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Therapeutic Potential of Mesenchymal Stem Cell-Derived Conditioned Medium for Diabetes Mellitus and Related Complications

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Diabetes mellitus (DM) is one of the most life-threatening metabolic disorders, with 9% of the global prevalence, and it is estimated to be rising to 12.2% in 2045. Currently, there is no definitive treatment for DM. Although life-saving, insulin administration to control blood sugar is not a cure for DM and is insufficient to prevent DM-related complications such as nephropathy, neuropathy, or retinopathy. For this reason, studies are continuing to develop treatments that will provide β-cell regeneration while suppressing autoimmunity. Mesenchymal stem cells (MSCs) are multipotent stem cells with a high proliferation capacity, immunosuppression, and immunomodulation ability. MSCs have gained therapeutic importance with these properties besides their differentiation ability. The immunosuppressive and immunomodulatory properties of the cells arise from the soluble and insoluble factors they secrete into the extracellular environment. Therefore, the culture medium where these cells grow has therapeutic value and is named conditioned medium (CM). In this context, CM obtained from MSCs can provide a similar therapeutic effect with fewer safety concerns. Furthermore, preconditioning of MSCs can improve the effectiveness of these cells and associated cellular products. So, this review summarizes the recent advances in MSC-derived CMs and their therapeutic potential for DM and related complications.

1. Introduction

Mesenchymal stem cells (MSCs), which are multipotent cells with self-renewal potential, were first identified by Friedenstein with the observation of the bone-forming abilities of the cells obtained from the mouse bone marrow (BM).[1] In the literature, different descriptive names, including mesenchymal stromal cells, mesenchymal progenitor cells, and multipotent stromal cells, have been used for MSCs.[2] There is no single biomarker for the characterization of these cells, so the International Society for Cellular Therapy proposed minimal criteria for the definition of MSCs in 2006, which include being able to adhere to a plastic substrate, differentiating into specialized cells, including osteoblasts, chondrocytes, and adipocytes and having cell surface expressions of the cluster of differentiation 90 (CD90), CD105, CD73 and not expressing CD45, CD34, CD14, CD79 and human leukocyte antigen (HLA)-DR cell surface proteins.[3–5] MSCs can be isolated from a wide variety of tissues such as BM, umbilical cord (UC), adipose tissue (AT), dental pulp, amniotic membrane, and placenta.[6–9]

MSCs, with their high self-renewal capacity, ability to differentiate into different cell lines, relative ease of isolation, and immunomodulatory properties, have come to the forefront as a new therapeutic potential for the treatment of a wide variety of diseases.[2] Paracrine interaction, transfer of extracellular vesicles (EVs) carrying RNAs and other molecules, mitochondrial transfer, and differentiating into the affected cell can be counted among the possible therapeutic mechanisms of MSCs.[10,11] MSCs are the most studied cell type in clinical trials. The findings of not reporting tumor development in the applications of MSCs in humans have encouraged the usage of these cells in a series of clinical trials for various clinical conditions.[12] Multifactorial characteristics of MSCs, easy manipulation, and availability from several sources have brought about more than 1150 clinical trials listed in www.clinicaltrials.gov by January 2023. Most of these trials are based on applications of MSCs as an alternative to conventional therapy to improve the quality of life and prolong the life expectancy of patients.[13] Even though promising results have been obtained in plenty of these

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adtp.202300216

DOI: 10.1002/adtp.202300216

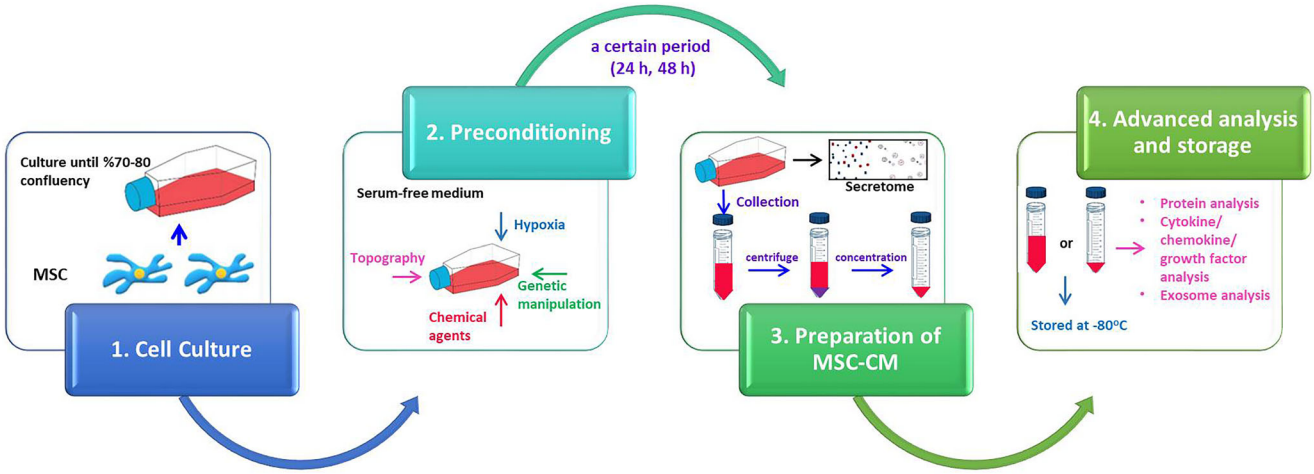

Figure 1. Conditioned medium (CM) preparation steps and the strategies for preconditioning cells for modification of CM content.

clinical trials, MSCs-based treatments have not been considered standard care at the clinic due to the lack of standards in isolation, ex vivo expansion, culture conditions and delivery route, dosage, frequency, and mode of infusion.[13] In preclinical studies, it was found that MSCs could show their recuperative effects as a distant immunomodulator besides their ability to homing to impaired sites.[14] In this sense, applications of MSCs intraperitoneally, intravenously, or directly into the affected area have not resulted in coherent results because most of the administered cells did not survive more than 24 h in the host tissue.[15,16] Furthermore, MSCs, regarding their "stemness," are expected to replace the injured cell by engrafting into the affected tissue. However, it was found that very few or none of the administered cells restore the functions by directly differentiating into the damaged cells.[16] In spite of their limited survival time and in vivo differentiation potential, observation of ameliorating effects following the application of MSCs has made the researchers consider the nonstem/progenitor cell properties of these cells.[2] Likewise, different studies have shown that the application of paracrine factors derived from MSCs had therapeutic effects at a similar level of direct administration of these cells.[17,18] At this point, the whole secretions containing EVs, cytokines, and growth factors, referred to as secretome, can be found in the medium where the cells are cultured and is called the conditioned medium (CM).[19,20] The preparation of the CM is summarized in **Figure 1**.

Cell-free CM has several advantages over cell therapies. The fact that it is easier to manufacture, freeze, thaw, package, and transport makes CM ergonomically advantageous.[21] CM could be frozen without toxic cryoprotectants like dimethyl sulfoxide (DMSO), unlike the cells, and stored for extended periods. In addition, the current Good Manufacturing Practice (GMP) rules that must be met during large-scale production for CM are much simpler than the cells and are analogous to conventional pharmaceuticals.[22] In addition, poor survival of cells after transplantation and differentiation capacity, localization, and secretion of cells in nontarget sites eliminate the possibility of cell-based therapy's success.[13] When the cells are transplanted into an unhealthy microenvironment, they may not exhibit their biological characteristics, and the expected therapeutic effect may not occur.[23] Instead, a more controlled and standardized treatment procedure can be developed with the CM, and the negativities of cell therapies can be ruled out. Since MSCs isolated from different sources may have different secretory contents[24] and even the secretome contents can be manipulated depending on the incubation conditions of the cells, such as hypoxia and three-dimensional (3D) scaffolds,[25] the CMs could be prepared as required by treatment of the target disease. Their reproducible manufacturing processes and analyzable contents increase the validation of the CMs and their possibility of usage as therapeutic products compared to MSCs themselves.[26] Moreover, there is no risk of rejection since donor-recipient matching is not required.[13] Therefore, the general paradigm related to exploiting the therapeutic potential of MSCs is shifting from their stem cell properties to their trophic effects, so that in this review it was aimed to summarize the recent advances in MSC-derived conditioned medium (MSC-CM) and their therapeutic potential for the treatment of diabetes mellitus (DM) and related complications (**Figure 2**).

