Considerations on the harvesting site and donor derivation for mesenchymal stem cells-based strategies for diabetes

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ABSTRACT

Mesenchymal Stem Cells (MSCs) possess important characteristics that could be exploited in therapeutic strategies for Type 1 Diabetes (T1D) and for certain complications of Type 2 Diabetes (T2D). MSCs can inhibit autoimmune, alloimmune and inflammatory processes. Moreover, they can promote the function of endogenous and transplanted pancreatic islets. Furthermore, they can stimulate angiogenesis. MSC functions are largely mediated by their secretome, which includes growth factors, exosomes, and other extracellular vesicles. MSCs have shown a good safety profile in clinical trials. MSC-derived exosomes are emerging as an alternative to the transplantation of live MSCs. MSCs harvested from different anatomical locations (e.g. bone marrow, umbilical cord, placenta, adipose tissue, and pancreas) have shown differences in gene expression profiles and function. Data from clinical trials suggest that umbilical cord-derived MSCs could be superior to bone marrow-derived MSCs for the treatment of T1D. Autologous MSCs from diabetic patients may present abnormal functions. BM-MSCs from T1D patients exhibit gene expression differences that may impact in vivo function. BM-MSCs from T2D patients seem to be significantly impaired due to the T2D diabetic milieu. In this review, we highlight how the harvesting site and donor derivation can affect the efficacy of MSC-based treatments for T1D and T2D.

CELL-BASED STRATEGIES FOR DIABETES

Glucose metabolism and glycemia are controlled by the secretion of insulin from pancreatic islet beta cells. Beta cells can be lost, can be impaired, or can become impaired due to very different mechanisms. The lack or insufficiency of their insulin release function leads to a group of diseases with characteristic pathological features: abnormal metabolism of carbohydrates and elevated levels of glucose in the blood and urine¹.

Type 1 diabetes (T1D) is a multifactorial chronic disorder that is characterized by the autoimmune destruction of insulin-producing pancreatic beta cells: the disease becomes clinically overt when the vast majority of beta cell lose function or are lost²-⁴. To date, there is no definitive cure for this disease and life-long exogenous insulin replacement is required⁵.

Differently, Type 2 diabetes (T2D) is characterized by insulin resistance, hyperglycemia and eventually dysfunction of the insulin-producing cells, and it is mainly caused by diet and lifestyle choices⁶.

Transplantation of cadaveric pancreas or pancreatic islets can correct diabetes-restoring normo-glycemia in T1D patients⁷-¹¹. Unfortunately, the low number of organs available for transplantation and the need for immunosuppression (often characterized by serious side effects¹²) limit these transplantation strategies¹³. The identification of an inexhaustible source of transplantable insulin producing beta cells is an important hurdle that still needs to be overcome¹³, but the recent activation of clinical trials testing the safety of embryonic stem cell-derived pancreatic progenitor cells represents an important milestone in that direction (NCT 02239354, ClinicalTrials.gov). Beta cell replacement would be extremely beneficial for T1D patients, and beta cell supplementation could be beneficial for a subset of T2D patients. Nevertheless, patients with T1D and T2D would
benefit from strategies that modulate immunity and inflammation, that protect or sustain beta cells, that improve islet transplantation and that stimulate angiogenesis. During the last decade, a cell population has catalyzed significant interest and has been tested in a number of clinical trials for diabetes: Mesenchymal stem cells (MSCs)

MSCs possess important characteristics that could be exploited in cell-based strategies for T1D and for complications of T2D. These cells showed a good safety profile in initial clinical trials for T1D and T2D. In order to maximize their therapeutic efficacy, important considerations related to the harvesting site and to donor derivation need to be made.

