

Harnessing the Immunomodulatory and Tissue Repair Properties of Mesenchymal Stem Cells to Restore β Cell Function

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Abstract Islet cell transplantation has therapeutic potential to cure type 1 diabetes (T1D), which is characterized by autoimmune-mediated destruction of insulin-producing β cells. However, current success rates are limited by long-term decline in islet graft function resulting partially from poor revascularization and immune destruction. Mesenchymal stem cells (MSCs) have the potential to enhance islet transplantation and prevent disease progression by a multifaceted approach. MSCs have been shown to be effective at inhibiting inflammatory-mediated immune responses and at promoting tissue regeneration. The immunomodulatory and tissue repairing properties of MSCs may benefit β cell regeneration in the context of T1D. This review will elucidate how MSCs can minimize β cell damage by providing survival signals and simultaneously modulate the immune response by inhibiting activation, and proliferation of several immune cell types. In addition, MSCs can enhance islet graft revascularization, maintaining long-term β cell viability and function.

Keywords Mesenchymal stem cells · Islet transplantation · Type 1 diabetes · Immunomodulation · β cell function · Immunomodulatory and tissue repair properties

Introduction

Mesenchymal stem cells (MSCs), also referred to as stromal cells or mesenchymal progenitor cells, are non-hematopoietic multipotent stromal cells that can differentiate into a variety of tissues [1]. Recently, MSCs have been investigated as a treatment for a wide range of diseases due to their capacity for self-renewal and unique immunosuppressive and regenerative properties [2]. For these reasons, MSCs have been studied as therapies in the context of immunopathologic disorders such as autoimmune encephalomyelitis (EAE), a model of human multiple sclerosis (MS) [3], arthritis [4], systemic lupus erythematosus (SLE) [5], Crohn's disease [6], and graft vs host disease [7]. In these models, MSCs have been shown to be effective at inhibiting immune inflammation and promoting tissue regeneration. As immunomodulators, MSCs can use both direct and indirect mechanisms to modulate the immune response in settings of inflammation, autoimmune diseases, and transplantation. In addition, the inherent regenerative properties of MSCs have been demonstrated to aid in tissue repair by assisting endogenous cell function and revascularization of damaged tissue. MSC-based therapeutic strategies are now under investigation to overcome the autoimmune destruction of insulin-producing cells in type 1 diabetes (T1D) and the inflammatory-mediated β cell dysfunction and insulin resistance in type 2 diabetes (T2D) [8].

T1D is characterized as an autoimmune-mediated destruction of insulin-producing β cells by pro-inflammatory autoreactive CD4⁺ and CD8⁺ T cells [9]. Prior to disease onset, a B cell immune response has taken place, producing antibodies to β cell antigens detected in the peripheral blood [10]. Together, these processes result in reduced and insufficient β cell mass to maintain glucose homeostasis, rendering patients dependent on exogenous insulin. In addition to

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insulin administration, other treatment options currently available to patients with T1D include whole pancreas transplantation and islet transplantation. Islet transplantation, primarily indicated in patients with unstable hypoglycemia, is advantageous compared with whole pancreas transplantation because it is relatively non-invasive. But significant challenges to islet transplantation still remain, including: revascularization of the islet cell graft, prevention of inflammation, rejection and autoimmune destruction of the graft, requirement for lifelong immunosuppression, which can be harmful to islet β cell function, and lastly, the limited supply of donor islets for widespread clinical therapies. In this context, MSC-based therapies may become an alternative option in the prevention of T1D disease onset and may also enhance tolerogenicity and engraftment of allo-islet grafts after transplantation.

This review first highlights the capacity of MSCs to modulate the autoimmune response during the pathogenesis of T1D and the alloimmune response in the setting of islet transplantation. Second, this review focuses on the role of MSCs in the repair of β cell mass and function in both T1D and T2D. Lastly, it illustrates the promises and potential obstacles for MSC therapies to become a clinically relevant approach to treatment of insulin dependent diabetes.