2. Secretome Content of MSC-CM

The ability of MSCs to produce and secrete paracrine factors was first demonstrated by Haynesworth et al.[27] Since then, the number of studies examining the secretome content of MSCs and the therapeutic effects of the secretome has increased, and CM that contains this secretome has begun to be analyzed. The secretome content of CM is composed of two distinct components secreted by MSCs: the soluble particles consisting mainly of cytokines, chemokines, immunomodulatory molecules, and growth factors, and the nonsoluble, vesicular particles composed of different types of vesicles that play a crucial role in the delivery of microRNAs and proteins for cell-cell communication[28] (**Figure 3**). The soluble components of the secretome can be separated from the vesicular fraction by centrifugation, filtration, and chromatography. Both of these components independently have therapeutic value and are used in tissue regeneration and tissue engineering studies.[29] However, to use this secretome effectively for therapeutic purposes, it is necessary to determine the factors in the content, reveal the differences in the secretomes of MSCs

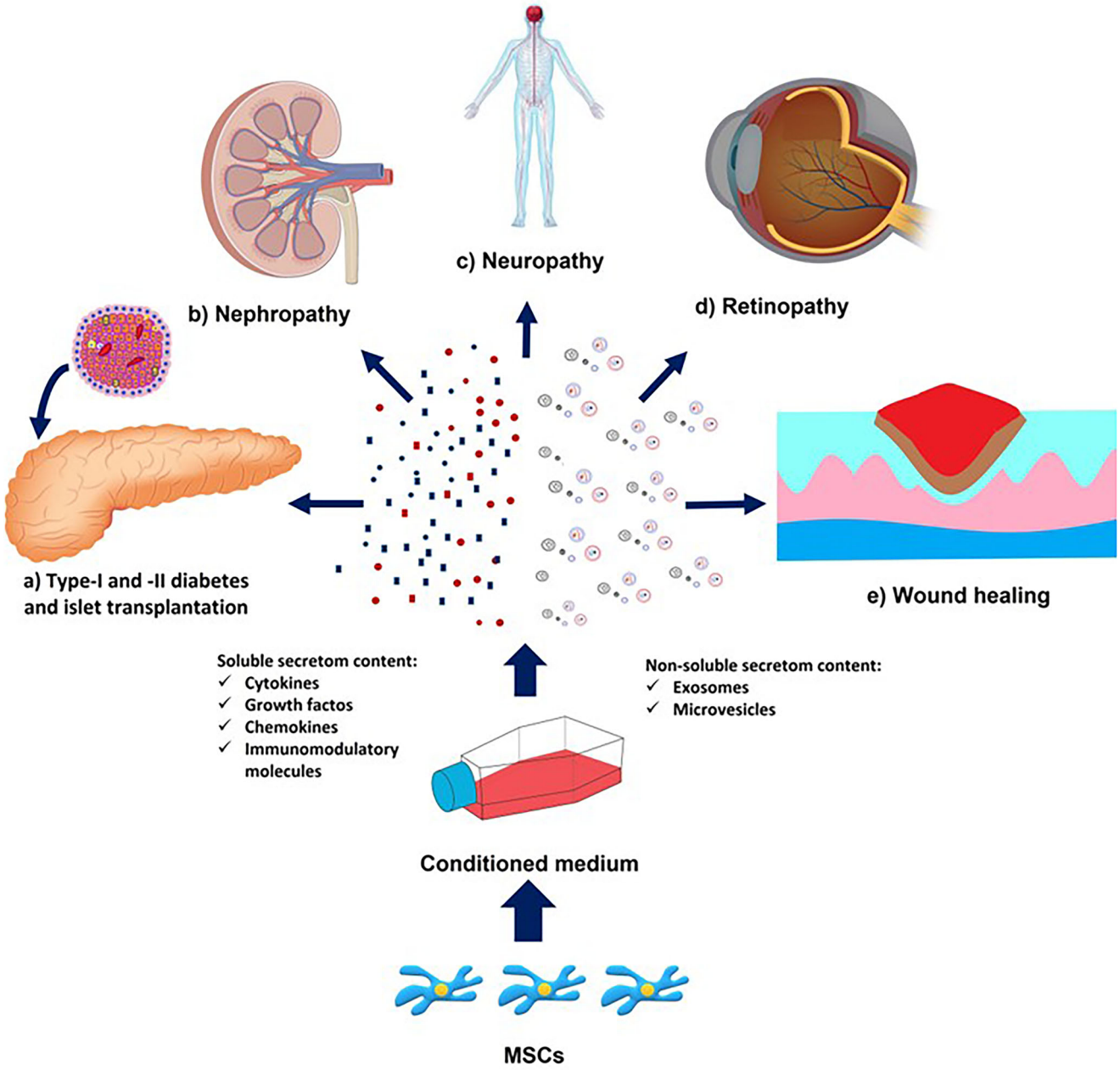

Figure 2. Therapeutic potential of MSC-derived conditioned medium for the treatment of diabetes mellitus and related complications.

isolated from different sources, and investigate the effects of different cultural conditions on the secretome content.

Initially, the ability of MSCs to secrete secretomes was thought to be intrinsic. Nevertheless, recent studies have shown that the variety and quantity of cytokines, growth factors, immunomodulatory molecules, chemokines, and vesicular particles secreted by MSCs can be regulated using different culture conditions such as the inflammatory environment, extracellular matrix components, hypoxia, and genetic manipulations.[30] Hypoxia, for instance, has been identified as a method to manipulate the secretion profile of MSCs.[31,32] Preconditioning MSCs with hypoxia positively affects their immune phenotype and paracrine secretion profile, enhancing their proliferation and migration ability. Besides, hypoxic conditions could improve the expression of pro-survival genes and various trophic factors in MSCs.[33,34] In addition to hypoxia, priming MSCs with inflammatory molecules like tumor necrosis factor α (TNFα), interferon γ (IFNγ), and lipopolysaccharide (LPS) also regulates the paracrine secretion of cells.[35–37] The tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) is secreted from MSC at deficient levels or not at all under normal circumstances. In contrast, its secretion elevates when MSCs are preconditioned with IFNγ or hypoxia.[38,39] Another approach to improve the MSC secretome involves utilizing a 3D microenvironment. Unlike two-dimensional

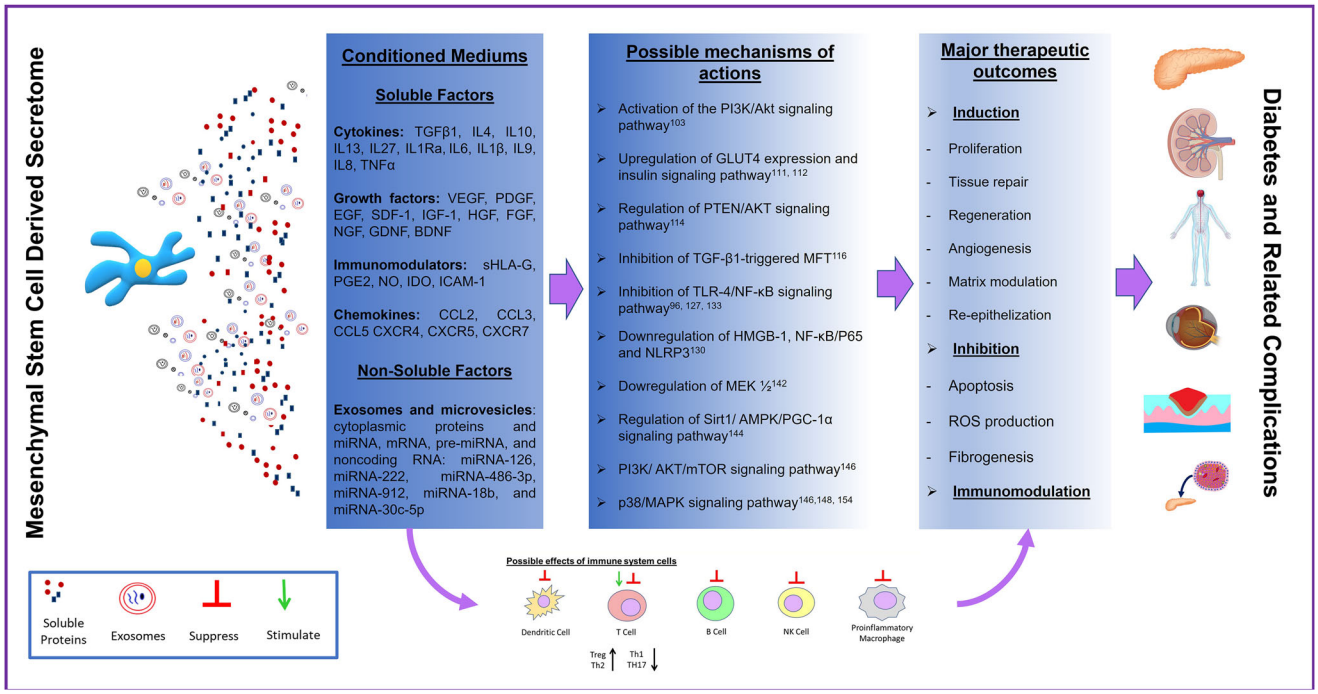

Figure 3. Soluble and nonsoluble composition of MSC-CM, their possible mechanisms along with major therapeutic outcomes.

