytes	Myocardial damage	[78]
Exosomal-miRNA cargoes	1. Restoration of mitochondrial antioxidant status and parkin-dependent and -independent mitophagy	1. Increased Parkin, PINK1, ULK1, BNIP3L, FUNDC1, LC3B, UCP2, and MnSOD genes in cardiac muscles 2. Regulated the pAKT, PI3K, hypoxia, VEGF, and NF-κβ signaling pathways	Non-alcoholic steatohepatitis-cardiotoxicities	[13]
miR-199b-3p miR-125a-5p	1. Induction of M2 macrophage-mediated angiogenesis 2. Activation of anti-inflammatory responses 3. Improved ultimate load, tensile modulus and stiffness of the repaired tendon 4. Increased endothelial cell migration, collagen deposition and maturation 5. Reduced risk of ossification	1. Modulated M1-to-M2 macrophage polarization 2. Upregulation of CD31, eNOS, and KDR genes in endothelial cells 3. Significantly increased VEGF expression	Tendon injury	[124]
BMSC-secretomes	1. Neurogenesis 2. Neuroprotective effects	1. Upregulation of neuronal markers like βIII-tubulin, paired box-6 (Pax6), MAP2a 2. Increase the number and expression of autophagy markers like Beclin, LC3a/b, and Atg7	Oxidative stress-induced loss of neurogenesis	[125]
MSC-derived exosomes	1. Formation of neo-epithelial cells, deposition of extensive collagen fibres, and ECM remodelling 2. Effectively promote keratinization	1. Regulated Wnt/β-catenin signaling pathway 2. Increased expression of cyclin D3 and N-cadherin	Diabetic wound healing	[126]
miR-125a and miR-29a	1. Differentiation of cartilage tissues 2. Inhibition of apoptosis 3. polarization of macrophages to the M2 phenotype	1. Inhibited IL-1β activities 2. Upregulation of M2 macrophage-related genes (*Arg-1, CD163, and CD206)* 3. Downregulated NF-κβ and NLRP3	Cartilage injury repair	[127]
Bio-active compounds	**Biological activities**	**Functional dynamics**	**Diseases**	**References**
miR-22-3p	1. Inflammation inhibition in podocyte 2. Ameliorate kidney injury	1. Attenuated the expression of IL1β, IL-6, Il-18, TNF-α 2. Depressed activation of NLRP3 inflammation	Diabetic kidney diseases	[128]
Bone morphogenic proteins (BMPs)	1. Suppress theca cell proliferation 2. Less androgen production 3. Suppression of inflammation	1. Upregulation of Caspase-3 and down-regulation of Bcl-2 in theca cells 2. Downregulate the expression of CYP17A1, CYP11A1, DENND1, and cAMP genes 3. Significantly suppress IL-1 and IL-6 markers	Poly cystic ovarian syndrome	[129]
(ACTB, AHSG, A1BG, APOA1, COL1A1, COL1A2, FN1, HP, HPX, ITIH4, PGLYRP2, TF and ALB) proteins	1. Genetic suppression of CYP11A1, CYP17A1 and DENND1A 2. Suppression of androgen production	1. Direct interaction with the MAPK (MEK) pathway and the SRC (proto-oncogene tyrosine-protein kinase) pathways	Poly cystic ovarian syndrome	[130]
LncRNA Y-RNA-1	1. Protect hepatopcytes from inflammation and apoptosis 2. Recruit Kupffer cells	1. Reduction in serum alanine, aspartate aminotransferase, alkaline phosphatase, and bilirubin levels 2. Suppression of EGF, SCF, Macrophage Inflammatory Protein-3β, Monocyte Chemotactic Protein-1 and 3, Interferon gamma-induced protein-10 (IP-10), and Interleukin-1α 3. Reduce expression of caspase 3/7	TNFα-induced hepatic failure	[11]

(*continued on next page*)

Table 5 (*continued*)

Bio-active compounds	Biological activities	Functional dynamics	Diseases	References
BMSCs-exosome ($CD81^+$, $CD63^+$ and $TSG101^+$)	1. Attenuate lung damage, inflammation and apoptosis	1. Decrease expression of TLR4 and NF-κβ	Lung injury	[131]
miR-539-5p	1. Block pyroptosis and ROS production	1. Suppression of NLRP3 and caspase-1 expression 2. Reduction of IL-1β, IL-18 and TNFα expression	Inflammatory bowel disease	[132]

biomaterials like nanoparticles, microparticles, microneedles, injectable hydrogels, and nano-scaffold patches for the prolonged, targeted and titratable release of BMSCs-secretomes to maximise therapeutic efficacy and limit off-target effects.

