Stem cell-derived exosomes: a novel vector for tissue repair and diabetic therapy

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Abstract

Exosomes are extracellular vesicles (EVs) secreted from a majority of cell types. Exosomes play a role in healthy and pathogenic intercellular interactions via the transfer of proteins, lipids and RNA. The contents and effects of exosomes vary depending on the properties of the originating cell. Exosomes secreted from some cell types, including stem cells, carry biological factors implicated in the protection, regeneration and angiogenesis of damaged tissues. Due to these properties, exosomes have attracted attention as a novel vector for regenerative therapies. Exosomes as a therapeutic tool could have applications for the treatment of many disorders characterized by chronic tissue damage. Exosomes derived from stem cells could be applied to repair or prevent damage from the complications of diabetes mellitus. The immunomodulatory and reparative properties of stem cell-derived exosomes could protect or even restore an early-stage type 1 diabetic patient's original islets from autoimmune destruction. Exosomes could also possibly suppress graft rejection of pancreatic islet transplants. Therefore, it is our recommendation that the treatment of diabetes mellitus using exosome-based therapies be further explored. Development of novel therapies using exosomes is slowed by a limited understanding of their mechanisms. This hurdle must be overcome to pave the way for clinical trials and ultimately the adaptation of exosomes as a therapeutic vector.

Key Words
- exosomes
- extracellular vesicles
- diabetes mellitus
- stem cells
- microRNA
- intercellular communication

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Introduction to exosomes

It has long been reported that cells release vesicles into the intercellular space (Simons & Raposo 2009, Schneider & Simons 2013). These extracellular vesicles (EVs) take several forms, characterized by size and the process by which they are formed. There are three known types of EVs: apoptotic bodies, microvesicles and exosomes. Apoptotic bodies (ABs) and microvesicles (MVs), collectively known as shedding vesicles or ectosomes, are released by budding from a cell's plasma membrane (Raposo & Stoorvogel 2013, Cocucci & Meldolesi 2015). Exosomes, a third type of EV, are released from cells via the exocytosis of intraluminal vesicles (ILVs). Exosomes are generally smaller than ectosomes and more uniform in shape (Corrado *et al*. 2013). This review primarily focuses on exosomes, although it must be noted that a significant overlap in characterization exists between exosomes and other EVs, causing the nomenclature to be inconsistent among publications (Raposo & Stoorvogel 2013). As a result, many of the studies cited in this article make no distinction between exosomes and other EVs.

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Exosomes were first identified in a study by Harding and coworkers in 1983. Pan & Johnstone also independently published findings identifying exosomes that same year (Pan & Johnstone 1983). These vesicles were initially thought to represent a novel mechanism for cells to dispose of unwanted proteins (Kalra *et al.* 2016). This changed in 1996 when Raposo and coworkers observed that exosomes released by antigen-presenting cells displayed MHC class-II capable of triggering a T-cell response (Raposo *et al.* 1996). In the following years, exosomes were linked to other forms of cell–cell communication in a variety of cell types (Simons & Raposo 2009, Schneider & Simons 2013, Yáñez-Mó *et al.* 2015). Notably, exosomes have been found to transfer functional RNA between cells (Deregibus *et al.* 2007, Valadi *et al.* 2007, Lee *et al.* 2012, Tomasoni *et al.* 2013). Studies have found that genetic material, particularly miRNA and mRNA, and proteins found within exosomes derived from certain stem cells can aid in the repair and protection of damaged tissues, generating interest in exosomes as a vector for regenerative therapy (Cantaluppi *et al.* 2012*b*, Lee *et al.* 2012, Tomasoni *et al.* 2013). In this review, we highlight the clinical significance of exosomes as novel therapeutic vectors for tissue repair and for the treatment of diabetes mellitus.

Exosomes are generated as an alternative endpoint of the endocytic recycling pathway. At the start of this process, an early endosome buds inward from the plasma membrane and is released into the cytoplasm of a cell. Next, intraluminal vesicles (ILVs) pinch inward from the endosomal limiting membrane and bud into the endosome, taking with them a sampling of material from the cytoplasm. The endosome is now a multivesicular body (MVB). Normally the MVB would fuse with a lysosome and its ILVs would be digested. Through unclear mechanisms involving certain Rab GTPases and SNARE proteins (Ostrowski *et al.* 2010, Bobrie *et al.* 2011, Raposo & Stoorvogel 2013), the MVB instead fuses with the plasma membrane, releasing its payload of ILVs into the extracellular medium as exosomes (Alvarez-Erviti *et al.* 2011, Harding *et al.* 2013) (Fig. 1).