MSC Characterization

Although rare, MSCs can be isolated from a variety of sources including: bone marrow, cord blood, dental pulp, adipose tissue, lung, placenta, tendons, synovial fluid, circulating peripheral blood, and fetal liver [11]. MSC isolation and purification was initially performed from bone marrow and defined by the colony forming unit-fibroblast (CFU-F) assay utilizing the tendency of MSCs to adhere to plastic [12]. Isolation of MSCs from solid tissue (i.e. fat, placenta) requires a collagenase digestion step. Isolated MSCs may be further purified prior to culture by fluorescence activated cell sorting (FACS), using monoclonal antibodies against common markers shared by all stromal cell precursors, such as STRO-1 [13–16].

In vitro, MSCs proliferate, display fibroblast-like morphology and are further characterized by their ability to differentiate into bone, cartilage and fat [17]. According to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, MSCs are identifiable based upon their ability to adhere to plastic and stain positive for the expression of surface markers CD105, CD73, and CD90 [18]. Although there is growing data suggesting that MSCs express additional surface markers, such as CD54, CD44, CD29, CD49, CD71, and CD271, the expression of these markers largely depends on culturing techniques, isolation methods, tissue sources, and species differences [2, 19, 20].

Historically, MSCs have been considered hypo-immunogenic because of their limited expression of MHC class II and costimulatory molecules and inability to stimulate T cell proliferation. However, MSCs are no longer considered immunologically silent [21]. Costimulation with IFN- γ can induce MSCs to express elevated levels of MHC class II, and it has been shown that MSCs express MHC class I and other receptors that interact with immune cells [2]. While the immunosuppressive effects of MSCs are well established, these findings demonstrate the greater complexity and bi-directionality of the interaction between MSCs and the immune system.

MSCs and T1D

MSC-based therapies may play a role in the prevention of T1D disease onset and in islet graft survival and function in the context of islet cell transplantation. MSCs have been shown to prevent and/or delay disease onset [22] and to reduce the infiltration of autoreactive T cells into the pancreas in both T cell-transferred and spontaneous diabetic mouse models [23]. In addition to their immunomodulatory properties (Table 1), MSCs may act as “repair cells” by regulating the expression of key anti-apoptotic and regenerative genes in β cells and reverse β cell damage (Table 2). Lastly, MSCs can increase islet graft β cell function by the secretion of trophic factors that can enhance insulin response and graft revascularization, minimizing the loss of transplanted islets during the peritransplant period. We will next discuss evidence for each of these potential mechanisms of MSCs as “helper cells” in the context of T1D.

Immunomodulatory Properties of MSCs In Vitro

Numerous studies show that MSCs primarily modulate the effector arm of the T cell immune response through the suppression of T cell proliferation and the inhibition of dendritic cell (DC) differentiation. Furthermore, MSCs may modulate NK cell cytotoxic activity, B cell proliferation, and immunoglobulin production.

MSC Interaction with Immune Cells

Direct Cell–Cell Contact. MSCs can exert immunomodulatory properties on T cells by direct cell-to-cell contact through the engagement of the inhibitory molecule programmed cell death 1 (PD-1) to its ligand PD-L1. In vitro, MSCs stimulated with recombinant IFN- γ showed upregulated expression of the surface molecule PD-L1 and suppressed autoreactive T cell proliferation; the suppression was reversed in the presence of a PD-L1 siRNA knockdown [24]. Similarly in vivo, Fiorina et al. showed that PD-L1

Table 1 Immunomodulatory molecules expressed or secreted by MSCs in the context of type 1 Diabetes