4. Therapeutic importance and functional dynamics of BMSC-secretomes in degenerative disorders: a giant growing

There is growing interested in BMSC-secretomes-based acellular therapy as a therapeutic alternative to MSC-based cellular treatments because of their potential lack of adverse effects. Other positives are eliminating the need for an invasive procedure to extract cells, the possibility of performing therapeutic dose and safety profiling, the simplicity of its application, and the ease with which its composition can be manipulated. The therapeutic potential and clinical applicability of MSC-secretomes are comprehensively illustrated in Table 4 and Fig. 4 and comparison with stem cell transplantation. Therefore, BMSC-secreted soluble factors, as well as exosomal-non-coding-RNAs, have recently emerged as attractive therapeutic targets in cardiovascular diseases, neurodegenerative diseases, liver diseases, lung diseases, wound healing, skeletal muscle degeneration, infertility, and immunotherapy (listed in Table 5).

4.1. Musculoskeletal disorders

4.1.1. BMSC-secretomes: a biological treasure against tissue damage and wound

It is well documented that BMSC-derived secretomes respond to internal and external signals to release various trophic factors. For example, high levels of IL-8, IL-6, IL-13, MCP-1, VEGF, TNFs, IL-1, connective tissue growth factor (CTGF), and MIP-1 were detected in the BMSC-secretomes under traumatic and degenerative conditions, recruiting diverse immune cells with enhanced longevity, growth rate, and tissue repair properties [18]. BMSC-secretomes protected myofibers against tissue injury and apoptosis by boosting the expression of pro-survival AKT molecules and lowering the expression of cytochrome-c, activated-caspase-3, and other pro-apoptotic indicators [71]. Further, BMSC-secretomes aided in the extension and ongoing replenishment of skeletal muscle regeneration cells such as satellite cells (SCs) and telocytes/$CD34^+$ stromal cells (interstitial nurse cells) to inhibit tissue damage [71]. Therefore, the secretomes of BMSCs aid in tissue repair and wound healing.

4.1.2. BMSC-secretome-derived reticulocalbin-2 and chemerin in osteoporosis: emerging crosstalk in bone health

Reticulocalbin-2 (RCN2), a highly conserved calcium-binding protein, is well distributed in the endoplasmic reticulum and bears the characteristics and function of the expressed protein from BMSCs. RCN2 binds to the neuropilin 2 (NRP-2) or integrin beta-1 (ITGB1) receptor complex. It activates the cAMP-PKA signalling pathway, thereby maintaining BM lipolysis and sustaining energy balance for osteogenesis and lymphopoiesis within BM. Hence RCN2 appears to be a key regulator of a local adipose-immune-osteogenic axis [72].

Chemerin is a regulatory adipokine highly expressed by BMSCs and adipocytes. Chemerin acts as a novel chemoattractant for BMSCs and is used as a powerful BMSCs trafficking tool into the target or damaged organs. Chemerin-facilitated MAPKs phosphorylation upregulated various BMSCs-homing specific TFs and genes, including signal transducer and activator of transcription 3 (STAT3), GATA3, CD44, and matrix metalloproteinase-2 (MMP-2) in injured organs [14]. Additionally, chemerin-mediated osteogenic differentiation of mouse BMSCs activates AKT and glycogen synthase kinase-3 beta (GSK-3 beta) phosphorylation at serine 9 in bone tissues, resulting in the inactivation of the Gsk3β/Axin/APC complex and activation of β-catenin (a biomarker of osteoblasts) to induce bone formation. Therefore, BMSCs-chemerin is a possible treatment approach for conditions requiring improved bone metabolism and homeostasis [73].

Contrarily, adipogenesis of the BM and increased chemerin levels are vital factors in obesity-related osteoporosis [74]. The adipocyte-mediated chemerin secretion inhibits Wnt/β-catenin and other osteoblast biomarkers. It induces osteoclastogenic genes like RANK, RANKL, and cathepsin K (CTSK) for inhibiting osteoblastogenesis and inducing osteoclastogenesis for osteoporosis—Moreover, long-term exposure to chemerin increases osteoclast physiology, tilting bone homeostasis towards demineralisation [75]. In essence, chemerin impacts bone formation; a causal relationship exists between chemerin and osteoporosis in obese patients [76].

4.2. Myocardial injury

4.2.1. BMSC-exosomal-non-coding RNAs in sepsis-induced cardiomyopathy and cardiotoxicity

Sepsis is characterised by oxidative stress, acute inflammation and organ dysfunctions, resulting in a high morbidity and mortality rate and a decreasing life expectancy. Among over 250 sepsis-biomarkers, sepsis-induced cardiomyopathy stands out for having a significant role in sepsis-related mortality [77]. BMSC-exosomal miRNAs and circular RNAs (circRNAs) were recently reported as

novel forms of non-coding and functional RNAs used for diagnosis or treatment or both for sepsis-induced cardiomyopathy. For instance, BMSC-exosome-derived 77 miRNAs were implicated in regulating the sepsis-induced cardiac inflammation and dysfunctions [6]. For example, miRNA-141 helped to treat sepsis-induced cardiomyopathy by lowering myocardial injury enzymes markers like creatine kinase MB (CK-MB) and lactate dehydrogenase (LDH), enhancing cardiac functions by inhibiting PTEN and raising β-catenin activity [12].