**Mesenchymal stem cells**

Mesenchymal Stem Cells (MSCs) were first described in the 1970s by Friedenstein et al. who isolated a population of cells from mouse bone marrow (BM) and showed these had the ability to form colonies. About twenty years later Caplan defined the corresponding terminology, and approximately ten years later MSCs were identified in human adult BM. MSCs are characterized by the adherence of plastic in culture, expression of a set of surface markers in the absence of lineage-specific marker expression, and potential to differentiate into multiple mesodermal lineages (osteoblasts, adipocytes, and chondroblasts). MSCs are potent immunomodulators, exerting suppressive functions on immune effector cells and orchestrating the action of other regulatory cells. MSCs are able to migrate to sites of inflammation and to regulate the traffic of different hematopoietic cells. Moreover, MSCs have been shown to promote repair and regeneration of endogenous and transplanted islets. Furthermore, they have shown a good safety profile in clinical trials, including a very limited risk of tumor formation.

The functional capacity of MSCs, together with their responsiveness to inflamed or damaged microenvironments, have made them an attractive potential agent for many regenerative, anti-inflammatory, and auto-immune applications for a wide range of disorders. MSC-based therapies for T1D are mostly focused on alleviating hyperglycemia by inhibiting autoimmunity, stimulating pancreatic beta cell regeneration and function. MSC-based therapies for T2D are more focused on the treatment of co-morbidities. The main therapeutic effect of MSCs seems to derive from their release of cytokines and soluble factors – molecules determine immunosuppressive, anti-inflammatory, pro-angiogenic and pro-regenerative changes. Alleviation of hyperglycemia seems to be the net result of a dampening of the immune responses, along with a stimulation of the survival and of the proliferation of pancreatic progenitors/beta cells. MSC transdifferentiation towards insulin producing beta cells is not considered a major therapeutic mechanism.

The pathways determining anti-inflammatory and immunosuppressive effects have not been completely elucidated, but direct contact with effector cells, the production of soluble mediators and the activation of regulatory cell subtypes, all may contribute to the MSC effect. MSCs can inhibit dendritic cells differentiation and maturation, suppress the proliferation of CD4+ and CD8+ T cells, impair the cytotoxic activity of cytotoxic lymphocytes, induce and expand T regulatory cells, and can balance T helper subsets. Thanks to the interaction of MSC receptors with ligands indicating inflamed environments, MSCs selectively home in inflamed tissues and promote tissue repair and regeneration. MSCs secrete several molecules (such as IL-6, IL-8, TGF-beta, TIMP-2, VEGF, HGF), which can stimulate tissue repair and act as chemo-attractants, recruiting macrophages and endothelial cells at the site of injury or inflammation. MSCs also appear to have angiogenic and trophic potential that improve, in a co-transplant setting, the ability of pancreatic islets to survive the first few days after transplantation.

In fact, several models of islet transplantation showed positive effects of MSCs in promoting engraftment and increasing survival and function of beta cells. MSCs from recipient rats mediated such an effect when co-transplanted with allogeneic islets, resulting in long term survival and sustained normoglycemia. The effect of MSCs in this model could be due either to an anti-inflammatory effect or an immunomodulatory effect, or to a combination of both.

The positive effect observed in this study was paralleled by increased neoangiogenesis at the implant site, a key observation that highlights the multiple mechanisms of action of MSCs.

In stringent models of transplantation in fully allogeneic recipients, the co-administration of MSCs with islets led to highly significant prolongation of
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The treatment was found to be safe; moreover, at one year after MSCs infusion, most of the patients treated with MSC transplantation showed the preservation of stimulated C-peptide secretion, a key marker of the insulin release from residual beta cells, whereas the control patients showed a decline in C-peptide levels. Patients in both the treated and control groups continued to require insulin therapy and there were no statistically significant differences in insulin requirements and glycated hemoglobin levels between the two groups.