While many of the general processes of exosome formation and secretion are well understood, many of the specifics remain under investigation (Rak 2010, Corrado *et al.* 2013). In particular, the molecular mechanisms driving cargo sorting have yet to be fully described. The endosomal sorting complexes required for transport (ESCRT) pathway is believed to play a principal role in this process (Bobrie *et al.* 2011, Colombo *et al.* 2013, Cocucci & Meldolesi 2015). There is also evidence of an ESCRT-independent cargo sorting mechanism requiring ceramide believed to govern the packaging of RNA and certain proteins (Colombo *et al.* 2013, Schneider & Simons 2013, Janas *et al.* 2015). The cargo sorting mechanisms of exosomes are believed to be highly selective as evidenced by the large expression overlap between exosomes of different cellular origins (Guduric-Fuchs *et al.* 2012).

Exosomes can be identified by their expression of markers associated with membrane transport and fusion, such as Rab GTPases, Annexins and ESCRT 0, I, II and III. MVB biogenesis–associated proteins Alix and TSG101 are also characteristically found within exosomes, along with the tetraspanins CD63, CD9 and CD81 (Zhang *et al.* 2015). The exosomal membrane is highly enriched

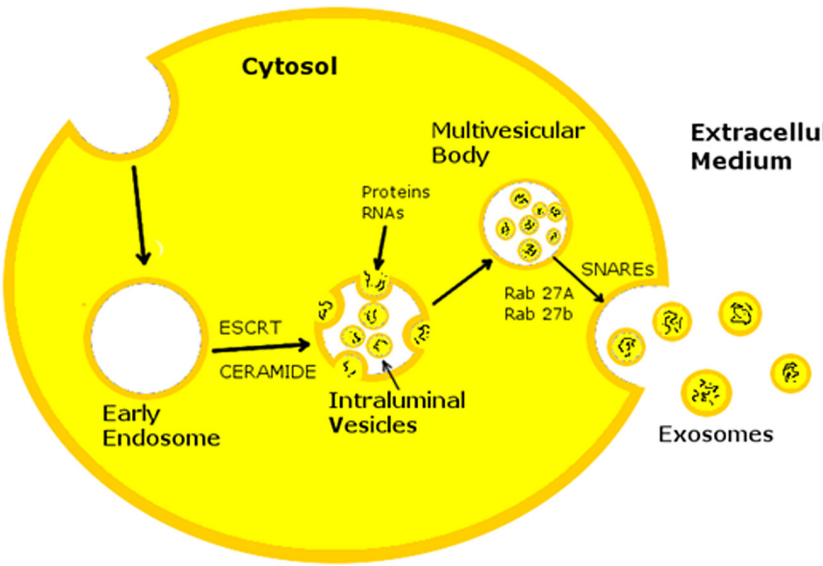

Figure 1
Exosome formation and secretion. The early endosome is formed when a segment of its parent cell's plasma membrane buds inward into the cytosol. Next, the ESCRT and ceramide pathways shuttle proteins and RNA from the cytosol into intraluminal vesicles as they bud into the endosome, forming a multivesicular body (MVB). Rab GTPases guide the MVB to the plasma membrane. SNARE proteins then fuse the MVB with the plasma membrane, releasing the ILVs into the extracellular medium as exosomes.

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in cholesterol, sphingomyelin, hexosylceramides and flotillin, making up structures resembling raft micro domains (Lee *et al*. 2012, Raposo & Stoorvogel 2013). Exosomes also commonly express protein and RNA factors unique to their parent cells (Corrado *et al*. 2013, Yu *et al*. 2014). For example, exosomes secreted by dendritic cells express MHC class-II molecules as well as CD80 and CD86 (Morelli *et al*. 2004, Robbins 2014). The expression profiles of exosomes are also reflective of the conditions of the parent cell, changing in association with a pathogenic state. For example, miR-146b and miR-155, miRNAs known to be upregulated in papillary thyroid cancer cells, can be found overexpressed in exosomes derived from papillary thyroid tumors (Rak 2010, Lee *et al*. 2015*a*,*b*). This attribute makes exosomes a topic of interest as a potential prognostic and diagnostic marker for diseases ranging from cancers to neurodegenerative disorders (Musante *et al*. 2014, De Toro *et al*. 2015). To this end, much ongoing research has been dedicated to identifying the specific cargo of exosomes from various sources. Online databases such as VESICLEPEDIA (http://www.microvesicles.org) and EXOCARTA (http://www.exocarta.org) have been established to allow researchers to post and compare their findings.