(2D) traditional cell culture, a 3D setup provides the necessary conditions for complex biological functions such as interactions with the extracellular matrix, migration, transcriptional regulation, and receptor expression. Moreover, it facilitates cell-to-cell interactions and provides space for MSC proliferation.[40,41]]

2.1. Soluble Secretome Content of MSC-CM: Cytokines, Growth Factors, Chemokines, Immunomodulatory Molecules

The secreted soluble factors from MSCs can be classified as cytokines, chemokines, growth factors, and immunomodulatory molecules. These components are pivotal in regulating vital biological processes encompassing anti-apoptotic, anti-microbial, antioxidant, and pro-angiogenic activities. MSCs exert their immunomodulatory and immunosuppressive effects through the processes involving these factors.[28] Cytokines, a subset of these soluble factors, are the signal proteins that play essential roles in the initiation, maintenance, and resolution of immune responses.[42] These proteins are predominantly produced by cells within the human innate and adaptive immune systems and are categorized into pro-inflammatory and anti-inflammatory classes.[43] Studies have shown that MSCs secrete anti-inflammatory cytokines, including transforming growth factor $\beta 1$ (TGFβ1),[44–47] IL4,[48] IL10,[49–51] IL27,[51] IL1Ra,[52,53] as well as pro-inflammatory cytokines, including IL6[47,54,55] and IL8.[21] Nevertheless, considering the available studies, the question of which of these cytokines are secreted by MSCs isolated from different sources and how effectively they are secreted without external stimuli remains a matter of debate and needs further clarification.[56] This is because there are studies indicating that cytokines are absent or observed at deficient levels in MSC secretome under normal conditions.[48,57] Indeed, it is widely acknowledged that the secretion of cytokines from MSCs notably rises in response to inflammatory circumstances.[58] Furthermore, research has revealed that the production of cytokines improves in the presence of external stimuli such as hypoxia and 3D culture conditions.[41,47,59]

MSCs also secrete growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), stromal cell-derived factor-1 (SDF-1), insulin-like growth factor I (IGF-1), hepatocyte growth factor (HGF), fibroblast growth factor (FGF),[60–63] neural growth factor (NGF), glial cell-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor (BDNF).[64] Growth factors are essential effectors, providing therapeutic benefits like tissue repair, protection, fibrogenesis suppression,[65] and angiogenesis induction.[66] Furthermore, they have regulatory impacts on MSCs.[67] For instance, it was shown that MSCs that are primed with genetically engineered hepatocyte growth factor–expressing MSCs had enhanced recuperative potential.[68]

The MSC secretome contains another important component that includes immunomodulatory molecules such as human leukocyte antigen-G (sHLA-G),[69] prostaglandin E2 (PGE2),[70–72] nitric oxide (NO), IDO,[73–75] intercellular adhesion molecule-1 (ICAM-1),[76,77] and chemokines such as chemokine ligand 2 (CCL2), CCL3, CCL5, chemokine receptor type 4 (CXCR4), CXCR5, and CXCR7.[78,79] The presence of these factors is significant for the therapeutic value of the secretome. Even if the cytokines that will take part in immunomodulation are not found at sufficient levels in the secretome of MSCs, it is seen that these immunomodulatory proteins increase the release of necessary cytokines by regulating T and B cell mechanisms and play a crucial role in treatment.[80] For instance, since the HLA-G

is a nonclassical HLA class I molecule that exhibits immunosuppressive properties by inducing differentiation of T regulatory cells (Tregs), it plays an essential role in the immunomodulatory potency of MSCs.[81] It is known that the PGE2 contained in the MSC secretome fulfills multiple functions. It induces macrophages to produce the anti-inflammatory cytokine IL10, which in turn suppresses Natural Killer cells (NK) and T helper cell proliferation. PGE2 affects macrophage metabolic status and plasticity.[82] Secretion of PGE2, IDO, TGFβ1, IL6, and NO by MSCs has been shown to strongly attenuate NK cytotoxic activity and proliferation.[70]

2.2. Nonsoluble (Vesicular) Secretome Content: Extracellular Vesicles

EVs are insoluble membranous vesicles secreted by MSCs and represent a significant mechanism of communication with other cells. EVs are the structures composed of cytoplasmic proteins, lipids, DNA, and RNAs (miRNA, mRNA, pre-miRNA, and other noncoding RNA) within a lipid bilayer containing transmembrane proteins first identified during the maturation of reticulocytes in 1983.[83] EVs are a cluster of nano-sized vesicles formed by different mechanisms with different sizes, surface charges, and markers.[84] Three types of EVs have been defined based on their sizes and mechanisms of release: exosomes (smaller sizes of 150 nm in diameter), microvesicles or shedding particles, and apoptotic bodies (both of which have sizes larger than 100 nm).[85] On the other hand, the current consensus investigates EVs as microvesicles and exosomes depending on their biogenesis while not excluding the presence of apoptotic bodies in EV preparations.[86] EVs formed by breaking off the cell plasma membrane through the process involving cytoskeleton reorganization and depending on the concentration of intracellular calcium, are called as microvesicles (or ectosomes or microparticles in the literature) and generally have 100–1000 nm in size.[83,87] Microvesicles contain a high amount of CD44 surface marker protein.[83] The other mechanism is the fusion of multivesicular bodies or endosomes formed within the cytoplasm with the plasma membrane, which leads to the release of secret molecules having 40–150 nm of diameter and called as exosomes.[87] The secretion of exosomes is regulated and controlled by p53 and the cytoskeleton activation pathway, respectively, and is not affected by calcium concentration in contrast to the former mechanism.[83] Characterization of exosomes can be executed by ultrastructural evaluation of their morphologies and determination of expressions of specific exosome-associated marker proteins like CD9, CD63, and CD81 tetraspanins, Alix, and tumor susceptibility gene 101 (Tsg101).[83] EVs can be isolated by different techniques, such as differential ultracentrifugation, size exclusion chromatography, ultrafiltration, immunoaffinity chromatography, flow cytometry, immunocapture, and commercial kits.[88]

Interaction of EVs with the recipient cells can occur in a paracrine, autocrine, or endocrine manner. EVs could be removed by macrophages or transported to detoxifying organs like the liver, while they could cross biological barriers like the blood-brain barrier through a mechanism that has not been thoroughly elucidated. Progression of EVs towards recipient cells could be modified by their interaction with extracellular and pericellular matrix enabling the specific engagement of EV-associated ligands with recipient cell receptors, which is one of the main mechanisms defining the tropism of EVs for certain cell types.[89] All EVs have surface proteins that permit them to be targeted to the recipient cell. They activate the signaling pathway by receptor-ligand association or deliver their contents into the cytoplasm by endocytosis or phagocytosis following their attachment to the target cell, which enables them to modify the physiological state of the recipient cell by leading to activation of certain signaling cascades, decoration of recipient cell surface and conferment of new functions.[89,90] However, the delivery or transfer of intraluminal and surface content of EVs needs to be better defined.

The modulation mechanism through EVs is still not fully understood. Based on the first observation of when BM cells started to produce albumin after incubating them with liver cells, the micro-array analysis showed that the phenotypic changes seen in BM cells occur through the transfer of tissue-specific mRNAs, miRNAs, and proteins via extracellular microvesicles.[91] MSCs-derived EVs have been shown to be involved in many physiological processes, such as immunomodulation, proliferation, angiogenesis, matrix modulation, homeostasis, and tissue regeneration.[92,93] On the other hand, it has been determined that the cytokine contents of EVs can vary dynamically according to the physiological state of the cells.[94] Fitzgerald et al. investigated distributions of 33 cytokines in 8 different biological systems and found that the types and quantity of cytokines in free or encapsulated EV forms differ in each system.[94] Furthermore, it was noted that EVs' contents, especially small RNA profiles, can show variations in 2D and 3D cell culture conditions.[95] In 3D cell culture conditions, small RNA next-generation sequencing analysis showed that EVs derived from cancer cells have higher similarity to the ones derived from cervical cancer patient plasma, which indicates that the contents of MSC-EVs can also be regulated or modified by changing or mimicking the environmental conditions in a desired manner. In addition to all, bio-engineering the MSC-EVs content in favor of the delivery of therapeutically effective exogenous nucleic acids or proteins to target tissue could provide a biological nano platform for clinical drug delivery.[96] It was found that RNA-binding protein synaptotagmin-binding cytoplasmic RNA-interacting protein (SYNCRIP) is a critical component of the exosomal miRNA sorting mechanism in hepatocytes. Knockdown of SYNCRIP leads to disrupted sorting of miRNA in exosomes. Moreover, it was discovered that certain miRNAs sharing a common extra-seed sequence (hEXO motif) are mostly enriched in the exosomes that are specifically sorted by SYNCRIP, which is a significant finding that could enhance loading of poorly exported miRNA into exosomes by embedding this hEXO motif into relevant miRNA.[97] In this sense, MSCs can be genetically modified to secrete EVs that have therapeutically effective mRNA information of associated genes in the cargo, whereby being rendered to home to the disease site so that being internalized by the targeted cells.[29]

Even though the considerable potency of EVs secreted by MSCs has been noted in preclinical studies, there are certain challenges that must be satisfied for the clinical translation of EVs-associated therapies. In this respect, defining standards for characterization, fulfilling the purity and sterility criteria, optimizing storage conditions, and improving their safety and

potency in accordance with the disease-related interrupted mechanisms can be counted as major issues that need to be clarified before clinical applications. In addition to all, it has been found that the concentration of these bioactive molecules in these vesicles cannot be determined by conventional assays such as the enzyme-linked immunosorbent method. This makes it necessary to reevaluate the concentration analysis of soluble proteins like cytokines or growth factors based on standard methods in physiological and pathological conditions by using new comprehensive methods.[94] The details of these limitations for the therapeutic application of EVs have been well discussed by Wiest and Zubair.[98]