Several clinical trials are currently testing MSCs transplantation in patients with T1D and T2D. So far, MSC transplantation has showed a good safety profile with a very limited risk of tumor formation. An open label pilot trial enrolled T1D patients with recent onset of diabetes. Twenty patients were randomized to the group receiving transplantation of autologous bone marrow-derived MSCs (BM-MSCs), or to the control group which only received insulin therapy. The treatment was found to be safe; moreover, at one year after MSCs infusion, most of the patients treated with MSC transplantation showed the preservation of stimulated C-peptide secretion, a key marker of the insulin release from residual beta cells, whereas the control patients showed a decline in C-peptide levels. Patients in both the treated and control groups continued to require insulin therapy and there were no statistically significant differences in insulin requirements and glycated hemoglobin levels between the two groups.

Ongoing clinical trials are also testing the safety and efficacy of MSCs transplantation in patients with T2D in order to treat common complications of diabetes such as ulcers, limb ischemia,
and nephropathy, and to improve metabolic con-
trol\textsuperscript{31,53,54}. Treatment with MSCs was reported to
be safe, it appeared effective in facilitating wound
closure of diabetic foot ulcers\textsuperscript{55} and in inducing
T reg cells in T2D patients\textsuperscript{56}. Allogeneic placenta
(PL)-derived MSCs were transplanted in 10 pa-
tients with T2D and the infusion was reported to be
safe. The patients experienced a reduction in daily
insulin requirement, showed a better control of
blood glucose fluctuations, and experienced impro-
vements in quality of life\textsuperscript{57}. Interestingly, a recent
meta-analysis on clinical reports highlighted that
the tissue source of the MSCs impacts the outcome
of the cell therapy\textsuperscript{58}.

**BOX 1. MSC-Derived exosomes and diabetes.**

Exosomes (EXOs) are nanoscopic (30-100 nm) biological entities that are secreted as vesicles in the extracellular environment
by many different types of cells\textsuperscript{59}, including MSCs. MSC-derived exosomes (MSC-EXOs) are emerging as a new important
paracrine mechanism for cell-to-cell communication, implicated in wound healing, injury and tissue repair. They are
known to contain proteins, mRNAs and microRNAs\textsuperscript{60,61}; moreover, they have immunostimulatory and immunoregulatory
functions\textsuperscript{59,62,63}. Certain EXOs and cargos present molecular signatures of pathological processes and could be implicated in
the pathogenesis of multiple pancreatic diseases, such as T1D, T2D, diabetic nephropathy, diabetic retinopathy, gestational
diabetes mellitus, and pancreatic cancer\textsuperscript{64,65}. EXOs can be easily isolated from different body fluids collected by non-
 invasive methods and therefore have the potential to be utilized for the analysis of disease biomarkers. EXOs can also be
easily collected from the supernatant of in vitro cell cultures. EXOs derived from MSC cultures were shown to promote
regulatory T cell (T reg) activity, inhibit Effector T cells, natural killer (NK) cells and dendritic cells (DCs) activities\textsuperscript{66,67}. The advantages of using EXOs instead of live cells are connected to their minimal immunogenicity (allowing an allogenic use),
low inherent toxicity\textsuperscript{68}, and potentially lower risk for tumor formation\textsuperscript{69}. Moreover, because of their chemical composition
and small size, EXOs may easily diffuse across the biological barriers reaching target cells. A common assumption in the
context of T1D is that imbalances between Effector T cells and T regs, as well as DC presentation of islet auto-antigens,
play a major role in the destruction of islet β cells\textsuperscript{70,71}. The beneficial effect of MSCs for the treatment of T1D derives largely
from their immune-modulatory and anti-inflammatory secretome. Therefore, MSC-EXOs might be employed as immune
modulators in MHC-mismatched recipients, overcoming the potential immunogenicity of MSC in an allogenic setting\textsuperscript{68}. EXOs/microvesicles derived from endothelial progenitor cells combined with islets can activate angiogenesis improving
revascularization and pancreatic beta cell function\textsuperscript{72}. The same study observed that EXOs/microvesicles also inhibited
endothelial-leukocyte interaction. MSC-EXOs could have important proangiogenic effects. Sheng et al\textsuperscript{73} showed the other
side of the coin: insulinoma-derived EXOs contain diabetes-triggering autoantigens that may stimulate autoreactive T cells
inducing inflammatory cytokine secretion and activating antigen-presenting cells. In accordance with this study, suggesting
that exosomes could serve as triggering factors for specific autoimmunity events leading to diabetes, also Rahman et al\textsuperscript{74}
and Lukic et al\textsuperscript{75} propose a possible causative role of the islet MSCs and their EXOs in triggering the islet-specific autoimmunity
in the NOD mouse strain. During beta cell apoptosis in the islet, MSCs might be activated or recruited in islets to repair the
damage and, therefore, could become a source of EXOs able to initiate autoimmune response\textsuperscript{74}.