Exosomes exhibit unique physical properties that also aid in their characterization. Exosomes range in size from 40 to 100 nm in diameter and appear in a cup-shaped morphology when viewed with negative staining. Although the latter property is caused by the fixation process, these attributes can be used to differentiate these vesicles from other EVs such as MVs and ABs, both of which are generally larger (50–500 nm for ABs, 100–1000 nm for MVs) and appear in various shapes when viewed under an electron microscope (Corrado *et al*. 2013, Raposo & Stoorvogel 2013). It must be noted however that overlap exists between vesicle types when it comes to size, shape and even protein content, so properly differentiating types of EVs poses a challenge even to modern techniques (Kastelowitz & Yin 2014). While electron microscopy, cell flow cytometry, differential centrifugation and immunoassays targeting exosome-specific proteins or lipids have been used to identify and/or isolate different EV types, the number of particles detected in a liter of serum can vary by as many as five orders of magnitude depending on the quantification method used. It is therefore important that standardized techniques be developed for the isolation and sorting of EV subpopulations. Until a standardized characterization technique can be adopted, the functional differences between exosomes and microvesicles will continue to be unclear, and the clinical application will remain difficult.

Exosomes as vehicles for cell–cell communication

Intercellular communication is a well-documented phenomenon essential for the regulation of a stable microenvironment within multicellular organisms. Traditionally, this process has been attributed to the transfer of extracellular proteins and soluble factors across the intercellular space (Gerdes & Pepperkok 2013). More recently, researchers have found that exosomes and other EVs also play a significant role in this process (Ciardiello *et al*. 2016). Exosomes act as shuttles: ferrying proteins, lipids and nucleic acids between cells. Receptors expressed on the exosomal membrane allow them to discriminately interact with specific cellular targets (Corrado *et al*. 2013) exosomal transfer of growth factor receptors, transcription factors and other regulatory proteins allows cells to influence the gene expression of their neighbors (Peinado *et al*. 2012, Montermini *et al*. 2015, Shabbir *et al*. 2015). Exosomes can also transfer functional RNA between cells (Valadi *et al*. 2007). mRNA transferred in exosomes can be expressed in target cells, altering the protein expression within those cells (Deregibus *et al*. 2007, Lee *et al*. 2012, Tomasoni *et al*. 2013). The transfer of miRNAs and siRNAs can also alter protein expression in target cells by binding to complementary mRNA in the cytoplasm, inhibiting translation (Guay *et al*. 2015, Lee *et al*. 2015*b*, Zhang *et al*. 2015). Intercellular transfer of exosomal cargo has been implicated in immunomodulation, initiation of cellular proliferation, angiogenesis, regulation of apoptosis, platelet activation and other phenotypic alterations in targeted cells (Rak 2010, Sheng *et al*. 2011, Corrado *et al*. 2013, Tomasoni *et al*. 2013, Rahman *et al*. 2014, Shabbir *et al*. 2015). Such alterations have been associated with both healthy and pathogenic cellular behaviors (Benito-Martin *et al*. 2015, Ciardiello *et al*. 2016).

Exosomes facilitate intercellular communication by acting as vectors for paracrine signaling (Tetta *et al*. 2013, Salido-Guadarrama *et al*. 2014). Exosomes are believed to be involved in cross-talk between glial cells and neurons, facilitating axon myelination, repair of neurological damage and other essential functions (Krämer-Albers & White 2011, Lopez-Verrilli & Court 2012, Sharma *et al*. 2013). Less beneficially, the transfer of toxic proteins within exosomes in the CNS has been implicated in the development of degenerative neurological disorders

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including Alzheimer's disease and amyotrophic lateral sclerosis (ALS) (Schneider & Simons 2013). Exosomes secreted from tumor cells are often implicated in cancer metastasis, transporting proteins and miRNAs to influence surrounding tissues to create a supportive microenvironment for the tumor (Hood *et al*. 2011, Bobrie *et al*. 2012, Hansen *et al*. 2014, Lugini *et al*. 2016).