Besides, circRNAs such as circRTN4 suppressed ROS, IL-1, IL-6, and TNF-α to improve cell survival and inhibit myocardial apoptosis. CircRTN4 directly interacted with miR-497-5p to upregulate MG53, a cell membrane repair protein, in cardiomyocytes to reduce myocardial damage [78]. Exosomal miRNAs may also mitigate non-alcoholic steatohepatitis-induced cardiotoxicity. These microRNAs increased Parkin, PTEN-induced kinase 1 (PINK1), uncoupling protein 2 (UCP2), FUN14 domain-containing protein 1 (FUNDC1), Unc-51-like kinase 1 (ULK1), BCL2/adenovirus E1B 19-kDa-interacting protein 3-like (BNIP3L/NIX), microtubule-associated proteins 1A/1B light chain 3B (MAP1LC3B), and manganese superoxide dismutase (MnSOD) genes, thereby modifying cardiotoxicity by regulating pAKT, VEGF, PI3K, hypoxia, and NF-kβ signalling pathways and restoring mitochondrial antioxidant status and Parkin-dependent and -independent mitophagy [13].

4.3. Brain and spinal cord injury

4.3.1. BMSC-exosomal miRNAs support the stroke recovery process under hypoxia

Let-7C–5P, miR-140-5P, and miR-126-3P are BMSC-exosomal miRNAs that play essential roles in neurotrophin and VEGF signalling, focal adhesion formation, leukocyte transendothelial migration, ECM-receptor interactions, cholesterol metabolism, protein digestion, and absorption in hypoxia. In addition, hypoxia-induced BMSC (miR409-3P, miR-186-5P, and miR-370-3P) downregulate platelet activation, endocytosis, and maintain homeostasis in cytoskeleton control, complement system, and metabolic pathways [79]. Following focal cerebral ischemia, these miRNAs further lengthened the microvasculature and increased branching point density to encourage human cerebral microvasculature endothelial cell proliferation, migration, and tube formation. This reduced brain atrophy and neuronal degeneration, thereby ensuring post-ischemic angiogenesis, remodelling, and neurological recovery [79].

In addition, exosomal long non-coding RNA ZFAS1 inhibits miRNA-15a-5P to increase superoxide dismutase (SOD) activities and decrease the levels of malondialdehyde (MDA), IL-6, IL-1, and TNF-α, repressing ischemic stroke-related inflammation and oxidative stress [80].

4.3.2. BMSC-exosomal miRNAs cargos against chemobrain: new sunshine

One of the unfavourable consequences of chemotherapy on the brain is chemobrain or chemofog. Axonal demyelination, altered hippocampal neurogenesis, and structural and functional changes in brain plasticity are its defining features [81]. A study described in greater detail the vital characteristics of doxorubicin (DOX)-mediated chemobrain, including (i) the presence of nuclear pyknosis, neural degeneration, and neural demyelination in the hippocampus and corpus callosum; (ii) decreased expression of opaline, oligodendrocyte transcription factor (OLIG2), doublecortin/DCX, BDNF, nuclear rRNA processing FCF-2, synaptophysin and fractalkine; and (iii) decreased activation/expression of hedgehog and β-catenin pathways resulting in hippocampal neurodegeneration and neural demyelination [10]. BMSC-exosome-derived miR-125b-5P, miR-21-5P, miR-199-3P, and let-7a-5P upregulate the expression of the NeuN gene, neural myelination factors, synaptic factors, and neurotrophic growth factors on neurons. These miRNAs also activate hedgehog and Wnt/signalling pathways and antioxidant glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD) proteins to reduce the activation of astrocytes and microglia, apoptosis, and inflammation, thereby enhancing neural re-myelination and neurogenesis [10]. Thus, the miRNA cargos of BMSCs can offer neuroprotection and hippocampus health for cancer survivors from DOX-derived chemobrain.

4.3.3. BMSC-exosomal miRNAs and spinal cord injury: a new intervention

Several studies have proven exosomal-miRNAs to be a promising therapy for spinal cord injury (SCI) and ischemic brain damage [82,83]. Exosome-derived miRNA-181c demonstrated considerable anti-inflammatory and cytoprotective effects during neuro-inflammation in rat models of SCI [8,84]. It has been suggested that miRNA-181c is a real player that targets PTEN mRNA to prevent PTEN-mediated dephosphorylation of phosphoinositide substrates from reducing microglial inflammation. miR-181c also inhibited apoptosis and inflammation by inhibiting the NF-κβ signalling pathway after SCI, dramatically decreasing the pathological changes in the spinal cord [8].