Molecule	Target cell	Effect	Reference
PD-1	Autoreactive T cells	Binds to PD-L1 and suppresses proliferation by direct cell contact	Jurewicz M et al. [24]
IDO	Activated T cells NK cells	Depletes amino acids and inhibits immune cell proliferation and function	Krampera ML et al. [38]
MMPs	Activated T cells	Reduces T cell responsiveness to IL-2	Ding YC et al. [43]
PGE2	Activated T cells	Inhibits IL-2 production and proliferation by direct action on T cells	Tse WT et al. [32]
	DC	Inhibits DC function and secretion of IL-12 and IFN- γ .	Chen L et al. [35]
	NK Cells	Suppresses IL-2–and IL-15–mediated cytotoxicity and cytokine production.	Sotiropoulou P A et al. [49]
NO	Activated T cells	Inhibits T cell activation and phosphorylation of Stat5	Sato K, et al. [40]
TGF- β	Activated T cells	Inhibits proliferation	Di Nicola M et al. [29]
	Regulatory T cells	Facilitates expansion and generation	Casiraghi et al. [65]
	NK cells	Inhibits IL-2-induced activation of NK proliferation	Aggarwal S et al. [34]
IL-6	DC	Inhibits differentiation and maturation	Jiang XX et al. [50]
	Regulatory T cells	Increases IL-10 secretion	Engela AU et al. [63] Crop MJ et al. [64]
HGF	Activated T cells	Suppresses proliferation synergistically with TGF- β	Di Nicola M et al. [29]
	NK cells	Suppresses proliferation, cytokine secretion, and cytotoxicity	Sotiropoulou PA et al. [49]
Galectins	T cells	Induces apoptosis	Sioud, M et al. [47]

PD-1 programmed death 1, *PD-L1* programmed death ligand 1, *IDO* 2,3-dioxygenase, *NK* cells natural killer cells, *MMPs* matrix metalloproteinases, *IL-2* interleukin-2, *PGE2* prostaglandin-E2, *DC* dendritic cell, *IL-12* interleukin-12, *TGF- α* transforming growth factor alpha, *IL-15* interleukin-15, *NO* nitric oxide, *TGF- β* transforming growth factor beta, *IL-6* interleukin-6, *IL-10* interleukin-10, *HGF* hepatocyte growth factor

expression levels were increased on MSCs that migrated to the pancreas of prediabetic NOD mice, delaying the onset of T1D by suppressing the proliferation of autoreactive T cells [22]. These results suggest that in the context of T1D, PD-L1 ligation on the surface of MSCs results in T cell immunosuppression.

Secretion of Soluble Immunomodulatory Molecules. A second mechanism for MSCs suppression of immune cell proliferation is the secretion of soluble molecules. In mixed lymphocyte reaction (MLR) cultures, MSCs down-regulated alloreactive T cell proliferation through soluble factors, including 2,3-dioxygenase (IDO), prostaglandin-E2 (PGE2), nitric oxide, and transforming growth factor- β (TGF- β) [25, 26].

TGF- β , IL-10 and HGF are the most commonly described MSC-secreted cytokines mediating suppression of T cell activation and proliferation both in vitro and in vivo [27–29]. TGF- β secretion was further increased in the presence of IFN- γ , supporting the notion that inflammatory signals enhance MSCs immunosuppressive activity [29, 30]. In the context of T1D, serum levels of TGF- β and IL-10 were increased in intravenous MSC-treated NOD mice [31]. β cell-specific T cells harvested from diabetic NOD mice also showed decreased proliferation in vitro in the presence of MSCs, and the effect was reversed by neutralization of TGF- β [31].

PGE2 is constitutively expressed and secreted by MSCs and may be associated with their immune suppressive effect in an MLR setting [32]. Whether the immune suppressive effects of MSCs are mediated by PGE2 has been debated; however, mounting evidence suggests a strong role of PGE2 in inhibition of T cell proliferation by MSCs [33–36], and the contradictory evidence [32] may be due to differences in isolation techniques [36]. A recent study by Duffy et al. established that MSCs inhibit Th17 cell proliferation and differentiation from naïve and memory precursors through PGE2 via the EP4 receptor [37]. In addition to the inhibition of T cell proliferation, MSCs inhibit DC differentiation and reduce DCs ability to produce IL-12 and IFN- γ [35].

MSCs secrete IDO, a tryptophan-catabolizing enzyme that causes amino acid depletion and the inhibition of proliferation and function of immune cells. MSCs do not constitutively express IDO; however, IFN- γ triggered by paracrine signaling induces upregulation of IDO expression by MSCs [38]. The upregulation of IDO expression has been shown to be partially responsible for MSCs suppression of T cell proliferation [39].