Exosomes and other EVs can be found in abundance circulating in bodily fluids such as blood, lymph and cerebrospinal fluid. It is believed therefore that exosomes may be able to exert their influence systemically as well as locally, facilitating endocrine communication between distant cells and tissues (McKelvey *et al*. 2015, Fais *et al*. 2016). This phenomenon has been well explored as a regulator of the immune response. Exosomes circulating in the blood and lymph are believed to play a significant role in antigen presentation via ingestion by antigen-presenting cells (Raposo *et al*. 1996, Théry *et al*. 2009, Alexander *et al*. 2015). There is also evidence that circulating exosomes may play a role in cancer development (Salido-Guadarrama *et al*. 2014). However, while circulating EVs, including exosomes, are plentiful, they have been found in multiple studies to possess very short half-lives of 2–5 min in the blood. It is thought that many of these EVs are quickly digested by macrophages and/or complement interactions or are processed by the spleen and liver (Willekens *et al*. 2005, Saunderson *et al*. 2014). The low persistence of circulating exosomes has made their role in non-immune cell endocrine signaling somewhat controversial; however, these same studies have also shown that subpopulations of EVs can persist for up to three hours post injection (Zhuang *et al*. 2011, Saunderson *et al*. 2014). In addition, intravenous injection of therapeutic exosomes has been successfully used to deliver treatment in animal models, even when not applied near the therapeutic target site (Graner *et al*. 2009, Cantaluppi *et al*. 2012*b*, Ibrahim *et al*. 2014).

Exosomes interact with their target cells through multiple mechanisms. These mechanisms typically begin with the binding of ligands expressed on the exosome's surface with compatible receptors on the membrane of the target cell (Hansen *et al*. 2014, Purushothaman *et al*. 2016). This interaction can trigger cascading downstream effects in the cellular target. The exosomes of antigen-presenting cells can present MHC-II complexes to primed T-cells in this manner to stimulate an immune reaction (Théry *et al*. 2009, Bobrie *et al*. 2011). Similarly, exosomes carrying MCH-I can trigger inhibitory signals in T-cells and natural killer cells, attenuating an immune reaction (McKelvey *et al*. 2015). In many cases, a receptor–ligand interaction facilitates the internalization of exosomes into the target cell via receptor-mediated endocytosis or plasma membrane fusion. RNA transfer via exosome requires internalization (McKelvey *et al*. 2015). In the case of endocytosis, internalization occurs when the exosome is engulfed within an endosome. To release its cargo, the exosome fuses with the limiting membrane, emptying its contents into the cytoplasm prior to lysosomal fusion and digestion (Tian *et al*. 2010, 2013). Such a mechanism is believed to govern the intake of exosomes by dendritic cells (DC). Through this mechanism, DCs process and present peptides introduced by exosomes to primed $CD4^+$ T-cells (Morelli *et al*. 2004). Alternatively, through mechanisms not completely understood, the exosome may directly fuse with the plasma membrane of the target cell, releasing its contents into the cell. This form of interaction was observed between melanoma cells and melanoma cell-derived exosomes by Parolini and coworkers (Parolini *et al*. 2009). A concise visual summary of the different mechanisms of exosome intake is provided in Fig. 2.

Stem cell-derived exosomes protect and support the regeneration of tissues

Stem cells have generated great interest in the research community due to their potent ability to home to and regenerate damaged tissues (Ilic & Polak 2011). In many cases, the exosomes secreted from stem cells have been found to mediate this effect. Like their parent cells, exosomes released from pluripotent and multipotent stem cells have been proven to stimulate angiogenesis and cellular regeneration, suppress apoptotic pathways and modulate immunity in chronically damaged tissues both *in vivo* and *in vitro* (Derkus *et al*. 2017). Unlike their parent cells, exosomes are not immunoreactive, can pass through biological barriers and pose no risk of maldifferentiation (Kunter *et al*. 2007). These properties give exosomes a great deal of potential utility in the field of regenerative medicine as an alternative to stem cell engraftment (George *et al*. 2011, Ilic & Polak 2011, Rahavi *et al*. 2015). Here, we will review some of the recent developments regarding the use of stem cell-derived exosomes as vectors for tissue repair.