3. Therapeutic Effects of MSC-CM on Diabetes and Related Complications

3.1. Type-1 Diabetes

Type-1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing β-cells in the pancreas by the immune system, leading to progressive insulin deficiency and consequent hyperglycemia.[99] Hyperglycemia brings complications such as cardiovascular diseases, blindness, and kidney failure in individuals.[100] In the brief pathogenesis of T1D, activation of T cells against pancreatic islets is the first step leading to β-cell destruction. As a result of the combination of genetic predisposition and environmental factors, the decrease in regulatory T cells (Treg) and the increase in auto-reactive CD8+ cytotoxic T (TC) cells initiate the autoimmune process. Proteins released from β-cells due to post-translational modifications are recognized as auto-antigens by T cells, and antigen-presenting cells present these autoantigens to CD4+ helper T cells (TH).[101] TH cells are activated by IL12 released from macrophages and dendritic cells. TH1 cells reduce TH2 cells and their protective effects by secreting IL2 and IFNγ, which trigger TC cells and cytotoxicity. TH17 cells that secrete IL17 in response to β-cell autoantigens contribute to the progression of this process. In addition, β-cells can be damaged by perforin and granzymes secreted by TC cells. Thus, activated macrophages, TH cells, and TC cells act synergistically in β-cell destruction, resulting in autoimmune T1D.[102] As a result, it is seen that the disrupted T cell mechanism and cytokine balance play a role in the pathogenesis of T1D.

Today, despite all the advances in insulin analogs and blood glucose monitoring, there is no cure for T1D, and life-threatening complications develop as the disease progresses. The strategy in treatment should be to prevent the destruction of β-cells by regulating the disrupted T-cell mechanism and to enhance the number of existing β-cells. In this context, the MSC-CM is a promising therapeutic agent for treating T1D due to its immunomodulator, anti-apoptotic, and cytoprotective components. The information regarding the secretome composition of MSCs, the signaling pathways affected by this secretome, and the resulting therapeutic benefits for ameliorating diabetes and associated complications are briefly summarized in Figure 3. In a study conducted for this purpose, it was emphasized that the effects of MSCs on T1D occur through paracrine mechanisms, and the effects of BM-MSC-CM on streptozotocin (STZ) damaged islets were studied in vitro and in vivo. Accordingly, after green fluorescent protein (GFP)-labeled MSCs were transplanted into diabetic mice, no staining was detected in any newly formed β-cells, thus indicating that MSCs did not exert their therapeutic effects through transdifferentiation. Subsequently, with the MSC-CM application, in vitro and in vivo examinations revealed an increase in islet and β-cell numbers. This application was shown to activate the phosphatidylinositol 3-kinase (PI3K)/ protein kinase B (Akt) pathway. Since this pathway is one of the potential regulators of β-cell regeneration, the importance of detailed evaluation of CM applications in treating diabetes has been pointed out.[103] In another study investigating the effects of MSC-CM on T1D, a diabetes model was formed by destroying all islet cells by applying Alloxan to Wistar Albino rats. Then, the CMs prepared from the human UC-MSC (hUC-MSC) were applied to these rats, and the new islet formation was evaluated histologically. After CM application, islets began to appear in pancreatic sections, and insulin-positive cells were shown in these islets. According to these results, it was stated that MSC-CM might be an important agent that can trigger endogenous islet formation in the treatment of T1D.[104] Nojehdehi et al. used AT-MSC-derived exosomes as therapeutics in the autoimmune experimental T1D model created in mice. They declared that exosome administration increased the Treg population among the spleen mononuclear cells and the level of IL4 and IL10 cytokines released from them. Histological evaluation of pancreatic sections revealed that the number of islets was higher in the exosome-treated groups. Biochemical findings also showed a significant decrease in blood glucose levels.[105] In another study, the effect of AT-MSCs and AT-MSC-CM on experimental T1D was evaluated by comparing routes of administration. The MSC-CM administered intravenously and intraperitoneally were evaluated regarding inflammation by examining Treg, IL4, IL10, IL17, IFNγ, and TGFβ, and β-cell regeneration by labeling pancreatic sections with insulin antibodies. Accordingly, both routes of administration have shown that CMs are effective in the T1D model, but the intraperitoneal route is more effective in immunosuppression.[106] Dias et al. used CM obtained by culturing AT-MSCs in 2D and 3D environments to treat T1D in a mouse model. 3D cell culture was performed for MSCs to form spheroids, and CMs were administered in two consecutive doses to the animals. The results of this study indicate that CMs do not lower the glycemic index or prevent β-cell apoptosis in the pancreas. Pdx1, a β-cell marker, was increased in the 2D-CM group, but this effect was not seen in the 3D-CM.[107] In our previous study, content analysis was performed on CMs produced with UC-MSCs cultured in 2D and 3D environments before examining their in vivo therapeutic effects. Scaffolds were used for the 3D culture, and the cells were grown following a specific topography. Then 2D-CM and 3D-CM were applied to the Sprague-Dawley rats with the experimental autoimmune T1D model within a specific treatment plan as 12 doses. When the results were evaluated in the context of immunomodulation and β-cell regeneration, it was seen that MSC-CMs started to regulate cytokine release by increasing Treg cells. β-cell regeneration was evaluated immunohistochemically with Nkx6.1 and Pdx1 markers and insulin labeling. According to the results, the number of β-cells increased significantly in the treatment groups, especially in the 3D group, and newly formed β-cells in exocrine pancreatic sections have been demonstrated.

These results suggest that UC-MSC-CM is effective in the context of immunomodulation and β-cell regeneration in the T1D model, and this effect could be enhanced by using 3D scaffolds.[48] Although studies are still in their infancy, the MSC-CM application appears to be a promising option for T1D. In addition, it is predicted that MSC-CMs may delay the onset of diabetes-related complications by showing protective effects on other tissues and organs, as well as their positive effects on immunomodulation and β-cell regeneration.

3.2. Type 2 Diabetes

Type 2 Diabetes (T2D), one of the most common metabolic disorders worldwide, is characterized by elevated blood glucose levels, which leads in progress of time to damage to the heart, vasculature, eyes, kidneys, and nerves. It is caused by defective insulin secretion by pancreatic β-cells and insulin resistance, which is the inability of insulin-sensitive tissues to respond to insulin.[108] Ideal therapeutic options for T2D include strategies to improve insulin resistance and promote β-cell regeneration, and MSC-CM may be an essential agent for the treatment of T2D.[109] Hao et al. investigated the effects of multiple BM-MSC infusions on the experimental T2D model, suggesting that paracrine effects of cells are important in therapy due to the low therapeutic role of MSC differentiation. Hyperglycemia, serum insulin, and c-peptide levels of BM-MSC-CM treatment were examined to observe the paracrine effects of MSCs. The results showed that MSC-CM effectively reversed hyperglycemia and improved serum c-peptide and insulin levels.[110] Sun et al. used exosomes isolated from the hUC-MSCs to treat T2D. This study revealed that intravenous single-dose exosome administration effectively decreases blood glucose levels and partially reverses insulin resistance. Exosomes restored the phosphorylation (tyrosine domain) of insulin receptor substrate 1 and protein kinase B in T2D, promoted the expression and membrane translocation of glucose transporter 4 in muscle (GLUT4), and increased glycogen storage in the liver to maintain glucose homeostasis. In addition, it was pointed out that exosome administration could be used to restore the insulin-secreting function of T2D by inhibiting STZ-induced β-cell apoptosis.[111] An in vitro study by Kim et al. concluded that UC-MSC-CM improved insulin resistance in C2C12 cells, and UC-MSC-CM improved glucose uptake by increasing membranous GLUT4 expression and insulin-signaling pathway. This study also showed the enhancement of mitochondrial contents and functions after the UC-MSC-CM application.[112] Another study demonstrated the antioxidant capacity of liver and adipose tissue-derived MSCs-CMs and their therapeutic applicability in T2D. Accordingly, it was observed that the biochemical data of the liver-MSC-CM group were better in terms of blood glucose, liver and renal function tests, and lipid and inflammation markers. At the same time, both CMs were effective in the regeneration of pancreatic islets, oxidative stress, and relief of insulin resistance.[113] Song et al. evaluated the association between BM-MSC-CM and T2D in the context of α-cells different from the other studies. For this purpose, palmitate-induced α-cell line (αTC1-6 cells) and high fructose diet (HFD)-induced mice were used. The glucagon secretory capacities of α-cells were analyzed in the supernatant, and the serum and the potential signaling pathways were investigated. BM-MSC-CM improved blood glucose levels and insulin tolerance. It protected against HFD-induced hyperglycemia and hyperglucagonemia and ameliorated HFD-induced islet hypertrophy, decreased α- and β-cell area. Consistent with in vivo, glucagon secretion from α-cells or primary islets was inhibited by BM-MSC-CM, accompanied by a reduction of intracellular phosphatase and tensin homolog deleted on chromosome 10 (PTEN) expression and restoration of AKT signaling. Moreover, miR-181a-5p overexpression was observed in BM-MSCs but this expression was prominently lower in αTC1-6 cells. Glucagon secretion in aTC1-6 cells was alleviated or aggravated via the PTEN/AKT signaling pathway due to overexpression or downregulation of miR-181a-5p, respectively. In this context, they suggest that miR-181a-5p derived from MSCs mitigates glucagon secretion of α-cells by regulating PTEN/AKT signaling, which gives us novel evidence revealing the potential of MSCs for treating T2DM.[114]