**Harvesting site**

An important open issue is represented by the site of
MSCs harvest (Figure 1). Historically, as it was men-
tioned before, the bone marrow has been long investi-
gated as a source of stem cells, and therefore also the
studies concerning MSCs in the context of diabetes
were conducted on BM-MSCs. However, the clinical
applicability of BM-MSCs is limited due to the rela-
tively invasive procedure required for sample collec-
tion as well as the marked reduction in cell number,
proliferation, and differentiation capacity with the age
of the donor\textsuperscript{76}. Thus, various different tissues have
been studied as alternatives sources of MSCs.

It is now accepted that MSCs can be harvested from multiple anatomical locations, and it has been
widely assumed that MSCs derived from different

**sources are largely equivalent, at least in terms of
surface marker expression and differentiation poten-
tial. On the other hand, evidence suggests differ-
ences in term of marker/gene expression profiles; these differences may have a profound impact on
MSCs function\textsuperscript{13,77,78} and clinical efficacy\textsuperscript{58}.

In recent years, multiple alternative sources of
MSCs have shown a great potential, including umbil-
icord (UC), umbilical cord blood (UCB), and adi-
pose tissue (AT). Cells derived from UC and UCB are
easily bankable and offer the theoretical advantage of
youth\textsuperscript{7}. The advantages of using MSCs from birth-as-


ased proliferative capacity in vitro, especially under hypoxic conditions, in comparison to certain MSCs populations obtained from adult tissues.

Another MSCs source that currently commands great attention is the adipose tissue (AT), which can be readily collected and processed for autologous use. AT-MSCs have been found to have proliferative ability and differentiation potential comparable to those of BM-MSCs \(^8^0\). Therefore, adipose tissue offers important advantages when compared to bone marrow, given its availability and ease of collection.

It is now evident that MSCs from these tissues and from BM are morphologically and immunophenotypically similar, but not identical\(^8^1\). UCB-derived MSCs form the fewest colonies and show the highest proliferative capacity, whereas AT-MSCs form the greatest number of colonies, and BM-MSCs have the lowest proliferative capacity. MSCs from AT and UCB\(^8^2\) may gain more popularity because of the versatility of the tissue sources and because of their great potential for a wide range of clinical applications.

Jeon et al\(^8^3\) isolated MSCs from the placenta and adipose tissue, and showed significant molecular differences in the properties of the MSCs according to their cellular source. The cytoskeleton proteins were abundantly expressed in BM-MSCs and in AT-MSCs, while the oxidative stress proteins and apoptosis proteins were abundantly expressed in PL-MSCs. Therefore, authors suggest that PL-MSCs may be more appropriate for treatments that aim to increase therapeutic ability.

In the context of diabetes, the source of the MSCs is considered important. Pancreas and pancreatic islet-derived MSCs (first isolated in 2001\(^8^4\)) could be considered a better option than other commonly used MSCs\(^8^5\). Pancreatic-islet derived MSCs may have the peculiar ability to enter the pancreatic endocrine differentiation path, although the level of transcriptional and functional maturation is still far from that expected of true beta cells\(^1^3\). The increasing interest in pancreas-derived MSCs is due to their potential use for the modulation of immune function, stimulation of angiogenesis, and potentiation of islet endocrine functions; moreover, these cells may differentiate into beta like cells with a yield superior to that of MSCs from different sources, without the need of additional genetic engineering – but this differentiation potential is still debated\(^8^5-8^9\). As stated, the tissue source of the transplanted MSCs seems to impact the outcome of the therapy in the clinical setting: importantly, UC-MSCs appeared to be superior to BM-MSCs in improving C-peptide levels in T1D patients\(^5^8\). The main characteristics of MSC harvested from different sources are summarized in Table 1.