The exosomes secreted by pluripotent stem cells hold great therapeutic promise. It has been observed that co-culturing various tissues with progenitor stem cells, such as endothelial progenitor cells (EPCs), has a regenerative and angiogenic effect on these tissues through

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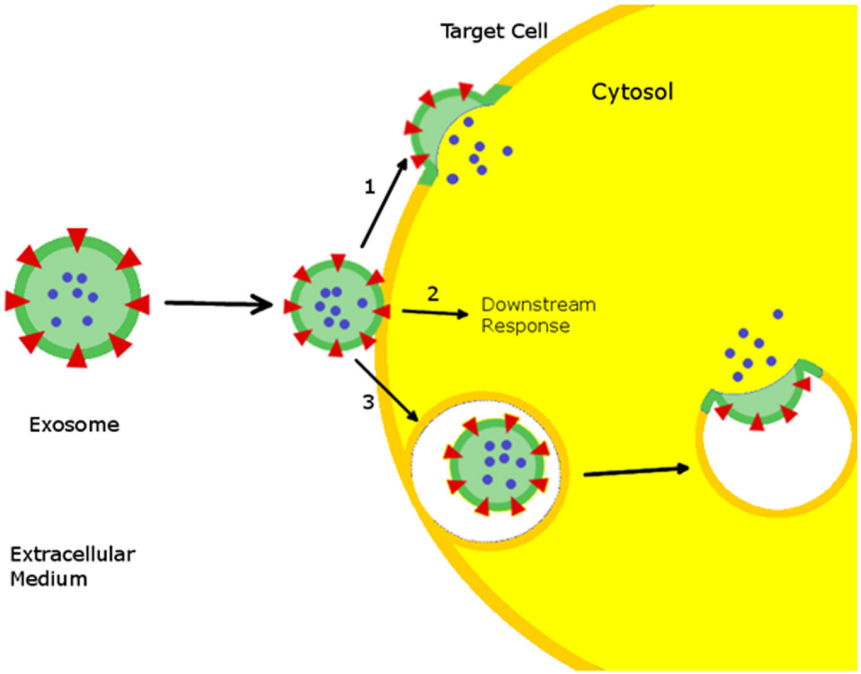

Figure 2
Mechanisms of exosome interaction with target cell. Ligands (triangles) on the surface of the exosomal membrane bind to receptors on the target cell. From there, the receptor–ligand interaction can trigger one of the following: (1) fusion of the exosomal membrane with the cell's plasma membrane, releasing cargo (small circles) into the cytosol, (2) initiation of a downstream signaling cascade in the target cell, (3) endocytosis of the exosome, followed by the fusion of the exosome with the endosome's membrane, releasing cargo (small circles) into the cytosol.

a paracrine signaling effect (Yang *et al*. 2010, George *et al*. 2011, Luo *et al*. 2011). Many recent studies suggest that exosomes and other EVs play a significant role in this phenomenon (Cantaluppi *et al*. 2012*a*, Lee *et al*. 2012, Tomasoni *et al*. 2013, Ibrahim *et al*. 2014, Tan *et al*. 2014, Shabbir *et al*. 2015). Deregibus and coworkers confirmed that EPCs stimulate the proliferation and angiogenesis of endothelial cells, at least in part, through the transfer of RNAs in EVs. In this study, endothelial cells were cultured with EVs taken from EPCs. The endothelial cells in the coculture displayed increased proliferation and reduced apoptosis *in vitro*. Angiogenesis was also observed both *in vitro* and *in vivo* after subcutaneous engraftment in SCID mice. However, pretreating the exosomes with RNase prior to the coculture removed their angiogenic and proliferative effects on endothelial cells (Deregibus *et al*. 2007). This is evidence that the angiogenic effect of EPCs is caused by the exosomal transfer of RNA. A subsequent study conducted by the same research group observed that EVs taken from EPCs had a similar effect on renal cells (Cantaluppi *et al*. 2012*b*). EPC EV injection prevented ischemia-reperfusion injury (IRI)–induced kidney damage in a rat model. The IRI-induced rats displayed reduced renal cell apoptosis, improved tubular cell proliferation and angiogenesis. It was found that this effect was due to the miRNA content of the MVs shifting the renal cells to a regenerative program (Cantaluppi *et al*. 2012*b*). miR-126 and miR-296, miRNAs known to be associated with angiogenesis (Wang & Olson 2009), were found to play a key role in the protective and angiogenic effect of EPC EVs (Cantaluppi *et al*. 2012*b*).

Certain adult stem cells, such as mesenchymal stem cells (MSCs), have also demonstrated a supportive effect on other tissues (Luo *et al*. 2013, Farini *et al*. 2014). Exosomes may be a mediator of such effects. Gatti and coworkers demonstrated that injections of MSC-derived EVs can protect rats from IRI-induced kidney damage. The injected EVs were found to reduce apoptosis and promote the regeneration of tubular endothelial cells. Treating the EVs with RNase prior to injection greatly reduced these effects, suggesting that RNAs within the EVs were the source (Gatti *et al*. 2011). In another study, Tan and coworkers were able to use exosomes derived from MSCs to promote the regeneration of hepatic tissue in mice poisoned with carbon tetrachloride. The exosomes stimulated proliferation of hepatocytes by triggering increased expression of IL-6, TNF-α and MIP-2, known priming factors for hepatic proliferation pathways. Proteins associated with these priming factors (IL6ST/gp130, TNFRSF1A/TNFR1 and CXCL2/MIP-2) were all detected within the MSC-derived exosomes. Human growth factor and hepatocyte growth factor receptor were also expressed in the exosomes. Both of these proteins have been previously reported to be powerful stimulators of hepatic regeneration (Tan *et al*. 2014).