3.3. Nephropathy

Diabetic nephropathy (DN) is a severe microvascular complication caused by DM)and is the most common cause of end-stage kidney disease (ESKD).[115] Characterization of DN includes specific morphological and functional alterations in the renal tissue. In the early period of DN progression, increased glomerular filtration rate (GFR), hypertrophy, microalbuminuria, thickened glomerular basal membrane (GBM), and mesangial expansion is noted, while the progressive decline in GFR, reduced creatinine clearance, increased fibrosis, and macroalbuminuria are the features of advanced stage DN.[116] When the microvascular nature of the DN is considered, the glomeruli and tubular areas are expected to be primarily affected due to the presence of glomerular and peritubular capillaries, respectively.[117] In the glomerulus, the integrity of the glomerular filtration barrier (GFB), which is composed of podocytes along with fenestrated endothelium of capillaries and its basal lamina, is compromised under diabetic conditions, which in turn leads to a slow decline in renal function.[118] Currently, available treatments, including the application of anti-hypertensive drugs and sodium-glucose co-transporter 2 protein inhibitors, slow the progression of the disease. However, they are not able to prevent the development of ESKD, which is the situation necessitating the development of novel therapies capable of stopping disease progression.[117] In this respect, the application of CM obtained from MSCs have promising results in in vitro and in vivo experiments. It was shown that human AT-MSC-CM reduced high glucose-induced apoptosis of podocytes and maintained expressions and arrangements of podocyte-specific synaptopodin and nephrin proteins via increased concentration of EGF.[119] In another study, treatment of mesangial cells with UC-MSC-CM following exposure to the high concentration of glucose led to decreased extracellular matrix depositions and fibrosis by inhibiting TGF-β1-triggered myofibroblast transdifferentiation (MFT) and cell proliferation and increasing the expressions of matrix metalloproteinase 2 (MMP2) and MMP9.[116] In two different diabetic kidney injury models induced by a high-fat diet and application of STZ, administration of MSC and MSC-CM have a similar level of ameliorating effect with respect to inhibition of exacerbated

albuminuria by each one of the two. They exert their therapeutic effects by inhibiting the expressions of pro-inflammatory cytokines and the formation of fibrosis in the tubular interstitium and by maintaining the expressions of tight junction proteins like zonula occludens (ZO-1) which are critical for the suppression of epithelial-to-mesenchymal transition (EMT) of tubular epithelial cells (TECs). In the same study, it was also noted that exosomes obtained from MSC-CM were injected into the subcapsular space of STZ-induced diabetic kidneys, improving the tubulointerstitial degeneration including inflammatory cell infiltration, vacuolization, atrophy, and loss of tight junction proteins of TECs.[18] Furthermore, incubation of TECs isolated from STZ-induced diabetic rats with exosomes derived from MSCs reduced diabetes-related apoptotic cell death and suppressed EMT of these cells.[18] Similarly, CM and specifically exosomes obtained from UC-MSC reduced the secretion of pro-inflammatory cytokines, including TNFα, IL1β, and IL6, and pro-fibrotic factor TGFβ in the high glucose treated renal TECs and glomerular endothelial cells.[120] In the study conducted by our group, it was demonstrated that CM obtained from UC-MSCs preconditioned with deferoxamine (DFX), a hypoxia mimetic agent, led to better improvement of DN compared to CM derived from unpreconditioned MSCs by restoring podocyte loss, reducing apoptosis in cortical tubular area, and regulating the autophagic activity.[54] Moreover, this improvement was found to be associated with more concentrated content of DFX-CM, specifically NGF and GDNF neuroprotective growth factors and pro-angiogenic factor VEGF all of which are crucial for the structural and functional integrity of podocytes and capillary endothelium.[117] This study is also important from another point of view that suitable preconditioning strategies which improve secretome content in favor of therapeutically effective paracrine factor could provide better recuperative results.

3.4. Neuropathy

Diabetic neuropathy (DNeu) is the most prevalent diabetic complication. DNeu is a group of clinical manifestations caused by damage to the peripheral and autonomic nervous systems and occurs in approximately half of the diabetic patients.[121,122] The most common form of DNeu is distal symmetric polyneuropathy, which manifests as loss of sensory function beginning in the distal ends of extremities in a "stocking and glove" distribution.[122] In addition, diffuse neuropathies, including disorders resulting from impairment of the sympathetic and parasympathetic nervous system, such as diabetic cytopathy and impotence, gastrointestinal dysmotility, and cardiac autonomic neuropathy, can occur secondary to diabetes. Focal neuropathies, which are less common, encompass injury of individual peripheral nerves or nerve roots, leading to isolated mononeuropathies and radiculopathy or polyradiculopathy, respectively.[122] In DNeu, multiple cells, including dorsal root ganglion neurons, neuronal axons, and associated Schwann cells, are primarily affected by hyperglycemia and dyslipidemia.[123] Even if glycemic control slows down the progression of the disease, especially in T1D patients, there is no definitive treatment due to the multifactorial etiology of DNeu.[122] In this respect, reduced expressions of neuroprotective, anti-inflammatory, and angiogenic growth factors in peripheral nerves have been implicated in the occurrence and progression of DNeu.[124,125] Unlike conventional pharmacological treatments, the secretome profile of MSCs with neuroprotective, immunomodulatory, angiogenic, and antioxidant factors could provide a multitarget recuperative solution for the treatment of DNeu.[126] Systemic application of CM-AT-MSCs ameliorated DNeu-related functional and structural impairments of transgenic mice (BKS db/db) that spontaneously develop T2D by ameliorating the thermal and mechanical sensitivity, restoring intraepidermal nerve fiber density, decreasing apoptotic deaths of neuron and Schwann cells, and reducing chronic inflammation in peripheral nerve and improving angiogenesis.[126] In another study, exosomes isolated from CM of mouse MSCs were applied to the diabetic peripheral neuropathy model of mice (BKS.Cgm+/+ Leprdb/J, db/db). The exosome application significantly improved thermal and mechanical hypoalgesia, nerve conduction velocity, and histopathological parameters, including the number of intraepidermal nerve fibers, axonal diameters, and myelin sheat thickness of sciatic nerves by shifting the macrophages from pro-inflammatory (M1) to anti-inflammatory (M2) phenotype, and suppressing the pro-inflammatory cytokines such as TNFα, IL1β, and interfering with the Toll-like receptor (TLR)−4/NF-κB signaling pathway.[127] These therapeutic effects of exosomes were found to be related to their enriched miRNAs content, specifically miR-17, miR-23a, and miR-125b involved in the regulation of macrophage phenotype and activation of inflammatory cascade.[127] At this point, exosome contents of MSCs can be engineered to amplify their therapeutic potential. The study conducted by Fan et al. showed that MSC-exosomes loaded with miR-146a, which is well known anti-inflammatory miRNA, have enhanced therapeutic efficiency on diabetic peripheral neuropathy compared to naïve exosomes by inhibiting (TLR)−4/NF-κB signaling pathway.[96] Diabetic cardiac autonomic neuropathy (DCAN) is another life-threatening condition in which parasympathetic nerves that innervate the heart are initially affected, leading to the relative predominance of sympathetic activity and increased risk of ventricular arrhythmia.[128] In the rat DCAN model, the application of MSCs and MSC-CM which contains a detectable concentration of Neurotrophin-3 (NT-3) and NGF, have a similar therapeutic effect on the recovery of parasympathetic and sympathetic nerve fiber density and imbalanced autonomic neural denervation. These findings also support the notion of MSCs showing their recuperative effects in a paracrine fashion.[128] Furthermore, studies conducted by our group showed that preconditioning of MSCs with hypoxia mimetic agents like DFX or dimethyloxallyl glycine (DMOG) led to an increased concentration of neuroprotective growth factors, including NGF and GDNF. Therefore, CMs obtained from MSCs which are preconditioned with hypoxia mimetic agents, could be potential therapeutic options for the treatment of diabetes-associated neuropathies.