BMSC-miRNA-126-mediated angiogenesis and neuroprotection in SCI rats presented a potential therapeutic approach to SCI treatment. miRNA-126 stimulated phosphoinositide-3-kinase (PI3K)/Akt signalling pathways, increased VEGF, and downregulated SPRED1 and PIk3R2 to promote spinal cord anti-inflammation, angiogenesis, and healing after SCI. After SCI, miRNA-126 inhibits Bax and caspase-3 and elevates SOX-2 and Nestin-neural stem cell markers in the spinal cord, stimulating neurogenesis and regaining functional strategies of the spinal cord [85].

During spinal cord ischemia-reperfusion (SCIR), exosomal miR-455-5p reduces caspase-3 and Bax expression, thereby inhibiting neuronal apoptosis and increasing Beclin-1 and LC3-II expression to induce neuronal autophagy after SCIR. In addition, exosomal miR-455-5p reduced the expression of Nogo-A (a neurite outgrowth inhibitor) to promote recovery of locomotor function after SCIR [86]. As a result, BMSC-derived miRNA cargoes have shown anti-inflammatory and healing potentials to lessen the pathological lesions of SCI.

4.4. Reproductive disorders

4.4.1. BMSC-secretomes against premature ovarian insufficiency (POI): a recent addition to female infertility and reproductive function recovery

A POI is characterised by the depletion of primordial follicles, ineffective synthesis of mature gametes, ovarian dysfunction, reduced-oestrogen levels, and raised gonadotropins. POI is one of the leading causes of poor oocyte quality and infertility [87]. POI is also strongly associated with the risk of breast cancer development [88,89]. Radiation-induced POI showed altered expression of caspase-3, Bax, Bcl2, TGF-β, proliferating cell nuclear antigen (PCNA), forkhead box 1/3 (FOX 1/3), follistatin (Fst), wingless-related integration site-2 (Wnt-2), Wnt-3a, yes-associated protein 1 (YAP1), cellular communication network (CCN), CCN2, TEA domain family member 1 (TEAD1), and β-catenin to inhibit granulosa cell proliferation, differentiation, and maturation [7].

Treatment with BMSC-secretomes increased PCNA, FOX, Fst, and anti-apoptotic markers to activate the Wnt/β-catenin pathway and to disrupt hippo pathways and TGF-β expression. BMSC-secretomes promote granulosa cell proliferation, increase primordial, secondary and functional follicle stock, repair hormonal alterations, and regain fertility in POI rats [7]. Moreover, BMSC-secretomes increased the expression of the FOXL2 gene and estrogen-producing steroidogenic enzymes, including stimulation of granulosa cell proliferation and functions in the chemotherapy-induced POI animal model [90,91]. In addition, several angiogenic indicators, including VE-Cadherin, VEGF, VEGF-A, and Endoglin, were also considerably upregulated in the ovarian tissues of POI after treatment with BMSC-secretomes compared to their corresponding controls [92]. Thus, BMSC-secretomes therapy may present a novel treatment modality for POI patients.

4.4.2. BMSC-secretome-derived SCF and VEGF: novel treatment potency in idiopathic male infertility

Despite the lack of curative therapies, idiopathic male infertility is common in developed countries. Given the potential that a shortage in the testicular microenvironment is implicated in the aetiology of idiopathic male infertility, using MSCs and MSC-secretomes is warranted [93]. The report indicates that sperm cells respond to intratesticular injection of MSC-derived SCFs by upregulating the expression of phosphoglycerate kinase-2, glycogen synthase kinase-3, and glyceraldehyde-3-phosphate dehydrogenase; activating AKT molecules, ATP production, and glycolysis cascades in sperm cells [94]. Moreover, MSCs and their secreted components restored the Sertoli cell pool and the Leydig cell's functions, thereby recovering spermatogenesis and germ cell function in rats [95]. In addition, MSC-secretome-derived VEGF was the surrogate marker for the testosterone production by Leydig cells in doxorubicin-induced male infertile mice [96].

4.5. Cellular senescence vs BMSC-secretomes: VIPs in ageing

Cellular senescence is associated with chronic diseases and age-related disorders. MSC-derived exosomes transfer the bioactive molecules, promote proliferation and reduce senescence in aged MSCs [97]. For example, MSCs-exosomes reversed senescence by decreasing the transcription of senescence markers (Lmnb1, Cdkn2d, and Cdkn2a) and senescence-associated secretory phenotypes (CCl7 and IL-6), resulting in the cell proliferation with less DNA damage in murine kidney primary tubular epithelial cells [98]. Moreover, aged MSCs' phenotype and capacity for differentiation are changed by NF-κβ activation, which is caused by oxidative stress. But this could be changed by administering young MSCs' derived secretomes in a conditioned medium, improving the activation of NF-κβ signalling in them and restoring the phenotype and differentiation potential [99]. In addition, EVs from senescent MSCs showed increased activation of p56, which was responsible for further activation of pro-inflammatory IL-6 and IL-8, leading to inflammation and cellular senescence. Therefore, MSCs-secretomes are now recognised as very important particles (VIPs) in cellular senescence and ageing [100].