Similarly, nitric oxide (NO) is not constitutively expressed by MSCs but is induced and secreted upon direct cell contact with activated T cells [40]. NO inhibits T cell proliferation by reducing phosphorylation of the tyrosine residues of the Stat5 transcription factor [41]. In this way, NO blocks the Jak3/Stat5

Table 2 Chemoattractants and regenerative molecules support β cell repair and function

Regenerative and repairing properties	Chemoattractant factor (cell of origin)	Target receptor/cell type/effect	Reference
Migration to sites of injury	CX3CL-1 (β cell) CXCL12 (islet EC)	CX3CR1/MSC/induces migratory activity CXCR4/MSC/attracts MSCs to islets	Sordi V et al. [72]
β Cell repair and protection	Protein/cytokine secreted by MSCs	Target molecule or receptor/effect	Reference
Anti-apoptosis	HO-1 IL-6	Free Heme/counteracts inflammatory reactions XIAP/inhibits caspase-3 and caspase-9	Lu Y et al. [81] Lu S et al. [76] Caja L et al. [77]
		Bcl-2/modulates Bax Bcl-xL/interacts with Raf-1	Lu S et al. [76] Park KS et al. [80]
β cell function	Unknown HGF	PDX-1/ β cell differentiation and function HGF/SF receptor (c-met proto-oncogene product)/ β cell growth and insulinotropic factor	Boumaza I et al. [62] Nakano M et al. [87]
Angiogenesis	VEGF TGF- β	VEGFR1 and VEGFR2/initiates vascularization TGF- β /angiogenesis through vascular cell receptors ALK-1 and AKL-5 and enhances VEGF synthesis	Park KS et al. [78] Figliuzzi M et al. [92] Park K, et al. [80]

CX3CL-1 chemokine (C-X3-C motif) ligand 1, *CX3CR1* CX3C chemokine receptor 1, *CXCL12* CXCL12 chemokine (C-X-C motif) ligand 12, *EC* endothelial cell *CXCR4* C-X-C chemokine receptor type 4, *HO-1* heme oxygenase-1, *IL-6* interleukin-6, *XIAP* X-linked inhibitor of apoptosis protein, *Bcl-2* B-cell lymphoma 2, *Bax* Bcl-2-associated X protein, *Bcl-xL* B-cell lymphoma-extra large, *PDX-1* pancreatic and duodenal homeobox 1, *HGF/SF* hepatocyte growth factor/scatter factor, *VEGF* vascular endothelial growth factor, *VEGFR1* vascular endothelial growth factor receptor 1, *VEGFR2* vascular endothelial growth factor receptor 2, *TGF- β* transforming growth factor-beta, *ALK-1* activin receptor-like kinase 1, *ALK-5* activin receptor-like kinase 5

signaling pathway, which locks the T cells in G0/G1 phase [42].

Matrix metalloproteinases (MMPs) secreted by MSCs may also play a role in inhibiting T cell proliferation. In vitro, MMP-2 and MMP-9 cleave CD25 from T cell surfaces rendering them unresponsive to IL-2 and thus impeding activation and expansion of alloreactive T cells [43]. In vivo, islets co-transplanted with MSCs into a diabetic mouse model showed rapid reversal of hyperglycemia, however MMP inhibitor administration overturned the protective effects afforded by the MSCs and resulted in islet graft failure [43].

MSCs secrete galectins, carbohydrate-binding proteins that contribute to MSCs immunosuppressive effects by inducing T cell apoptosis and interfering with immune cells activation and secretory function [44]. Galectin-1 induces T cell apoptosis by binding to CD45, CD43, and CD7 [45]. Galectin-3 induces immune cell apoptosis by binding to either CD7 and CD29 or CD45 and CD71 [46]. The secretion of galectins by MSCs affects T cell development and activation, apoptosis, cytokine secretion and regulatory T cell function [47].