Regenerative treatments using exosomes are also generating promising results in cardiac repair. Ibrahim and coworkers leveraged exosomes from cardiosphere-derived

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cells (CDCs) to induce a proliferative and angiogenic effect on the damaged myocardium of mice afflicted with myocardial infarction. Furthermore, CDCs treated with a sphingomyelinase to prevent exosome formation had no observed proliferative or angiogenic effect on the damaged myocardium when cultured together *in vitro*. This strongly suggests that exosomes are the method by which CDCs can repair wounded myocardial tissue. CDC-derived exosomes were highly enriched in miR-146a. Direct injection of a mimic of this miRNA had a proliferative effect on rat cardiomyocytes. Additionally, exosomes treated with a miR-146a hairpin inhibitor had a reduced proliferative effect compared to ordinary CDC exosomes when delivered to damaged cardiac tissues. These results suggest that miR-146a was partially responsible for the effect the CDC exosomes (Ibrahim *et al*. 2014).

Exosomes taken from bone marrow stem cells contain protein and genetic factors that are capable of healing and protecting the tissues of various organs both *in vitro* and *in vivo*. There are a number of advantages to using exosomes instead of whole stem cell engraftment for regenerative therapies. Unlike whole cells, exosomes are not immunogenic, can cross biological barriers, and can be easily stored without loss of function. Exosomes also carry no risk of long-term maldifferentiation, a serious concern with stem cell engraftment (Kunter *et al*. 2007). Thus, exosomes hold great promise as a treatment vector for many illnesses characterized by chronic tissue damage.

Stem cell-derived exosomes and diabetes mellitus

Due to their demonstrated potential as vehicles for regenerative therapies, exosomes have become a topic of great interest for many labs dedicated to the treatment of chronic illnesses. One illness that stands to benefit from advances in exosome research is diabetes mellitus. Diabetes mellitus is an incurable disease characterized by chronic hyperglycemia due to a patient's reduced ability to produce (type 1) or utilize (type 2) the hormone insulin. Diabetes is becoming increasingly prevalent worldwide, and its complications carry significant morbidity and mortality (Brody 2012, WHO 2016). Controlling the symptoms of diabetes mellitus most often requires daily administration of insulin and other medications for the duration of the patient's life (Brody 2012). This comes at a significant financial burden to patients and to the healthcare establishment (Petersen 2016). Despite advances in treatment, more long-term solutions such as pancreatic islet transplantation pose significant difficulties, making them impractical for most patients (Khosravi-Maharlooei *et al*. 2015). Findings by various labs in the past decade have suggested that exosomes may be instrumental to the development of novel diabetic treatments. The regenerative and immunomodulatory properties of stem cell derived exosomes could be leveraged as a treatment for serious diabetic complications such as diabetic nephropathy and CNS damage (Jiang *et al*. 2016, Venkat *et al*. 2017). Stem cell-derived exosomes may also be the key to protecting the pancreatic islets of type-1 diabetic (T1D) patients from autoimmune targeting, slowing the disease's progression (Mokarizadeh *et al*. 2012, Bu *et al*. 2015). Exosomes could also aid in the angiogenesis and survival of transplanted pancreatic islets, enhancing the efficiency and success rate of the treatment (Cantaluppi *et al*. 2012*a*, Kordelas *et al*. 2014).