5. BMSC-secretome-mediated immunoregulation against immunological disorders

5.1. Secretome of BMSCs nurture LLPCs in BM against the coronavirus disease of 2019 (COVID-19)

BMSC-derived bio-active factors, including IL-6, 14-3-3 proteins, fibronectin (FN-1), enolase 1 (ENO1), a proliferation-inducing ligand (APRIL), and hypoxic conditions, contribute significantly to BM-LLPCs survival and maturation [101]. FN-1 plays a crucial function in anchoring plasma cells to the BM extracellular matrix through its interactions with integrin α4β1 [102], also inducing mTOR signalling-mediated autophagy and metabolism of BM-LLPCs [101]. The 14-3-3 proteins/YWHAZ protein inhibited proapoptotic BAD, BAX, and NOXA proteins from blocking the anti-apoptotic myeloid cell leukaemia-1 (MCL1) protein to control the mammalian target of rapamycin (mTOR)-AMP-axis, insulin signalling, and autophagy pathways in BM-LPCs [103,104]. In addition, ENO1, APRIL and hypoxia are critically important for the BM-LLPC's survival through an adaptation program, representing a central concept that the local BMSCs paracrine factors facilitate ongoing LLPC maturation.

The primary purpose of BM-LLPCs is to act as a permanent reservoir of already-formed, protective antibodies and the most robust biomarker of long-lasting immunity against bacterial and viral infections, including COVID-19. According to research with 77 SARS-CoV-2-infected individuals, the virus strongly stimulated short-lived BM-derived plasma cells (PCs), the ancestors of BM-LLPCs, to secrete anti-SARS-CoV-2 serum antibodies. Hence, BM-LLPCs were characterised as original non-proliferative long-lived memory plasma cells specific for the receptor-binding domain (RBD) of SARS-CoV-2 in BM. The number of these PCs was relatively stable between 6 and 12 months of infection. Moreover, the reactivity of BM-LLPC-derived SARS-CoV-2 antibodies and neutralising functions of these RBD-specific PCs or memory B cells remained the same during this period [105]. These anti-SARS-CoV-2 memory B cells

responded six months post-infection and are based on the accumulation of antibody somatic mutations and the production of monoclonal antibodies with enhanced neutralising breadth and potency against SARS-CoV-2 RBD mutations [106,107]. Based on the above data, the BM-secretome has full credit for its vital immune-protective role against SARS-CoV-2 by supporting the survival and maturation of BM-LLPCs in BM.

5.2. BMSC-secretomes and exosomal-miRNA-146a-5p: regulate ILC2 during allergic airway inflammation

In vivo, MSC-derived SCFs and IL-7 increased the expression of GATA3 and ID2, two transcription factors critical to ILC2 development and differentiation. SCFs significantly increase the expression of IL-4, IL-5, IL-9, IL-9r, IL-17RB, IL-13, and TGFs, which are essential for ILC2's effector functions in homeostatic conditions [108,109]. In contrast, MSC-derived Runx/Cbfβ molecules are required under allergic conditions to regulate the functional state of ILC2s. For example, ILC2s lacking Runx have a decreased inflammatory response and increased IL-10 and T cell immunoreceptor expression with Ig and ITIM domains (TIGIT) proteins, known markers of exhausted T cells, to attenuate airway inflammation [110]. In addition, MSC-secretome-induced IL10^{+}ILC2 further activates Treg cells via the ICOS-ICOSL interaction to suppress Th2 inflammation in the lungs [111]. Besides, after systemic delivery of MSC-exosomes, miR-146a-5p stimulates IL10^{+}ILC2 cells to suppress effector ILC2 cells, Th2 cytokines, inflammatory cell infiltration, mucus production, and airway hyperresponsiveness in a mouse model of asthma [112]. Therefore, BMSC-secretome and exosomal-miRNAs may regulate the development, proliferation, and phenotypic maintenance of ILC2 cells during airway inflammation.

Interestingly, IL-10^{+}ILC2 has been a central player in allergen immunotherapy (AIT), iterative immunotherapy for allergic disorders like seasonal rhinitis, and home dust mite allergic rhinitis [16,113]. In IL-10^{+}ILCs-based AIT, KLRG1^{+}CRTH2^{-}CD117^{+}ILC2 precursors differentiate into mature IL-10^{+}ILC2 in the presence of GATA TFs, vitamin A metabolites and IL-33. This IL-10^{+}ILC2 successfully induced the RDH10, RARA, and DHR3 genes of the retinol metabolism pathway; the IL-10RA gene of the cytokine-cytokine receptor pathway, and the JAK1, STAT3, and SOCS3 genes of the JAK-STAT pathway to initiate anti-inflammatory pathways and to restore the integrity of the allergen-breached nasal epithelial barrier in AIT [16]. Additionally, zonula occludens-1 (ZO-1), NF-κβ1, Myc, EGR1, cytotoxic T-lymphocyte-associated protein 4 (CTLA4), and IL-2RA (CD25) protein expression and functions were restored by IL-10^{+}ILC-2s to maintain the barrier's integrity and establish an anti-inflammatory state by reducing CD4^{+}T cell proliferation and Th2 response. Therefore, scientists are currently exploring IL-10^{+}ILC2 as a novel cellular biomarker in the context of the AIT [113].