MSCs also have a suppressive effect on B lymphocyte proliferation, chemotactic behavior, and immunoglobulin production. Soluble factors involved in B lymphocyte inhibition are secreted by MSCs upon a paracrine signal from B cells, as shown in a transwell assay by Corcione et al. [48]. In addition, MSCs interact with NK cells through a combination of direct cell-to-cell contact and soluble molecules (ie, TGF- β , PGE2).

The MSC-NK cell interaction has immunosuppressive effects resulting in decreased NK cell proliferation, IFN- γ production, and cytotoxicity activity [49].

MSC Interaction with Dendritic Cells (DC)

DC Maturation. DC and macrophage infiltration initiates general immune responses and perinsulinitis of the pancreas in which β cell mass is decreased. MSCs have suppressive effects both on the maturation and function of DCs, suggesting that MSCs may be beneficial in an islet-MSC co-transplant setting, by protecting the β cells from DC infiltration. MSCs may suppress the generation of inflammatory DCs through IL-6 secretion [24]. In vitro, MSCs have been shown to inhibit the differentiation of monocytes into immature DC [21, 50, 51]. In the context of islet transplantation, DC isolated from allo-islet-MSC co-transplant recipients showed inhibited maturation, impaired antigen-presentation capabilities, and suppressed IL-12 secretion, which plays an important role in DC maturation and function [52]. In addition to inhibiting DC maturation, MSCs may modulate the secretory function of DCs. Co-culture of DCs and MSCs resulted in changes in the cytokine secretion profile of DC with upregulated expression of regulatory cytokines (IL-10) and reduced expression of inflammatory cytokines (TNF- α , IFN- γ , IL-12) [51, 53, 54].

DC Migration. In the islet allograft setting, co-administration of MSCs may also enhance graft survival by minimizing DC migration. DCs are involved in initiating cell-mediated immunity by migrating to local lymph nodes and presenting allo-islet antigens to the T cells. DC migration is controlled in part by upregulation of CCR7, a chemokine receptor important for migration to lymph nodes, and downregulation of tissue anchoring proteins such as E-cadherin [55]. English et al. demonstrated in vitro that co-culture of DC and MSCs resulted in low CCR7 expression on DC and decreased DC migration in response to CCL19 (chemokine for CCR7) [55]. Thus the colocalization of MSCs with an islet graft may reduce DC migration and recruitment to the peripheral draining lymph nodes, limiting allogeneic antigen presentation and immune rejection.

MSCs Expand Regulatory T Cells

MSCs are known to induce regulatory T cell expansion both in vitro and in vivo [34, 56]. The differentiation and generation of regulatory T cells is dependent in part on TGF- β [57, 58]. TGF- β gene therapy was shown to enhance β cell function in diabetic NOD mice [59–61], and this protection may be partially attributed to the effect of TGF- β on generation of regulatory T cells. MSCs administered into a rat model of streptozotocin-induced β cell injury also shifted peripheral T cells toward a Th2 phenotype with IL-10/IL-13 production and higher frequencies of CD4⁺/CD8⁺ Foxp3⁺ regulatory T cells [62]. The MSC-mediated upregulation of IL-10 secretion [63, 64] may facilitate T cell differentiation toward a tolerogenic regulatory T cell phenotype [65].

In an NOD T1D model, Fiorina et al. observed only a marginal increase in regulatory T cells in the pancreatic draining lymph nodes and no significant increase in these cells in the spleens of NOD mice treated with MSCs compared with control NOD mice [22]. The MSC-mediated shift toward the generation of regulatory T cells is more consistently confirmed when MSCs are co-administered with the islets rather than injected systemically and allowed to migrate to the β cells. This is supported by the finding that diabetic non-human primate recipients presented with increased numbers of regulatory T cells in their peripheral blood following allo-islet-MSC co-transplantation [66].

Similarly, Ding et al. demonstrated MSCs ability to modulate immune cells in a mouse islet allograft model in which MSCs, co-localized with the islets, prevented islet allo-graft rejection [43]. Additionally, T cells isolated from the spleen of islet-MSC co-transplant mouse recipients showed low levels of IFN- γ and TNF- α secretion upon ex-vivo activation compared with T cells isolated from islet alone transplant recipients [67••]. The mechanism of MSCs' ability to regulate the cytokine profile of inflammatory cells remains under investigation as the suppression of T cell responses

either at T1D onset or after islet transplantation will be important to protect β cell mass and function.