3.5. Retinopathy

Diabetic retinopathy (DR) encompasses a series of hyperglycemia-associated pathological changes, including inflammation in the optic nerve, aberrant vascular permeability, decomposition of the blood-retinal barrier, and glial hyperplasia is one of the main reasons for vision loss in older adults.[129]

It was noted that increased expressions of pro-inflammatory cytokines such as IL18, IL1β, and caspase-1 in the retinas of DM rats could bring about apoptotic death of retinal cells. In one study, administration of exosomes obtained from MSCs inhibited the production of inflammatory cytokines and reduced vascular endothelial injury by significantly downregulating high glucose-induced expressions of high mobility group box 1 (HMGB-1), NF-κB/P65 and NOD-like receptor family pyrin domain containing 3 (NLRP3).[130] The same study also showed that MSC-derived exosomes with enhanced expression of miRNA-126 have a similar regulative effect on the expression of HMGB-1 and the activity of NLRP3 in human retinal endothelial cells.[130] In another study, rabbit AT-MSC-derived exosomes improved retinal damage by delivering bioactive factors, including miRNA-222, which is important for the inhibition of abnormal neovascularization in advanced DR by regulating signal transducer and activator of transcription 5A (STAT5) protein expression.[131] Müller cells are the major type of glial cells in the retina and provide homeostatic, metabolic, and structural support to retinal neurons.[132] In an in vitro study, it was shown that application exosomes derived from BM-MSCs inhibited inflammation, oxidative stress, apoptosis, and increased proliferative capacity of the Müller cells treated with high glucose via suppression of TLR4/NF-κB axis by their enriched content of miRNA-486-3p.[133] Similarly, different study groups have shown that EVs have therapeutic effects like delaying DR development exerting anti-apoptotic, and anti-inflammatory effects through the delivery of miRNA-912, miRNA-18b, and miRNA-30c-5p separately.[134–136] In each of the above studies,[131,134–138] ameliorative effects of one specific miRNA (some of which expressions were enhanced by transfection) oligonucleotides in the EVs have been focused on and evaluated. At this point, it would be a good idea to evaluate all the aforementioned miRNAs following enhancing their expressions in vesicles, which could amplify the observed therapeutic effect in a synergistic manner. On the other hand, when the uptake and distribution of intravitreally administered EVs by the cells of the retina is investigated in Sprague Dawley rats, it was found that EVs were not able to get deeper than the inner nuclear layer.[139] Therefore, it is also necessary to modify the surface of EVs for more efficient delivery through the whole retina thickness, enabling better therapeutic results.

3.6. Diabetic Wound

Diabetic wound healing has been the most studied area in the literature regarding the relationship between MSC-CM and diabetes. In particular, diabetic foot ulcer is a common complication of diabetes and may result in amputation following infection after the nonhealing wound. It is one of the most common causes of limb loss.[140] Wound healing is a complex cellular response to injury coordinated by numerous growth factors and cytokines which are secreted from keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets.[141] Numerous growth factors and cytokines released by these cell types are necessary to coordinate and heal wounds. Since MSCs secrete growth factors, cytokines, and chemokines that increase angiogenesis, reduce inflammation, and stimulate fibroblast migration and collagen production, the CM obtained from these cells is considered a crucial therapeutic for wound healing. Proper keratinocyte migration and proliferation during re-epithelialization are interrupted in diabetic individuals; therefore, Li et al. investigated the effects of MSC-CM on the migration and proliferation of keratinocytes in vitro by forming a diabetes-like microenvironment by the application of high glucose medium and LPS. The administration of MSC-CM improved the migration and proliferation of rat keratinocytes by reducing ROS overproduction, reversing the downregulation of MAPK/ERK kinase (MEK) 1/2 and extracellular signal-regulated kinase (Erk) 1/2 phosphorylation, which were induced by high glucose and/or LPS stimulation.[142] In another study, pulse-wave low-level laser therapy with intraperitoneal application of BM-MSC-CM was used to treat open skin wounds in diabetic rats and wound healing was evaluated by biochemical parameters. The results of this study revealed that laser therapy and BM-MSC-CM, both separately or together, improved the biochemical parameters in the wound healing process, but laser therapy was statistically more effective.[143] In a study examining the possible effects of hUC-MSC-CM on diabetes-related epithelial dysfunction, an in vitro and in vivo evaluation was performed using human umbilical vein endothelial cells (HUVEC) grown in high glucose medium and STZ-induced diabetic rat model. When they investigated the mechanism underlying the positive effects, it was concluded that the mitochondrial bioenergetics was improved through the silent information regulator 1 (Sirt1)/ AMP-activated protein kinase (AMPK)/ peroxisome proliferator initiated receptor gamma and coactivator 1 alpha (PGC-1α) signaling pathway, resulting in an amelioration in epithelial dysfunction.[144] Keratinocytes are used as autologous or allografts for wound healing, especially in burn patients, and hence effective cryopreservation of epidermal stem cells is required. A study in which AT-MSC-CM was used for keratinocyte culture and cryopreservation aimed to create a xenograft-free transplantation opportunity by removing the feeder layer required for keratinocyte culture, thus paving the way for safer and cheaper clinical applications. According to the results, keratinocytes treated with MSC-CM rather than commercial solution expressed stem cell and differentiation markers similarly to those treated with the commercial solution and had similar proliferation and migration abilities.[145] In another study, VEGF overexpressed MSC-CM was shown to ameliorate palmitate-induced diabetic endothelial dysfunction through the PI3K/ protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathways and p38 mitogen-activated protein kinase (p38/MAPK) signaling pathway.[146] In a study on diabetic wound healing, photobiomodulation therapy, and BM-MSC-CM were used separately and in combination to treat infected wound models in diabetic rats. The evaluation was performed in terms of stereological parameters and expression of basic FGF (bFGF), hypoxia-inducible factor (HIF-1α), and SDF-1α. The findings showed a decrease in macrophages and neutrophils and an increase in fibroblast counts and angiogenesis in all treatment groups compared to the control group. bFGF, HIF-1α, and SDF-1α expressions were higher in the photobiomodulation therapy group in most cases. At the same time, stereological evaluations revealed that the best results were in the photobiomodulation therapy and BM-MSC-CM group.[147] In another study, as diabetes causes intervertebral disc degeneration, the extracellular

matrix production of MSCs was investigated in the nucleus pulposus following hUC-MSC-CM applications. For this purpose, nucleus pulposus-MSCs were induced with high glucose in vitro, and then the effects of hUC-MSC-CM on these cells were investigated. High glucose triggered apoptosis in cells, decreased aggrecan and collagen II synthesis, and increased p38 MAPK phosphorylation. After the hUC-MSC-CM application, these effects were reversed in nucleus pulposus MSCs. In conclusion, UC-MSC-CM alleviated high glucose-induced extracellular matrix degeneration via p38 MAPK.[148] Saheli et al. examined the effects of BM-MSC-CM on human dermal fibroblasts. In vivo administration of BM-MSC-CM improved wound healing kinetics in diabetic animals; in particular, the rate of wound closure was significantly increased. At the same time, inflammatory response, remodeling, angiogenesis, EGF, and bFGF expressions increase with the application of MSC-CM. Consistently, in vitro findings showed that fibroblasts in high glucose medium treated with MSC-CM showed higher viability, proliferation, and migration, as well as an increase in bFGF gene expression.[149] Hendrawan et al. investigated the effects of CM prepared from hUC-MSCs under hypoxic conditions on wound healing of diabetic rats along with the growth and collagen production of rat fibroblasts. Accordingly, CM prepared under hypoxic conditions contained higher growth factors associated with wound healing. In vitro, results showed that hUC-MSC-CM increased fibroblast cell growth and collagen synthesis, although statistical significance was not observed. In vivo, re-epithelialization and collagen production were increased using hUC-MSC-CM.[150] MSC-CM applications seem promising for wound healing models. However, as an alternative to systemic application, which is the predominant route of administration in the literature, it is thought that topical applications or the development of a CM-containing patch may be beneficial for accelerating wound healing.

3.7. Effects of MSC-CM During Islet Transplantation

The effect of MSC-CM is not restricted to the systemic treatment of diabetes; it could also be effective in vitro or in vivo for other diabetic treatments, such as islet transplantation. Islet transplantation is considered to be an effective treatment option, but its applicability is low due to the lack of donor number, insufficient cell formation after transplantation, and the risk of immune reaction.[151] Implantation difficulties can occur for several reasons, including ischemic injury during removal and delivery of the donor pancreas, enzymatic digestion during islet isolation, and reperfusion injury in the recipient after transplantation.[152] In this regard, the proliferative, angiogenic, and anti-apoptotic properties of MSCs can increase survival, and cell formation after transplantation, and their immunosuppressive properties may reduce the likelihood of rejection. It was indicated in a study that protein fractions secreted by MSCs could activate preserved islets. So, protein fractions concentrated as 3, 10, 30, 50, and 100 kDa from the CM prepared from adipose tissue MSCs were used, and their effects were evaluated by adding them to the preservation solutions of the isolated islets. According to the results, some islets were seen with relative luminescence intensities above 150% of the baseline values by four days. Using these factors, it could be possible to return islets to their pre-culture conditions.[152] Hypoxia is one of the crucial factors reducing the survival of islet cells after transplantation. The angiogenic and protective effects of MSCs can be exploited to increase their chances of survival in the hypoxic microenvironment in which they are transplanted. In another study, CM obtained from MSCs preconditioned under hypoxic conditions increased the recovery and survival rates of the human islets exposed to hypoxia-induced damage compared to the one obtained under normoxic conditions. In addition, mRNA analyses showed that the islets treated with hypoxia preconditioned CM had lower pro-apoptotic Bax and higher anti-apoptotic Bcl2 expressions, all of which indicated that the transition from a pro- to an anti-apoptotic islet phenotype and this could be resulted from the increased secretion of VEGF by the cells exposing to hypoxia.[153] In a study aiming to increase post-transplant survival by protecting islets from hypoxia, MSC-derived exosomes were used, and β-cells (BTC-6) were cultured in the presence and absence of exosomes under 2% oxygen. The results showed that high miR-21 levels in exosomes inhibit ER-stress proteins (GRP78, GRP94, p-eIF2α, CHOP) and p38 MAPK phosphorylation, thus increasing cell viability and reducing apoptosis in islet cells.[154] In another study, when treated with the CM from porcine adipose tissue MSCs, islet cells were activated, and the intracellular ATP concentration increased by 160% compared to the initial level.[155] These studies suggest that the secretome of MSCs can be utilized to increase the survival of islets during islet transplantation. MSC-derived secretome could increase transplantation success, especially by protecting the islets from hypoxia.