5.3. BMSC-secretome and intestinal immunity: a repair tool for intestinal inflammation

Intestinal ILC3-mediated IL-22 secretion, a crucial cytokine for healthy intestinal epithelial cells, is a potential component of intestinal immunoregulation and allogeneic stem cell transplantation. During co-culture conditions, BMSCs-secreted IL-7 cytokines enhanced ILC3 proliferation and IL-22 production by increasing the link between ILC3 and aryl hydrocarbon receptor ligands. Reciprocally, ILC3s induced IL-7-dependent ICAM- 1 and VCAM-1 expression in the MSCs [114]. Therefore, the BMSCs-ILC3 axis regulates intestinal stem cell maintenance and contributes significantly to wound repair and healthy homeostasis in treating inflammatory intestines through intestinal transplant (IT). However, ILC3-mediated immunoregulatory mechanisms and the phenotypes and composition of ILC3 subsets in IT could be more precise. Researchers discovered a decrease in the number of protective NK44^{+}ILC3 subsets and an increase in the number of proinflammatory NK44^{-}ILC-1 subsets in allografts transplanted more than six months prior, resulting in an unfavourable interaction between the NK44^{+}ILC3 and NK44^{-}ILC1, damaging cytokine storms, and allospecific inflammations following IT. Less NK44^{+}ILC3 leads to a reduction in IL-22 expression, IL-22-mediated STAT phosphorylation, and AMP production, which affects intestinal regeneration and fails to protect the intestinal mucosa, resulting in intestinal injury, bacterial translocation, organ failure, and IT allograft rejection [17,115].

HO-1-treated BMSC-exosomal MiR-200b was reported to alleviate intestinal injury. MiR-200b expression was significantly increased in HO-1/BMSC-exosome-treated intestinal epithelial cells, which could reduce intestinal damage by downregulating phosphorylated c-Jun NH2-terminal kinase (JNK) and high mobility group box 3 (HMGB3) [116]. Accordingly, the cargoes of BMSC-exosomal miRNAs downregulated caspase-3, cyclo-oxygenase-2, mucin-2, and cytokeratin-20 in inflamed intestinal epithelial cells, alleviating colitis by decreasing epithelial apoptosis and increasing epithelial repair and organoid formation *in vitro* [117]. As a result of their ability to interact with the intestinal milieu, BMSC-secretomes have been identified as a potential acellular therapeutic intervention for intestinal inflammation.

5.4. BMSC-exosomal miRNAs against PANoptosome: a new innovative approach against inflammatory cell-death

PANoptosis is a newly recognised proinflammatory programmed cell death (PCD), consisting of apoptosis (apoptosome-mediated and caspases-executed PCD), necroptosis (RIPK3-mediated and MLKL-executed PCD), and pyroptosis (inflammasome-mediated and gasdermins-executed PCD) [118]. PANoptosis is controlled by a cytoplasmic multimeric protein complexes with a molecular platform named PANoptosome [118]. PANoptosome is made up of the caspases 1–8, the apoptosis-associated speck-like protein with a CARD (ASC), the Z-DNA binding protein (ZBP1), the NOD-like receptor (NLR) family pyrin domain-containing 3 (NLRP3) protein, and the receptor-interacting serine/threonine kinase (RIPK)-1/3 [119]. Additionally, gasdermin D (GSDMD), mixed lineage kinase domain-like protein (MLKL), and caspases 3/6/7 are likewise PANoptosome components [120]. Hence, the COVID-19 pathogenesis is heavily influenced by PANoptosis, which may also shed light on the connection between the disease's symptoms, tissue damage, and

Table 6
Limitations of BMSC-secretome-based therapy in clinical applications.

Risk factor	Adverse effects	Proposed solution	References
Donor-related: age, obesity, cell source and cell passage	Variability of the secretome compositions MSCs are unpredictable	Immortalized MSC-derived secretome	[138,139]
Epigenetic modifications e.g. viral infection	Composition of the MSC-secretome, development, maintenance, and progression of inflammatory diseases		[140]
Pro-tumor effects	MSC-tumor cell interaction by exchanging secretome, aggressive tumor development and inflammation-related tissue injury	Reciprocal exchange, and inducing biological markers	[141,142]
Exosomal-enzymes like primarily MMPs and their regulators, dysregulated ECM	Pre-metastatic niche formation, tumor neovascularization, fibroblastic switching and mesenchymal mode acquisition, and development of ecto-5′-nucleotidase activity	In-depth study required before application	[143,144]

dysregulated immune responses [121].