Stem cell-derived exosomes can regenerate tissue damage from diabetic complications

Diabetes mellitus is often accompanied by severe and potentially lethal complications. Diabetic nephropathy affects 30–35% of all patients suffering from diabetes mellitus and is associated with a 20–40 times increase in mortality (Thomas & Karalliedde 2015). Diabetic nephropathy is characterized by accumulating hyperglycemia-induced damage of the renal glomerular capillaries. If left unchecked, this damage results in kidney failure necessitating transplantation. This condition is also associated with high blood pressure and a greatly increased risk of heart failure (Schena 2005). There is evidence that exosomes may be able to aid in the treatment of diabetic nephropathy. Studies have found that stem cells have a reparative effect on renal tissues in both diabetic nephropathy and other kidney diseases (Zhang *et al*. 2013). As previously discussed, the paracrine signaling effect of exosomes may aid in stem cell–mediated regenerative processes, including the repair of renal tissue. In one such study, exosomes secreted from human urinary stem cells (USC) were isolated and injected into the kidneys of nephritic rats. The research group observed a notable decrease in urinary albumin over the course of the experiment, reduced podocyte apoptosis and enhanced glomerular endothelial cell proliferation. The presence of growth factor, transforming growth factor-β1, angiogenin, and bone morphogenetic protein-7, all factors that are known to enhance angiogenesis and cell

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survival, were detected within the USC-derived exosomes (Jiang *et al*. 2016). These data suggest that stem cell-derived exosomes have strong therapeutic potential as a treatment for diabetic kidney damage.

Also, among the complications of diabetes mellitus is a significantly increased occurrence of damage to the CNS (Wrighten *et al*. 2009). Often this damage is the result ischemic stroke, a condition that diabetic patients are two to six times more likely to experience due to cardiovascular complications. In addition to being more common, strokes experienced by diabetics are often more severe, with a poor prognosis and rate of functional recovery under current treatment protocols (Ergul *et al*. 2013). MSCs have been found to mediate the functional repair of damaged neuronal tissue, including damage from a stroke, via the transfer of regenerative paracrine factors (Chen *et al*. 2014). Exosomes derived from MSCs have been demonstrated to have a similar reparative effect on neuronal stroke damage in animal models, transporting miR-133a to promote axonal remodeling and neuronal outgrowth (Xin *et al*. 2014, Zhang & Chopp 2016). A recent abstract published by Venkat and coworkers suggests that isolated exosomes derived from MSCs may be an effective treatment for improving the outcomes of diabetic stroke victims specifically (Venkat *et al*. 2017). In this study, rat models of T2D were subjected to transient middle cerebral artery occlusion (MCAo) to induce ischemic strokes. Rats injected with exosomes isolated from MSCs three days after the stroke experienced reduced hemorrhaging and significantly improved long-term recovery of neurological function and white matter density compared to untreated controls (Venkat *et al*. 2017). In addition to damage caused by strokes, diabetic patients have a significantly increased likelihood of developing cognitive impairment due to structural damage of the CNS. This damage is thought to be a result of vascular abnormalities and oxidative stress in the CNS caused by chronic hyperglycemia (Wrighten *et al*. 2009). In a study published by Nakano and coworkers, exosomes derived from MSCs were found to ameliorate CNS symptoms in diabetic rats. Diabetic cognitively impaired, mice injected with the exosomes displayed improved cognition, learning and synaptic density within days of exosome injection compared to the untreated diabetic controls. It was concluded that the exosomes repaired oxidative damage in neurons and astrocytes to restore cognitive function in the diabetic animals (Nakano *et al*. 2016). These results demonstrate the potential of exosome-based therapies as a treatment for CNS damage prevalent in diabetic patients. Treating CNS damage has long been a challenge for even the most advanced treatment techniques. Novel therapeutic strategies using exosomes to improve patient outcomes would be of tremendous benefit to both diabetic and nondiabetic patients suffering from CNS injury.

Stem cell-derived exosomes may improve treatment outcomes for T1D patients

Diabetes mellitus type 1 (T1D) is an autoimmune disorder characterized by immune-associated destruction of pancreatic islet β-cells, resulting in greatly reduced insulin secretion and hyperglycemia. Sufferers of this heritable disorder are often dependent on frequent insulin injections to prevent hyperglycemia, along with other medications to manage T1D's often debilitating symptoms (Atkinson *et al*. 2014, Miller *et al*. 2015). Exosomes derived from stem cells and certain antigen-presenting cells (APCs) have shown the ability to reduce immune activity by promoting the proliferation of regulatory T-cells and the apoptosis of autoreactive T-cells (Mokarizadeh *et al*. 2012, Robbins 2014). These immunoregulatory properties have been successfully applied to treat various autoimmune disorders such as myasthenia gravis in pre-clinical models (Bu *et al*. 2015). These findings suggest that it may be possible to utilize stem cell-derived exosomes to slow or stop the progression of T1D, preserving pancreatic islet cells and the patient's insulin independence. There is even a possibility that the reparative properties of stem cell-derived exosomes could cause functional recovery of the islets. Multiple clinical trials were able to achieve this effect through the engraftment of whole hematopoietic stem cells in early-stage T1D patients (D'Addio *et al*. 2014). Stem cell-derived exosome injections could potentially be equally effective, while not requiring immunosuppressants to prevent graft rejection. However, near total destruction of pancreatic β cells is quick to occur (Atkinson *et al*. 2014), so such treatments would have to be administered very early in the disease's progression if insulin function is to be retained.