### AUTOLOGOUS OR ALLOGENIC

Another important matter of debate is whether autologous or allogenic MSCs are more suitable for therapeutic strategies in T1D and T2D. Under pathological conditions, MSCs can become functionally compromised. Autologous MSCs may present abnormal functions due to the autoimmune process in T1D, or due to the diabetic microenvironment in

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**Table 1.** Main characteristics of MSC harvested from different sources.

<table>
<thead>
<tr>
<th>MSC Source</th>
<th>Main characteristics</th>
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</thead>
<tbody>
<tr>
<td>BM</td>
<td>Long investigated. Invasive procedure for sample collection. Yield may be limited in aging individuals.</td>
</tr>
<tr>
<td>AT</td>
<td>Morphologically and immunophenotypically similar to BM-MSC. Proliferative ability and differentiation potential similar to BM-MSC. Easy accessible, highly available, easily bankable, no invasive procedure for sample collection. Important advantages for autologous applications.</td>
</tr>
<tr>
<td>UC, UCB</td>
<td>Morphologically and immunophenotypically similar to BM-MSC. Increased proliferative capacity. Increased expression of oxidative stress proteins. Easy bankable, no invasive procedure for sample collection.</td>
</tr>
<tr>
<td>PL</td>
<td>Morphologically and immunophenotypically similar to BM-MSC. Increased proliferative capacity. Increased expression of oxidative stress proteins. Easy bankable, no invasive procedure for sample collection.</td>
</tr>
<tr>
<td>P, PI</td>
<td>Features and differentiation capacity in line with those of MSC from other sources; potential for the stimulation of islet-specific functions, potentially easier differentiation into beta cells.</td>
</tr>
</tbody>
</table>

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**Abbreviations:** BM, bone marrow; AT, adipose tissue; UC, umbilical cord (including Wharton’s Jelly); UCB, umbilical cord blood; PL, placenta; P, Pancreas; PI, Pancreatic Islets.
both T1D and T2D. The main characteristics of autologous BMMSCs isolated from T1D and T2D patients are summarized in Table 2. Allogeneic MSCs may be recognized and may be rejected by the competent immune system of the recipient, may transmit donor-derived infections or diseases.

Studies addressing potential abnormalities in MSCs derived from patients with autoimmune or inflammatory disorders are scarce and somewhat contradictory. To date, available evidence is still not strong enough to support a recommendation, and more studies should be performed in order to fully establish advantages and weaknesses of autologous or allogeneic MSCs.

Thus, studies that investigate characteristics of autologous MSCs isolated from both T1D and T2D patients are essential to improve the knowledge of the effect that the host environment has on stem cell function, and therefore to guide future clinical applications.

**Autologous T1D-MSCs**

Recent studies analyzing functions of T1D BM-MSCs demonstrated that T1D and healthy BM-MSCs exhibit no differences in terms of morphology, immune-suppressive activity, and migration capacity. However, some studies revealed differential expression of genes related to cytokines, immunomodulation, and wound-healing potential, which would be important to investigate further.

A study by Yaochite et al. evaluated the in vitro properties and the in vivo therapeutic efficacy of BM-MSCs isolated from newly diagnosed (6 weeks, corresponding to early stages after clinically overt disease) T1D patients. T1D BM-MSCs showed morphology, immunophenotypic profile, and adipocyte differentiation capacity comparable to healthy MSCs. MSCs in inflammatory environments develop immunosuppressive functions by molecules of acute phase inflammation, especially tumor necrosis factor alpha (TNFα) and interferon gamma (IFN-γ), or toll-like receptor (TLR) ligands. In the study by Yaochite et al., microarray analysis was performed and no significant differences were observed in the expression of immunomodulatory genes (PDL1, NOS2, IL10, PTGES, TGFβ1, PDL2, HLAG, and TGS6) and licensing-related genes (IFNGR2, TNFR1, IFNGR1, TNFR2, TLR4, and TLR3). However, the HGF gene was significantly downregulated in T1D BM-MSCs.