Regenerative and Repairing Properties of MSCs as a Therapeutic Approach for T1D Treatment

Migration to Site of Injury

MSCs selectively migrate to sites of injury and participate in repair as shown in lung injury [68] and myocardial infarction [69]. In vitro, Lin et al. used a microfluidic device to show that freshly isolated islets secrete attractant signals that encourage MSC migration towards them, resulting in improved islet cell survival and function [70]. In T1D, MSCs may use their migratory properties to localize at the site of islet cell damage and aid in cellular repair as shown by Lee et al. [71]. Similarly, in a streptozotocin-induced β cell injury rodent model, MSCs injected intravenously appeared in the pancreas within 7 days and reversed hyperglycemia [62]. These observations suggest that in vivo recruitment of MSCs to injured pancreatic islets might contribute to β cell repair. Some of the functional chemokine receptor/ligand pairs involved in MSC migration were identified as CX3CL1-CX3CR1 and CXCL12-CXCR4 [72].

Supporting β Cellular Regeneration and Function at the Site of Injury

Once the MSCs have reached the site of islet cell injury, they may aid in islet regeneration, as shown in experimental animal models. Human MSCs injected into NOD-SCID mice reduced hyperglycemia by increasing pancreatic islet β cell mass [71, 73]. Furthermore, Lee et al. found that new islets formed off of pancreatic ducts, suggesting that MSCs promote islet regeneration [71]. As well, Ezquer et al. observed a significantly increased β cell mass in streptozotocin-induced diabetic mice treated with a single injection of MSCs compared with non-treated animals [74]. Although MSC-treated recipients did not perform better against a high glucose challenge, the increased number of insulin-producing cells suggests that MSC treatment contributes to newly regenerated β cells through induced proliferation and differentiation of endogenous progenitors. Conversely, recent data from Si et al. demonstrated that human MSCs injected into streptozotocin-induced diabetic mice resulted in higher β cell numbers as a consequence of selective homing in injured islets, resulting in the prevention of β cell death rather than proliferation [75]. Furthermore, β cell protection and repair was observed preferentially when MSCs were injected near (day 7) to the streptozotocin injection rather than later (day 21), suggesting that MSCs' impact on β cell mass may vary depending on the experimental methods.

Another potential mechanism for MSCs' therapeutic effect in T1D is the modulation of islet gene expression. Islets co-cultured with MSCs showed increased β cell expression of anti-apoptotic signaling molecules XIAP [76, 77], Bcl-2, and Bcl-xL [78••]. In addition, islets isolated from streptozotocin-induced diabetic animals treated with MSCs expressed high levels of PDX-1, a transcription factor that regulates insulin gene expression and plays a role in pancreatic development and differentiation [62]. The expression and activation of PDX-1 can potentially stimulate growth, survival, and differentiation of β cells, resulting in enhanced β cell function.

MSCs secrete many bioactive growth factors and cytokines with paracrine and autocrine activities that may be responsible for the observed increase in expression of PDX-1 and anti-apoptotic molecules [79]. Freshly isolated islets were susceptible to β cell loss from apoptosis, but when co-cultured with cord blood-derived MSCs, these islets showed improved viability and function [80]. This enhanced viability and function was due to anti-apoptotic proteins and active trophic agents secreted by the MSCs [80], such as HO-1 [81], IL-6, hepatocyte growth factor (HGF), and SDF-1 [62, 80]. IL-6 may induce expression of β cell anti-apoptotic signaling molecules Bcl-2 and Bcl-xL [82–85]. HGF, a β cell growth factor [86], may prevent primary non-function in islet grafts by inhibiting apoptosis, inducing β cell proliferation [87], and improving β cell insulin response to high glucose [88]. SDF-1 is involved in islet generation and differentiation from endocrine stem cells within the pancreas [89] and enhances the β cell regeneration potential provided by HGF. The secretion of trophic molecules may be a key mechanism in the ability of MSCs to minimize β cell loss during T1D onset and protect islet cell engraftment after transplantation.

