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Exosomes for drug delivery — a novel application for the mesenchymal stem cell

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ABSTRACT

Exosomes are the most extensively characterized class of secreted membrane vesicles that carry proteins and RNAs for intercellular communication. They are increasingly seen as possible alternatives to liposomes as drug delivery vehicles. Like liposomes, they could deliver their cargo across the plasma membrane and provide a barrier against premature transformation and elimination. In addition, these naturally-occurring secreted membrane vesicles are less toxic and better tolerated in the body as evidenced by their ubiquitous presence in biological fluids, and have an intrinsic homing ability. They are also amenable to in vivo and in vitro loading of therapeutic agents, and membrane modifications to enhance tissue-specific homing. Here we propose human mesenchymal stem cells as the ideal cell source of exosomes for drug delivery. Mesenchymal stem cell transplantation for various disease indications has been extensively tested and shown to be safe in numerous clinical trials. These cells are also prolific producers of immunologically inert exosomes. Immortalization of these cells does not compromise the quantity or quality of exosome production, thus enabling infinite and reproducible exosome production from a single cell clone.

Contents

1. Introduction

Exosomes are a class of secreted lipid membrane vesicles. Secreted membrane vesicles are classified as microvesicles, ectosomes, membrane particles, exosome-like vesicles, apoptotic bodies, prostasomes, oncosomes, or exosomes, according to their biogenesis pathway, size,

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flotation density on a sucrose gradient, lipid composition, sedimentation force, and cargo content (see review (Duijvesz et al., 2011; Thery et al., 2009)). However, the parameters used in this classification are generally not definitive or exclusive leading to much confusion in our understanding of the biology and functions of the different classes of secreted membrane vesicles. This is further exacerbated by the lack of proper qualification in describing a particular class of membrane vesicle. It is not clear if all of the above mentioned vesicles represent exclusive entities. A nomenclature standard to define each class of membrane vesicle unambiguously is therefore urgently needed to facilitate knowledge sharing and exchange (Simpson and Mathivanan, 2012).

Secreted membrane vesicles are known to be produced by many cell types. These vesicles have attracted much interest from the biomedical research community because of their potential as sources of biomarkers for diseases, therapeutic agents or vehicles for drug delivery. Secreted membrane vesicles carry cargoes of proteins and nucleic acids that reflect their cell sources and the pathophysiological states of the cell sources such that these proteins or nucleic acids could be used as biomarkers to diagnose, prognose or predict disease and its progress. In addition, these vesicles with their cargoes could be internalized by other cells as a form of intercellular communication making these vesicles potential therapeutic agents or delivery vehicles of therapeutic agents.

Generally, secreted membrane vesicles could be broadly divided into two classes: 1) vesicles formed by inward budding of endolysosomal vesicles and released through exocytosis; and 2) vesicles which are shed from the plasma membrane. Exosomes and microvesicles are the two most representative vesicle types from the two classes respectively.

1.1. Microvesicles

Microvesicles (MVs) are formed by the outward budding and fission of the plasma membrane. MVs are highly variable in size with diameters that range from 100 to 1000 nm. Most cell types are known to produce MVs either constitutively or when stimulated during apoptosis or activation. EBV-transformed epithelial cells (Piccin et al., 2007), B cells (Quah et al., 2008), and immature dendritic cells (Piccin et al., 2007) were reported to secrete MVs constitutively while platelets (Zwaal et al., 1992), HEK293 cells (MacKenzie et al., 2001), monocytes (MacKenzie et al., 2001), tumor cells (Kim et al., 2002) and endothelial cells (Jimenez et al., 2003) were reported to secrete MVs upon activation. MVs are unlikely to be a homogenous class of membrane vesicles and it remains to be determined if MVs could be further differentiated by the plasma membrane microdomains from which they budded, their protein or nucleic acid cargos, their surface markers, membrane lipid composition, density, etc.

MVs have been reported to have many diverse functions and this could be attributed to the apparent heterogeneity in this class of secreted membrane vesicles. For example, MVs have been reported to be involved in blood clotting (Zwaal et al., 1992), interleukin-1β (IL-1β) secretion (MacKenzie et al., 2001) tumor invasion and metastasis (Sidhu et al., 2004), promotion of tumor growth (Skog et al., 2008), induction of apoptosis of immune cells protection of the developing fetus from maternal immune recognition (Abrahams et al., 2004), reprogramming of hematopoietic progenitor (Ratajczak et al., 2006) and shuttle functional mRNAs between cells (Collino et al., 2010). These reported functions of MVs suggest that MVs are vehicles for intercellular communication. However it remains to be determined if the MVs reported in these studies are similar entities sharing a common biogenesis and biophysical or biochemical properties. For example, platelets were reported to secrete two types of membrane vesicle, MVs and exosomes, and that these two membrane vesicles have different functions (Heijnen et al., 1999). The lack of a definitive criteria for MV classification impeded the development of standardized isolation and assay protocols leading to the preclusion of MVs as a potential class of natural drug vehicles.