Two studies have focused on BMSC-sourced 58 miRNA cargos and their propensity for binding to the 3′UTR of the PANoptosome genes to attenuate the cytokine storm, coagulation, and cell death in acute COVID-19. For instance, BMSCs-derived miRNA-125a-3p binds to the 3′UTR of the TNF-α, IFN-γ, CXCL10, IL-2, IL-10, and IL-15 genes, while the miRNA-125b-1-3P targets the IL-10, IL-17A, IL-18, IL-33, TNF, IFN, gasdermin E (GSDME), and CXCL10 of PANoptosis-associated genes to improve the clinical outcomes of severe COVID-19 [15,122]. Additionally, miRNA-202-3p and miRNA-769-3P might reduce inflammation, tissue damage and cell death by inhibiting TNF-α and IFN-γ synthesis [15]. Therefore, BMSCs-exosome-derived-miRNAs may slow PANoptosis-mediated harmful cellular processes in inflammatory disorders, including COVID-19. Nevertheless, more studies are needed to determine the efficacy of BMSC-derived miRNA cargos in treating COVID-19.

6. BMSCs and parabiosis: need more study

Parabiosis is a standard physiological study method in which two organisms are surgically joined to form a single physiological system. When young and old mice are surgically connected, the older mouse keeps some of the young mouse's features while the young mouse becomes functionally older. Scientists use this method to study physiological processes and interactions in stem cell research, endocrinology, anti-ageing, and immunology. BMSCs-derived microvesicles have been applied in the co-culture method that re-capitulates parabiosis. For example, exosome-derived PAX and PPMIF miRNAs were reported for mobilising young peripheral blood to rejuvenate old hematopoietic systems by elevating MYC and E2F pathways and decreasing p53, resulting in anti-ageing and anti-inflammatory effects hallmarks and balanced lymphoid: myeloid ratios. In addition, restoring the old hematopoietic system prevented dormant breast cancer cells from forming in vivo [133]. More studies are urgently needed to establish the therapeutic application of BMSCs-secretomes in parabiosis.

7. BMSC-secretomes and organoids bioprinting: a unique platform for medical research

Human organoids are self-organised, multicellular and functional 3D cell cultures derived from pluripotent stem cells or a patient's biopsy and the unique platform for studying human diseases like infections, genetic disorders, and cancers. Cancer organoids accurately recapitulate the biological heterogenicity of inter- and intratumoral research and drug screening in patient-specific cancer studies [134,135]. BMSC-derived secretomes have shown a positive impact on different organoids. A study with intestinal organoids and animal models showed that treatment with MSC-secretomes upregulated Wnt/Notch signalling pathways by increasing the expression of β-catenin, Wnt4 and Wnt7 after irradiation in vivo and *in vitro*, thereby facilitating the structural restoration of irradiated crypt-villus and the regrowth of intestinal stem cells [136]. In cerebral organoids, treatment with BMSC-derived secretomes increased four-fold of MOR protein and two-fold of β-Tub III protein expression compared to the control group, resulting in the secretome-mediated modulation of opioid receptors and the enhancement of the maturation of glial cells, respectively. Moreover, after treatment with secretomes, dopamine release from cerebral organoids was not changed, which indicated an alternative treatment option for pain management by delivering BMSC-secretomes [137].

8. BMSC-secretome-based therapy and their limitations

Due to the ease and safety of the cell-free immunomodulatory method, research into the clinical applications of BMSC-secretome is essential. However, the potential risks associated with MSC-secretome use must also be considered. It has been noted that MSC-secretome-based therapy can have several undesirable side effects, and Table 6 summarises these concerns.

9. Conclusion

Despite BMSCs' promising immunoregulatory and immunomodulatory benefits, cell-based treatments have significant drawbacks in inflammation management. BMSCs-based therapies are threatened by repeatability, graft rejection, thrombosis, unfavourable immune responses, and bioactive factor evaluation. BMSC-derived immunomodulatory and anti-inflammatory secretomes lead to cell-free therapies and overcome cell-based treatments' limitations. This is spurring scientific research into its medicinal properties for