Angiogenesis

MSCs secrete angiogenic and arteriogenic cytokines including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), angiopoietin-1 (Ang-1), and transforming growth factor- β (TGF- β). While each of these signaling proteins plays a role in angiogenesis, MSCs may promote islet vascularization primarily through VEGF secretion [90]. Increased VEGF expression at the islet transplant site significantly increased graft survival and function [91]. Similarly, a marginal islet cell mass that reversed hyperglycemia within 2 weeks in mice co-transplanted with islets and MSCs showed enhanced expression of VEGF [92]. In diabetic rodent models the co-transplantation of MSCs with pancreatic islets improves graft function significantly through islet remodeling and revascularization mediated by the MSCs [93, 94].

Johansson et al. utilized MSCs to enhance islet revascularization by co-culturing MSCs and endothelial cells with islets in vitro [95]. The MSCs may promote islet engraftment by initiating the formation of vessel-like structures through secretion of proteases that degrade the islet extracellular matrix and allow the migration of endothelial cells into the islet. The ability for MSCs to encourage revascularization might allow for exploration of alternative sites for islet transplantation. Currently, the preferred transplantation site is the portal vein of the liver, which is not optimal due to venous hypoxemia and the potential risk of thrombosis [96]. Islet transplantation in the subcutaneous site would be more accessible and minimally invasive; however, the lack of early vascularization of the graft may result in loss of function and inability to restore normoglycemia due to poor graft oxygen supply [97–100].

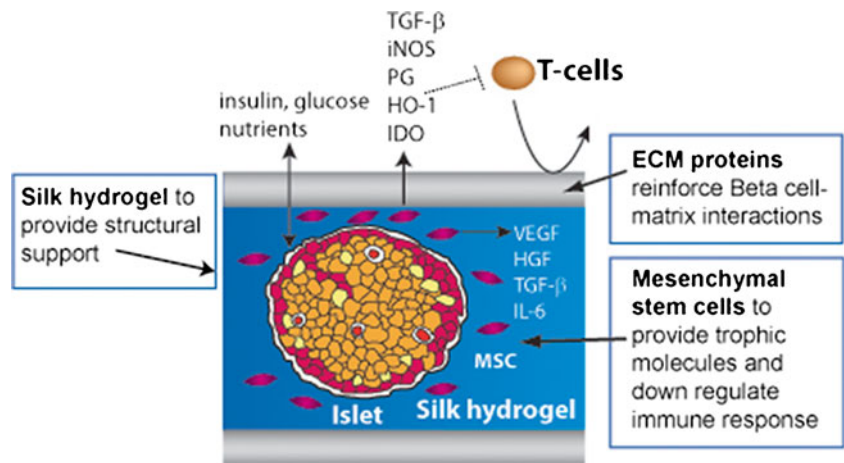
An emerging therapeutic strategy involves the use of biomaterials to encapsulate islets and overcome these obstacles. Biomaterials may enhance islet function by providing a three-dimensional cellular support and delivering proteins, growth factors, and immunosuppressive agents [101, 102]. Some approaches are focused on islet encapsulation platforms with prevascularization of the device prior to islet implantation [103]. Our laboratory is developing a silk-based hydrogel biomaterial in which islets are encapsulated with ECM proteins (laminin and collagen IV) and bone marrow-derived MSCs to enhance islet cell graft revascularization survival and function (Fig. 1). The use of a biomaterial-based approach in MSC-islet co-transplantation aims to re-establish the islet microenvironment, enhance islet function and provide the protective and angiogenic effects of MSC therapy.

MSC and T2D

T2D is characterized clinically by uncontrolled hyperglycemia resulting from both insulin resistance and pancreatic β cell dysfunction [104]. Interestingly, inflammation seems to play a role in impairing β cell insulin response to high glucose [105]. The immunomodulatory properties of MSCs may be beneficial for the treatment of patients with T2D [8]. Recent randomized T2D clinical trials showed a beneficial effect of bone marrow-mononuclear cells containing MSCs on glycemic control [75]. Hemoglobin A1C levels were markedly reduced following intra-arterial MSC infusion into the vasculature of the pancreas. This study supports that the pancreatic infusion of MSCs directly improved β cell function in T2D [106].