One of the most promising treatments for T1D is pancreatic islet transplantation. This procedure consists of the transplantation of healthy pancreatic islets from recently deceased donors into T1D patients. This therapy has allowed many patients to regain temporary insulin independence (Kuise & Noguchi 2011). However, many challenges remain before this treatment can become suitable for widespread use. Pancreatic islet β-cells tend to rapidly undergo necrosis and apoptosis *in vitro* upon

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harvest and often continue to do so after engraftment in a new host. This is thought to be brought about by the disruption of the islet's proper microenvironment and a lack of vascular support. Host immune rejection is also a significant aggravating factor (Rother & Harlan 2004). To compensate for the high rate of islet loss, at least two donors are required for each procedure. Even then, most recipients lose insulin independence within five years (Rother & Harlan 2004, Jiang & Morahan 2014). Our lab has found that co-culturing islets with BMSCs can greatly improve islet vascularization and reduce apoptosis, resulting in increased survival and function of islet β-cells both *in vitro* and after engraftment in animal models (Luo *et al.* 2011, 2013, 2015). There is evidence that stem cell-derived exosomes mediate this effect. Cantaluppi and coworkers used EVs taken from EPCs to promote angiogenesis and regeneration when cultured with pancreatic islets *in vivo* and *in vitro*. These EPC-derived EVs contained miR-126 and miR-296, two miRNAs that had been previously described by the same research group to promote angiogenesis and proliferation of renal endothelial cells. When these EVs were treated with RNase, the angiogenic effect on the islet endothelium was reduced, suggesting that RNAs contained within the EVs were responsible for the observed angiogenesis (Cantaluppi *et al.* 2012*a*). Furthermore, limited clinical evidence shows that exosomes derived from MSCs can act to suppress immune targeting of allogeneic grafts, effectively treating graft vs host disease (Kordelas *et al.* 2014). Based on these findings, it is likely that exosomes released by stem cells are at least partially responsible for the supportive effect whole stem cells have on islet β-cells. Therapies incorporating exosomes such as these, which contain anti-apoptotic, regenerative, angiogenic and immunomodulatory factors could make islet transplantation a more practical and appealing option for patients suffering from T1D, extending the functional life of an islet graft while reducing the need for immunosuppressive treatments.

Conclusions and future perspective

With a more complete understanding of the mechanisms driving exosome formation, exosomes could be engineered as vectors for the targeted delivery of molecular therapies. In 2011, Alverez-Erviti and coworkers published a study demonstrating the potential of such a technique (Alverez-Erviti *et al.* 2011). Exosomes have the advantage of being nonimmunogenic, highly resilient and capable of acting both locally and systemically. These advantages make exosomes a potentially safer and more effective alternative to conventional viral vectors (Lee *et al.* 2012). Proteins and RNA found to aid in tissue regeneration could be packaged into specially engineered exosomes expressing ligands facilitating attachment to the therapeutic target. Injection of these exosomes would, in theory, allow for the discriminate delivery of medicine directly into specific target cells. Such therapies could one day be administered to diabetic patients suffering from nephropathy or CNS damage to stimulate cellular regeneration. It could also be of benefit as a supportive treatment for pancreatic islet transplant recipients, lengthening insulin independence and reducing the patients' dependence on immunosuppressive medication. The long-term efficacy and safety of such a treatment would first have to be established.

Exosome research is still in its early stages. No full-scale clinical trials using exosome-based regenerative therapies have yet been conducted. Progress is dependent on the development of a greater understanding of the mechanisms governing exosome formation and signaling. Gaps in mechanistic knowledge, inconsistent nomenclature, and a lack of standardized characterization techniques remain a challenge for exosome research going forward. Overcoming these obstacles will pave the way for clinical trials and will allow for a wide range of new discoveries and treatments for a variety of disorders. Exosomes have the potential to provide all the benefits of stem cell–based regenerative therapies without the dangers of maldifferentiation or immune rejection. Such treatments could revolutionize the field of regenerative medicine with applications for the treatment of many common disorders, including diabetes mellitus.

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