When administered to diabetic mice, both T1D-MSCs and healthy donor-derived MSCs showed equal contribution to improving β-cell mass, increasing insulin production and glucose tolerance. Therefore it seems that T1D-MSCs do not present functional abnormalities.

Accordingly, Dong et al. reported that MSCs isolated from diabetic rats decreased blood glucose levels and prevented body weight loss when transplanted into diabetic animals, suggesting that diabetes does not influence MSCs properties and supporting the use of autologous MSCs in the treatment of T1D patients.

On the contrary, Fiorina et al. supported the hypothesis that transplantation of MSCs derived from nondiabetic donors, rather than autologous MSCs, would be the best option for the treatment of T1D; in fact, they reported that MSCs isolated from non-obese diabetic (NOD) mice were unable to delay the onset of diabetes when administered to pre-diabetic NOD mice and did not reverse hyperglycemia with already established diabetes.

Studies have demonstrated the beneficial role of MSCs on in vivo and in vitro induction/proliferation of Treg cells, but neither the study conducted by Yaochite et al. nor the study by Fiorina et al. observed significant modifications. Opposite results were reported by Madec et al. Yaochite suggested that their analyses were performed 35 days after MSCs administration, which may repre-

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**Table 2. Main characteristics of autologous BM-MSCs isolated from T1D and T2D patients.**

<table>
<thead>
<tr>
<th>Autologous T1D BM-MSCs</th>
<th>Autologous T2D BM-MSCs</th>
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<tbody>
<tr>
<td>No differences compared to healthy BM-MSCs in term of morphology, immune-suppressive activity, and migration capacity</td>
<td>No differences compared to healthy BM-MSCs in term of phenotype, morphology, and multilineage differentiation potential</td>
</tr>
<tr>
<td>Differential expression of genes related to cytokines, immunomodulation, and wound-healing potential</td>
<td>Decreased potency, these cells appear to be terminally differentiated</td>
</tr>
<tr>
<td>Dysfunctional secretome composition, affecting pro-angiogenic functions</td>
<td>Several oxidative stress-dependent dysfunctions</td>
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sent too long a period of time to detect alterations in Treg cell frequency. Therefore, on the one hand further experiments should be performed earlier after cell transplantation, and on the other hand the beneficial effects promoted by administration MSCs are not related to late or long-standing expansion of Treg cells93.

Another recent study by Davies et al91 investigated whether BM-MSCs from T1D patients offer a therapeutic cell source equivalent to healthy donors BM-MSCs. Differences in gene expression were observed between healthy and late-stage T1D donors in relation to cytokine secretion, immunomodulatory activity, and wound-healing potential - suggesting a state of disease memory in these cells. Long-term exposure to the diabetic environment has been suggested to induce disease memory in BM-MSCs100. Despite differential gene expression, T1D-MSCs did not demonstrate a significant difference from healthy controls in immunosuppressive activity, migratory capacity, or hemocompatibility. Therefore, the authors concluded that MSCs from T1D donors are phenotypically and functionally similar to healthy control MSCs indicating their suitability for use in autologous cell therapy91.