4. Limitations of the CM Studies on Diabetes

The studies summarized above show that systemic administration of MSC-CM is promising for the treatment of diabetes and related complications. However, to create an effective CM and a treatment plan accordingly, studies should be progressed by considering some issues. The first of these is content analysis, which is one of the essential advantages of CM treatments over cell therapies. Before the therapy plan is made, the factors in the CM content should be determined and revealed in detail, the factors that may be important for treating diseases should be focused on, and the content balance should be studied. However, as used in most studies, techniques like ELISA and antibody arrays to characterize MSC-CM offer only a limited view of the secreted factors. Proteomic methods would offer a more comprehensive understanding of the MSC secretome's complexity. This approach seeks to create well-defined profiles of MSC-CM, ensuring tailored usage based on each CM's distinct profile. At this point, artificial intelligence (AI) can play a significant role in the secretome profiling of MSCs. Secretome data can be integrated with other omics data, such as transcriptomics and genomics. AI techniques like machine learning and deep learning can analyze these complex multidimensional datasets to identify patterns and relationships and to uncover the biological pathways, networks, and cellular behavior that are influenced by the secreted proteins, all of which may enable to fully unleash the therapeutic capacity of the MSCs.

The half-lives of these factors in CM are another critical aspect that must be taken into consideration for treatment success. Determining the half-life is important both to understand how long CMs can be used effectively after preparation and to know how

Table 1. Summary of studies on MSC-CM and MSC-exosome therapy in diabetes and related complications.

Condition/disease model	Subject	Route	Source of CM	Medium/vessel	Culture condition	Outcome	Ref. number
Streptozotocin-induced diabetes model	STZ-damaged islets/ Six-week-old C57BL/6J mice	Intravenous	Mouse-bone marrow MSC	Conditioned medium	Normal	Islet cell proliferation and an increase in pAkt and pErk expression by islets in vitro/ Increase in the number of islets, β-cells, and insulin-positive β-cells	[103]
Alloxan monohydrate-İnduced T1D model	Male Wistar rats	Intramuscular	Human-umbilical cord MSC	Conditioned medium	Normal	New islet formation, increase in the number of insulin-positive cells	[104]
Streptozotocin-induced autoimmune diabetes model	6- to 8-week old C57BL/6 male mice	Intraperitoneal	Mouse- adipose tissue MSC	Exosomes	Normal	Increase in Treg population and number of islets	[105]
Streptozotocin-induced autoimmune diabetes model	8-week-old C57Bl/6 male mice	Intraperitoneal-Intravenous	Mouse- adipose tissue MSC	Conditioned medium	Normal	Increase in Treg population and anti-inflammatory cytokine levels, decrease in pro-inflammatory cytokine levels. Increase in the number of islets and insulin-positive islets after intraperitoneal administration.	[106]
Streptozotocin-induced T1D model	2–3 months old Swiss mice	Intraperitoneal	Mouse- adipose tissue MSC	Conditioned medium	2D and 3D culture environment	2D-ASC-CM decreased the glycemia, and the pancreatic islets showed a higher expression of the transcription factor PDX-1	[107]
Streptozotocin-induced autoimmune diabetes model	10–12-week-old Sprague-Dawley-type male rats	Intraperitoneal	Human umbilical cord-MSC	Conditioned medium	2D and 3D culture environment	Increase in regulatory T cells, increase in β-cell numbers in islets, β-cell formations in the exocrine pancreas	[48]
T2D model using high fed diet+Streptozotocin	8-week-old Sprague-Dawley-type male rats	Intravenous	Rat-bone marrow MSC	Conditioned medium	Normal	Reversing hyperglycemia and improving serum c-peptide and insulin levels	[110]
T2D model using high fed diet+Streptozotocin	8-week-old Sprague-Dawley-type male rats	Intravenous	Human-umbilical cord-MSC	Exosomes	Normal	Restoration in the phosphorylation (tyrosine domain) of insulin receptor substrate 1 and protein kinase B in T2D, promotion in the expression and membrane translocation of GLUT4 and increased glycogen storage in the liver to maintain glucose homeostasis	[111]
Palmitate-induced insulin resistance model in C2L12 cells	C2L12 cells	-	Umbilical cord-MSC	Conditioned medium	Normal	Improved insulin resistance in C2C12 cells, and enhanced glucose uptake by increasing membranous GLUT4 expression and insulin-signaling pathway	[112]
Streptozotocin-induced T2D	Sprague-Dawley-type male rats	Intravenous	Rat- adipose tissue MSC	Conditioned medium	Normal	Improved biochemical data in terms of blood glucose, liver and renal function tests, and lipid and inflammation markers. Regeneration of pancreatic islets, oxidative stress, and relief of insulin resistance	[113]

(*Continued*)

Table 1. (Continued)

Condition/disease model	Subject	Route	Source of CM	Medium/vessel	Culture condition	Outcome	Ref. number
High fat-induced hyperglucagonemia	Palmitate-induced αTC1-6/Six-week-old male C57BL/6J mice	Intraperitoneal	Rat-bone marrow MSC	Conditioned medium	Normal	Mitigation in glucagon secretion of α-cells by regulating PTEN/AKT signaling	[114]
High glucose induced podocyte injury model	Mouse podocyte clone 5 (MPC5)	In vitro culture	Human- adipose tissue MSC	Conditioned medium	Normal	Reduction of podocytic apoptosis and injury induced by high glucose concentration	[119]
High glucose induced mesengial cell injury model	Mouse mesangial cell line (SV40-MES-13)	In vitro culture	Mouse- umbilical cord MSC	Conditioned medium	Normal	Decreased extracellular matrix depositions and fibrosis by mesangial cells	[116]
High fat diet-induced T2D-associated kidney injury	Eight-week-old male C57BL/6J mice	Intravenous	Rat- bone marrow MSCs	Conditioned medium	Normal	Inhibition of exacerbated albumunuria, and pro-infllammatory cytokine expression and fibrosis in tubular interstitium.	[18]
Streptozotocin-induced diabetes associated kidney injury	57BL/6-Tg (CAG-EGFP) (GFP-transgenic; GFP-Tg) mice	Intravenous	Rat- bone marrow MSCs	Conditioned medium	Normal	Inhibition of exacerbated albumunuria, and pro-infllammatory cytokine expression and fibrosis in tubular interstitium.	[18]
Streptozotocin-induced diabetes associated kidney injury	57BL/6-Tg (CAG-EGFP) (GFP-transgenic; GFP-Tg) mice	Administration into the renal subcapsular space	Rat-bone marrow MSCs	Exosomes	Normal	Improvment in tubulointerstitial degeneration	[18]
Streptozotocin-induced diabetic nephropathy model	10-12 week-old Sprague Dawley rats	Intraperitoneal	Human-umbilical cord MSCs	Conditioned medium	Normal and DFX preconditioned cell culture	CMs obtained in either conditions restored podocyte loss and damage. Better improvement in albumin-creatinine ratio for CM obtained from DFX preconditioned MSCs.	[64]
Diabetic polyneuropathy model	18 week-old diabetic BKS db/db mice	Intravenous	Human- adipose tissue MSCs	Conditioned medium	Normal and DFX preconditioned cell culture	Improvement in thermal and mechanical sensitivity	[126]
Diabetic peripheral neuropathy	20 week-old mice (BKS.Cgm+/+ Leprdb/J, db/db)	Intravenous	Mouse- bone marrow MSCs	Exosomes	Normal	Improvements in thermal and mechanical sensitivity, nerve conduction velocity, and histopathological parameters	[127]
Diabetic peripheral neuropathy	20 week-old mice (BKS.Cgm+/+ Leprdb/J, db/db)	Intravenous	Engineered mouse- bone marrow MSCs derived exosome carrying miR-146a	Exosomes with enriched miR-146a content	Normal	Improved nerve conduction velocity, and thermal and mechanical sensitivity	[96]

(*Continued*)

Table 1. (Continued)