The MSC mechanism to improve metabolic control was elucidated in a recent study by Si et al. in a rat model of T2D, which promoted β cell viability and function [75]. Importantly, MSCs improved insulin sensitivity in the

Fig. 1 Silk hydrogel-based biomaterial. Islets are co-encapsulated in a silk hydrogel with extracellular matrix proteins (ECM) and with bone marrow-derived mesenchymal stem cells (MSC) to enhance islet cell graft revascularization survival and function



peripheral tissue (adipose and skeletal muscle tissue) by upregulating GLUT4 protein expression and translocation to the cellular membrane. In T2D, reduced translocation of GLUT4 protein to the cell membrane is associated with insulin resistance and impaired glucose metabolism [107]. In light of these results, MSC-based therapies could be considered in clinical trials to improve insulin sensitivity in patients with T2D.

Future Challenges

MSCs have been studied as therapies in many immunopathological disorders, including: autoimmune encephalomyelitis, a model of human multiple sclerosis, arthritis, systemic lupus erythematosus, Crohn's disease, graft-vs-host-disease, and T1D [2]. MSCs are now in clinical trials as therapies for more than 30 indications (<http://clinicaltrials.gov>). But some key issues need to be addressed before MSC based therapies become a safe and viable option.

Most importantly, the standardization of MSC isolation, characterization, and culture *in vitro* needs to be addressed, as MSC characteristics may vary according to culture conditions and passage number [25]. Even though cultured MSCs show progressive senescence and growth arrest without tumor transformation [108], they may still acquire genetic abnormality and become tumorigenic *in vivo* [109]. In the context of islet transplantation, it is unclear if co-transplanted MSCs engraft and differentiate at the implantation site. Thus the long-term stability of MSC activity and function *in vivo* after transplantation needs to be assessed and safety criteria need to be defined prior to transplantation.

The majority of research to date has focused on bone marrow-derived MSCs, but MSCs derived from other tissue sources, such as umbilical cord and adipose, may also have immunomodulatory properties [110, 111]. The regenerative and immunomodulatory properties of MSCs most likely will

vary with the tissue source. For example, adipose-derived MSCs show a greater angiogenic potential than bone marrow-derived MSCs in a prevascularized biomaterial implanted in the subcutaneous tissue of diabetic rats [112]. In addition, the question of appropriate donor source needs to be clarified on whether autologous or allogeneic MSCs should be used. The ability to transplant autologous MSCs, if viable, may be advantageous to further prevent an allo-immune response against MSCs [21]. But in the case of T1D, autologous MSCs are less functional and of less therapeutic value, as observed with allogeneic rather than autologous MSCs reversing hyperglycemia in an NOD mouse model [22]. Thus the criteria for choosing a specific tissue and/or donor MSC source may differ with the indication and whether the treatment is aimed at modulating the autoimmune disease or enhancing pancreatic islet engraftment and vascularization.

Conclusions

MSC have the potential to aid in the treatment of T1D and overcome some of the current limitations to islet transplantation. The immunomodulatory properties of MSCs may assist in reducing inflammatory damage to the islets in the early peritransplant period. MSCs may also attenuate autoimmunity through their immunomodulatory properties while secreting regulatory cytokines to control autoreactive T cells and alloreactive effector CD4+ and CD8+ T cells. Thus MSCs may be a viable alternative to harmful immunosuppressive drugs that can damage islets. The ability of MSCs to secrete trophic and angiogenic factors may also prevent early islet damage and assist in engraftment. Together, MSCs may potentially establish a microenvironment that stimulates growth, survival and differentiation of β cells and minimizes apoptosis and necrosis. The multiple beneficiary roles that MSCs play could help in alleviating donor shortages by reducing the number of islets needed per transplant, decreasing early islet cell death and maintaining long-term graft function.

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