In another recent study by de Lima et al92, BM-MSCs from newly-diagnosed T1D patients (within 6 weeks from diagnosis) were compared with those from healthy individuals for morphological characteristics, immunophenotypical characteristics, differentiation potential, and gene expression profile. T1D-MSCs and control MSCs showed similar morphology, immunophenotype, and multipotent differentiation, as reported by others, but T1D-MSCs showed an increased migratory capacity. Importantly, T1D-MSCs showed abnormalities in mRNA expression, including a downregulation of the immunomodulatory molecules VCAM-1, CXCL12, CCL2, CCL24, CXCL5, of the pro-regenerative molecule HGF, of the stemness-related EGFR and FGFR, along with the activation of sympathetic nervous system and JAK STAT signaling92. This gene expression profile suggests that human T1D-MSCs may have impairments in their interactions with immune/hematopoietic cell populations and in their ability to suppress immune effector functions. In accordance with what Davies et al91 had found, the study by de Lima et al92 also confirmed the down-modulation of HGF in T1D-MSCs. HGF is associated with angiogenesis and cell survival101,102, it can stimulate kidney and liver regeneration. Moreover, HGF is believed to be a protective factor for pancreatic β cells, and consequently its downregulation may indicate a decreased potential for the stimulation of pancreatic islet regeneration. Additionally, EGFR and FGFR were also found downregulated in T1D-MSCs: these receptors regulate stemness, inhibit senescence, are essential for cell growth, tissue repair, and homeostasis103,104; a downregulation of EGFR signaling may determine the downregulation of HGF103,105,106. This study analyzed MSCs after in vitro culture; therefore, the abnormalities found could be influenced by culture conditions beyond the exposure to the altered diabetic bone marrow milieu. Further functional experiments will be required in order to better elucidate how these gene expression alterations may affect therapeutic efficacy of autologous MSCs in T1D patients92.

**Autologous T2D-MSCs**

The studies focused on autologous T2D BM-MSCs suggest that long-term exposure to the disease-related inflammatory and hyperglycemic environment affect their functions.

Shin and Peterson107 examined the influence of T2D on the therapeutic potential of endogenous BM-MSCs, showing that the diabetic mice had BM-MSCs occurring in lower numbers, with impaired proliferation and survival in vitro.

The study conducted in 2009 by Phadnis et al108 investigated the characteristics of BM-MSCs derived from T2D patients. As it was described by the articles cited in this review about T1D91-93, also T2D-MSCs appear similar to healthy MSCs in phenotype, morphology, and multilineage differentiation potential. However, the diabetic environment seems to have an impact on MSCs: C-peptide and insulin transcripts can be detected in T2D-MSCs108. Kojima and colleagues had previously observed that hyperglycemia, with or without established diabetes, activates insulin gene transcription and proinsulin production in multiple extrapancreatic and extrathymic tissues109.

However, unlike in β-cells, MSCs from T2D exclusively produced proinsulin and very little mature insulin, and did not contribute significantly to insulin production in vivo108. Although high glucose concentration induces proinsulin transcription, it also stimulates the secretion of cytokines such as interleukin1, which cause β-cell apoptosis in vitro and in vivo110. Kojima et al109 hypothesized that the-
se cells may mediate the ill effects of hyperglycemia, and may contribute to chronic diabetic complications such as diabetic neuropathy.

Although the amount of proinsulin produced by the BM cells exposed to hyperglycemia in vivo was extremely small, the appearance of proinsulin-producing cells outside the pancreas may represent the body’s attempt to reverse hyperglycemia. Thus, chronic exposure to hyperglycemia may be important for the decreased potential of these BM-MSCs, precluding them for autologous stem cell therapy in T2D patients. In fact, these cells appear to be terminally differentiated, therefore leading to a loss of stemness and failure of further propagation.

Furthermore, the persistent hyperglycemic milieu in T2D is also associated with several pathological complications, mostly related with compromised vascularization and/or aberrant angiogenesis. By releasing growth factors and cytokines such as IGF-1, BM-MSCs stimulate endothelial cell migration, inhibit endothelial apoptosis, stimulate angiogenesis, promote neovascularization and tissue regeneration. The influence of T2D on the secretome and pro-angiogenic genes of BM-MSCs deserves thorough investigations. Ribot et al. analyzed the impact of T2D on BM-MSCs secretome and functions, hypothesizing that in the diabetic milieu these could have different composition and properties. The results obtained provided the evidence that short-term T2D alters the BM-MSC secretome composition and promotes angiogenic capabilities.