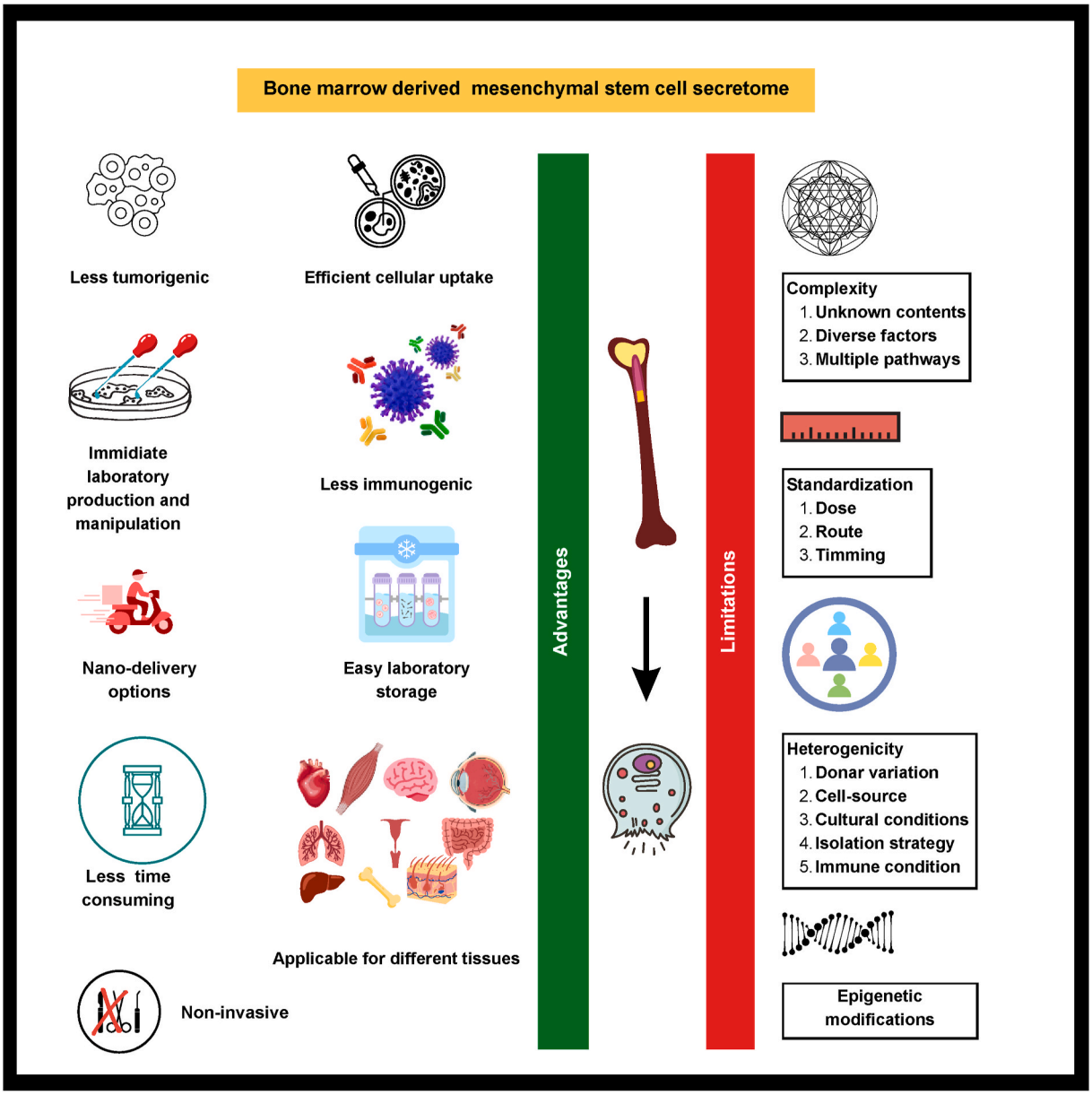

Fig. 5. Advantages and significant considerations for therapeutic applications based on BMSC-secretomes.

replacing cellular therapies. In sum, the cell-free alternative biological strategy of BMSC-secretome therapies may broaden the therapeutic avenue for degenerative and inflammatory illnesses in humans. Fig. 5 depicts the advantages of BMSCs-secretome-based medicinal applications and critical factors to consider. The secretome must be fully characterised for therapies to be understandable and reproducible. Methods used to precondition BMSCs to stimulate their biological properties, such as priming with hypoxia, inflammatory factors, gene therapy, and biomaterials, significantly modify the contents and therapeutic effects of BMSCs-secreted secretomes. Many approaches have also been developed for isolating and purifying the complete secretomes or EVs; however, output and expandability must be optimised. BMSCs-secretomes deliver genetic materials, cytokines, chemokines, growth factors, and immunomodulatory factors to target cells, activating pro-survival and anti-apoptotic pathways to enhance tissue regeneration and repair. Therefore, BMSC's secretome, either through MSC-conditioned medium or isolated MSC-secreted EVs, is currently being applied in various degenerative and inflammatory diseases.

10. Recommendations

Although pre-clinical research employs various animal studies and cultured cells, experiments strongly support the use of cell-free

BMSC-secretome in treating multiple human diseases; however, human investigations are still in their infancy. Standardised secretome separation, purification, storage, quality assurance processes, and specified doses and routes are further needed for demanding human applications.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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