Condition/disease model	Subject	Route	Source of CM	Medium/vessel	Culture condition	Outcome	Ref. number
Diabetic cardiac autonomic neuropathy	6 week-old male Sprague-Dawley rats	Injection into left ventricular myocardium	Rat- bone marrow MSCs	Conditioned medium	Normal	Balanced autonomic neural innervation by the recovery of parasympathetic and sympathetic nerve fiber density	[128]
Retinopathy in streptozotocin-induced diabetes model	Wistar rats	Intravitreal injection	Engineered human-umbilical cord MSCs derived exosome carrying miR-126	Exosomes with enriched miR-126 content	Normal	Reduced hyperglycemia-induced retinal inflammation	[130]
Retinopathy in streptozotocin-induced diabetes model	Male rabbits	Intravenous or Subconjunctival (SC) or intraocular (IO) injection	Rabbit- adipose tissue MSCs	Exosomes	Normal	SC or IO injection of the exosomes restored organisation of retinal layers	[131]
High glucose induced Müller cell injury model	Mouse Müller cells	In vitro culture	Bone marrow MSCs	Exosomes	Normal	Inhibition of inflammation, oxidative stress, apoptosis, and increased proliferative capacity	[133]
In vitro wound healing model in high glucose and LPS microenvironment	Sprague-Dawley rats' keratinocytes	In vitro culture	Rat- bone marrow MSCs	Conditioned medium	Normal	Counteraction of the effects of HG and LPS. Decreased HG- and/or LPS-induced ROS overproduction. Reversed HG and/or LPS-induced downregulation of phosphorylation of MEK1/2 and Erk 1/2	[142]
T1D-related wound healing	4 months-old Wistar male adult rats,	Intraperitoneal	Human-Bone Marrow MSC	Conditioned medium	Normal	Increased biomechanical parameters within the healing wounds	[143]
Diabetic endothelial dysfunction	High glucose-induced HUVECs	In vitro culture	Human-umbilical cord MSCs	Conditioned medium	Normal	Protective effects on endothelial cells, concerning glucotoxicity, by ameliorating mitochondrial dysfunction via the PI3K/Akt/Sirt1 pathway, and Sirt1 potentiated mitochondrial biogenesis, through the Sirt1/AMPK/PGC-1α pathway.	[144]
Keratinocyte application for wound healing	Human abdominal skin's keratinocytes	In vitro culture	Human- adipose tissue MSCs	Conditioned medium	Normal	Similar results to commercially used culture media	[145]
Palmitate-induced diabetic endothelial dysfunction	The islet microvascular endothelial cell line (MS-1)	In vitro culture	VEGF over-expressing mouse- bone marrow MSCs	Conditioned medium	Normal	Amelioration in palmitate-induced diabetic endothelial dysfunction through the PI3K/AKT/mTOR pathways and p38/MAPK signaling pathway	[146]

(*Continued*)

Table 1. (Continued)

Condition/disease model	Subject	Route	Source of CM	Medium/vessel	Culture condition	Outcome	Ref. number
T1D-related wound healing	Wistar male adult rats	Intraperitoneal	Human- bone marrow MSC	Conditioned medium	Normal	Acceleration in the healing process by the induction of anti-inflammatory and angiogenic activities	[147]
Diabetes-related intervertebral disc degeneration model in vitro	High glucose-induced nucleus pulposus MSC	In vitro culture	Human-umbilical cord MSC	Conditioned medium	Normal	Alleviation in high glucose-induced extracellular matrix degradation via the p38 MAPK pathway	[148]
Diabetic wound healing model in vitro/ in vivo	High glucose induced-human dermal fibroblasts/ Wistar male rats	In vitro culture/ intraperitoneal	Human- bone marrow MSC	Conditioned medium	Normal	A significantly higher cell viability/proliferation, migration, and bFGF gene expression, in vitro/Upregulation of EGF and bFGF genes, improvements in the healing kinetics of diabetic wound, in vivo	[149]
Diabetic wound healing model in vitro/ in vivo	Rat fibroblasts/14 week-old Sprague–Dawley rats	In vitro culture/ intradermal	Human-umbilical cord MSC	Conditioned medium	Hypoxic conditions	Higher re-epithelialization and collagen production	[150]
Islet transplantation preparation	Luc-Tg rat islets	In vitro culture	Rat- adipose tissue MSCs	Conditioned medium	Normal	Activation of preserved islets	[152]
Islet transplantation preparation	Human islets	In vitro culture	Human- adipose tissue MSCs	Conditioned medium	Hypoxic condition	Increased survival and in vitro function of hypoxic human islets	[153]
Hypoxia-induced β-cell apoptosis	B-cells (βTC-6)	In vitro culture	Human-umbilical cord MSCs	Exosomes	Normal	Protection of β-cells against apoptosis induced by hypoxia, alleviating ER stress and inhibiting p38 MAPK signalling.	[154]
Islet transplantation preparation	Porcine islets	In vitro culture	Porcine-derived adipose tissue MSC	Conditioned medium	Normal	Activation of islet cells, 160% increase in the intracellular ATP concentration compared to the initial level	[155]

often to repeat the dose in vivo. Secondly, the in vivo outcomes of CM therapies should be explained by associating them with the factors in the CM content and revealing the mechanisms. As these studies increase, more effective CMs could be produced. Another critical issue is the stem cell source of CMs. It should always be considered that different MSCs may secrete different secretomes. The type, age, and physiological state of the stem cell source will be important in terms of the secretome. The studies in the literature highlight the variations in MSC secretomes based on their cellular origins.[24,156,157] Consequently, prior to the clinical implementation of MSC secretome-based products, it becomes crucial to identify the cell sources that hold the most significant promise for specific applications linked to tissue regeneration. This targeted approach ensures optimal utilization for each regenerative context. A wise choice regarding the source of stem cells would have a significant impact on the target disease.

The optimal number of cell passages used in MSC-CM production is also a critical consideration. Initial passages exhibit considerable heterogeneity, while a more homogeneous population of MSCs is obtained as the passage progresses, which improves CM content quality. However, late-passage cells might undergo senescence and differentiation, altering the secretome composition. Hence, precise definition and standardization of cell passage numbers are crucial in CM preparation. This guarantees consistent quality, safety, reproducibility, and efficiency, aligning with intended outcomes. In addition, preconditioning or priming strategies which have been a hot topic recently to enrich the therapeutic potential of MCS-CM could be a very promising but it must also be kept in mind that such manipulation could bring their own side-effects along with the expected improvement in recuperative effect that is on focus, which is the situation that imposes the more elaborate and comprehensive analysis is imperative following the MSC-CM application.

The availability of cost-effective large-scale production of conditioned medium with defined standards is still a problem that needs to be finalized for utilization of the therapeutic potential of MSCs-derived CMs for therapeutic purposes. As MSC-CM studies progress, our knowledge of optimizing the secretion properties of MSCs will increase and thus the GMP-compliant procedures necessary to create large-scale MSC-CM will be developed. Apart from production, storage and transport processes are also important. It is vital in preserving the qualities and functions that the content of MSC-CMs does not change at any of these stages.

5. Conclusion and Prospects

In this review, the current status of the therapeutic potential of MSC-derived soluble and nonsoluble paracrine factors, collectively found in the CM, was evaluated in the context of a treatment option for diabetes and its complications (**Table 1**), which imposes unequivocally a major health challenge in the last two decades. At this point, when the limited implantation time and survival of MSCs and the potential ameliorating effects of a wide range of bioactive factors produced by MSCs in the regulation of various physiological processes are taken into account, the secretome content of MSCs has been a focus point for the clinical implementation of these cells in regeneration and repair of diabetes-related damage, dysfunction and failure of the organs. Studies indicate that the secretome of MSC reverses the decrease in Treg in the pathogenesis of diabetes, and the cytokine profile changes depending on the modifications of the immune system cells. These findings mark a significant stride toward treatment as Tregs are essential for suppressing autoimmunity that destroys β cells. Furthermore, significant discoveries have emerged from studies aiming to restore diminished β cell mass and reverse the damage in the kidney, retina, skin, and nerve tissue that develops due to complications related to diabetes. This progress is attributed to secretome factors that trigger cell growth, tissue repair, and regeneration. In addition, reduction of ROS production and suppression of apoptosis due to MSC secretome also contribute to this cellular recovery. Nevertheless, despite the potential demonstrated by MSC-CM in in vitro and in vivo research to meet the various treatment needs of diabetes and its complications, a definitive therapeutic approach has not yet been established. One of the reasons for this predicament is that the mechanisms of the progression of diabetes-related complications are not fully deciphered, which hampers the determination of active pharmaceutical ingredients that could be possibly obtained from MSCs. Moreover, the secretory profiles of MSCs with respect to soluble and insoluble components show variations depending on many parameters like sources of the MSCs and under which culture conditions they are obtained, which is the situation that imposing the necessity of forming a repertoire of secretome profile. Preconditioning strategies are crucial in this context to enhance the concentration of key proteins identified within the secretome, particularly those pivotal for effective treatment. In this way, the most therapeutically effective matching of disease-secretome content can be achieved. In conclusion, unveiling the regulation of secretory mechanisms of MSCs in correlation with their in vivo paracrine effects is of paramount importance for the development of prospective treatments that can readily be used in clinic.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. In the graphical abstract image, figures, and schemes some elements were adopted from www.humanbiomedia.org.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

B.I. and S.O. contributed equally to this work. B.I, S.O., and M.K. have conceptualized the review and contributed to the acquisition, analysis, and interpretation of data and the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Keywords

conditioned medium, diabetes mellitus, extracellular vesicles, mesenchymal stem cells, secretome

Received: June 21, 2023
Revised: September 1, 2023
Published online: September 20, 2023

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