Angiogenesis-related genes are differentially expressed in BM-MSCs from diabetic fatty rats (ZDF, a T2D model) when compared with lean animals (control). In particular, several pro-angiogenic genes were found to be overexpressed, while anti-angiogenic genes were downregulated. The up-regulated genes included IGF-1 and TIE1, which are critical regulators of angiogenesis, MCP-1 and IL-6, homing factors for BM-MSCs and endothelial cells/endothelial progenitor cells, and IL-6 and TNFa, critical mediators of the inflammatory process. Moreover, proteomic analysis of the T2D BM-MSC secretome showed decreased levels of ab-crystallin, a chaperone for VEGF-A, and increased levels of LTBP1 and LTBP2, regulators of TGF-b availability, as well as of OSTP and FMOD, which are components of the extracellular matrix and might be involved in the paracrine action of T2D BM-MSCs on endothelial cells. In addition, the proteomic analysis of T2D BM-MSC demonstrated a specific secretory phenotype of extracellular matrix remodeling and glucose metabolism, showing overexpressed proteins involved in extracellular matrix homeostasis and remodeling-related molecules; in contrast, proteins involved in the metabolism of glucose (such as ALDOA, LDHA, KPYM, G6P, PTMA, OAS2, ALD1, and IBP2) were secreted at lower levels.

Functional impairment of T2D MSCs is evident from preclinical and clinical studies that have been performed to determine their efficacy in the treatment of peripheral arterial disease (PAD). PAD is frequently associated with diabetes, hypertension, atherosclerosis, and aging - all of which could damage the regenerative function of stem cells and progenitor cells. Yan et al. have shown that experimental T2D causes hyperinsulinemia-induced oxidant stress in murine MSCs, a stress that restricts their multipotency and impairs their capacity to promote neovascularization.

The same authors observed that MSCs harvested from T2D mice show several dysfunctions deriving from oxidative stress. Rather than increasing post-ischemic neovascularization and limb blood flow, injection of MSCs from T2D mice impaired blood flow recovery. Should human MSCs display similar oxidative stress-induced impairment of function, these findings recommend a therapeutic approach aimed maximizing the potential of MSC transplantation, particularly in the increasingly common setting of diabetes or other cardiovascular risk factors. The authors propose that either in vivo systemic treatment with an antioxidant and/or ex vivo treatment of MSCs with antioxidants could significantly increase the intended clinical benefit.

A recent study by Rezabakhsh et al. investigated the impact of T2D sera on the angiogenic differentiation capacity of primary healthy BM-MSCs. The study showed that T2D serum decreased the angiogenic properties of MSCs via direct effect on angiogenesis pathways or via induction of autophagy signaling.

Taking all these considerations together, the pathophysiology of T2D and the associated changes in the bone marrow microenvironment seem to affect multiple aspects of BM-MSCs biology and function. T2D seems to exacerbate the impairment of these stem cells to an extent greater than T1D. It is however still largely unknown whether distinct mechanisms underlie BM-MSCs dysfunction in T1D compared to T2D.
De Vyver et al\(^6\) argued that strategies focused on restoring stem/progenitor cells mobilization in autologous cell therapy are limited in that stem cell damage can occur at the bone marrow niche before mobilization into the peripheral blood. This hypothesis was also confirmed by an observation by Januszyn et al\(^3\), who affirmed that the pathogenesis of both T1D and T2D may deplete specific subpopulations of BM-MSCs and this defect cannot be corrected by restoring glucose homeostasis. In addition to affecting BM-MSCs viability and functional capacity, long term exposure to the pathological bone marrow niche environment can induce a certain degree of disease memory in MSCs\(^8\)\(^9\). Future studies are required to provide a strict assessment of the efficacy of MSCs transplantation in T1D, T2D, and related complications.

**CONFLICT OF INTEREST**

The Authors declare that they have no conflict of interests.

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