1.2. Exosomes

Presently, exosomes are the most clearly defined class of secreted membrane vesicles reported to date. They are the only class of membrane vesicles known to originate from the endosomes. They are formed by the invagination of endolysosomal vesicles to form multi-vesicular bodies. Exosomes are released to the extracellular space when the multi-vesicular body fuses with the plasma membrane. They have a narrower diameter range of 40–100 nm, float at 1.1–1.18 g/ml in sucrose density gradient and their membranes are enriched in cholesterol, sphingomyelin and ceramide and are known to contain lipid rafts (de Gassart et al., 2003; Wubbolts et al., 2003). The presence of phosphatidylserine on the outer face of exosome membranes remains to be confirmed as there are contradicting reports for (Keller et al., 2009; Zakharova et al., 2007) and against (Carmo et al., 2003; Heijnen et al., 1999) this presence. Exosomes are reported to contain both proteins and RNAs (Chen et al., 2010; Lai et al., 2010a). Most exosomes have an evolutionary conserved set of protein molecules including tetraspanins (CD81, CD63, CD9), Alix, Tsg101 but they also have unique tissue/cell type-specific proteins that reflect their cellular source (Simpson et al., 2008). Richard Simpson and his colleagues have set up Exocarta, a freely accessible web-based compendium of proteins and RNAs found in exosomes (http://www.exocarta.org) (Mathivanan and Simpson, 2009).

1.2.1. Exosome functions

Exosomes were first discovered as a vehicle for discarding unwanted transferrin by maturating sheep reticulocytes (Pan and Johnstone, 1983). For many years, exosome research was a highly specialized and relatively obscure domain until Raposo and her colleagues observed that B-lymphocytes stimulate T cell proliferation (Raposo et al., 1996) and suppress tumor growth (Zitvogel et al., 1998a) by secreting exosomes that express functional MHC I, MHC II and T-cell costimulatory molecules. More recently, exosomes were found to contain mRNA (Skog et al., 2008) and miRNA (Rabinowits et al., 2009; Skog et al., 2008; Taylor and Gercel-Taylor, 2008) that could be transferred into recipient cells to modulate protein synthesis. Together, these studies suggest that the function of exosomes extends beyond the disposal of unwanted proteins and may mediate intercellular communication through protein–protein interactions and exchange of proteins and genetic materials. For a more extensive discussion on exosome function, the reader may refer to this review (Lai et al., 2011).

Since most of the earlier studies observed the secretion of exosomes by in vitro cultures of different cell types such as B cells (Raposo et al., 1996), dendritic cells (Zitvogel et al., 1998a), mast cells (Raposo et al., 1997), T cells (Peters et al., 1989), platelets (Heijnen et al., 1999), Schwann cells (Fevrier et al., 2004), tumor cells (Wolfers et al., 2001), mesenchymal stem cells (Lai et al., 2010a), human embryonic kidney cells (Sokolova et al., 2011), various cancer cell lines (Clayton et al., 2011) and sperm (Sullivan et al., 2005), they were initially suspected to be culture artifacts. However, the subsequent discovery of exosomes in physiological fluids including bronchial lavage fluid (Admyre et al., 2003), human urine (Caby et al., 2005; Pisitkun et al., 2004) and human blood (Caby et al., 2005), helped establish exosomes as physiological cellular products. The large diversity in exosome-secreting cell types and the presence of exosomes in different physiological fluids indicate that secretion of exosomes is a general cellular function. It also suggests that exosomes play a significant role in the intercellular communication by transferring both proteomic and genomic materials between cells. This intercellular communication role of exosomes generated a lot of interest in the scientific community as exosomes could be used as natural drug delivery vehicles if they could be loaded with the desired therapeutic molecule.

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2. Exosomes as drug delivery vehicles

Currently, the preferred pharmaceutical vehicles for drug delivery are the phospholipid membrane vesicles or liposomes. Liposomes have been used for the delivery of anti-cancer drugs (Goncalves, 2003; Harrington, 2001; Johnston and Gore, 2001; O'Shaughnessy, 2003; Perez et al., 2002; Schmidinger, 2001; Schwonzen et al., 2000; Seiden, 2004; Skubitz, 2003; Symon, 1999; Wollina, 2003), anti-fungal drugs (Sundar, 2003), analgesics (Grant, 2004), etc. They have many positive attributes that are pivotal in their function as drug delivery vehicles (reviewed (Torchilin, 2005)). They help provide a protective barrier against premature transformation and elimination. With a phospholipid membrane, liposomes could readily incorporate hydrophilic and/or hydrophobic drugs in their aqueous core or within the hydrophobic membranes, respectively. They could also breach the plasma membrane to deliver their drug cargo. Liposomal membranes can also be modified to display ligands or antibody fragments that bind to specific cell types and enhance cell type-specific drug delivery. They can be coated with inert polymers such as PEG to reduce liposome recognition by opsonins and clearance of the liposomes.

Despite significant advances in the design and efficacy of synthetic liposomes as drug delivery vehicles (Torchilin, 2005), the ideal liposome vehicle that could evade host immune system, be internalized specifically by the target cells, have sufficiently long circulating half-life, minimal toxicity and are amenable to loading with varied therapeutic payloads remains elusive. Secreted membrane vesicles being essentially nature-derived liposomes could potentially overcome some of the limitations of synthetic liposomes such as the toxicity of their lipid membranes. Among the different secreted membrane vesicles, exosomes being the most clearly defined are the most suited for development as a drug delivery vehicle.

Exosomes have many features of an ideal drug delivery vehicle. Firstly, the presence of protein and genetic materials in exosomes imply that such biological materials could be loaded into exosomes. Secondly, exosomes are well tolerated in the body as evidenced by their wide distribution in biological fluids such as blood (Caby et al., 2005), urine (Zhou et al., 2006) and breast milk (Admyre et al., 2007). Therefore, exosome-derived drug delivery vehicles will likely be as well tolerated leading to a longer circulating half-life and improved efficacy. Thirdly, exosomes have been shown to cross the plasma membrane to deliver their cargo into target cells. For example, dendritic cell-derived exosomes can transfer peptide loaded MHC class I and II complexes to other dendritic cells to modulate immune response (Andre et al., 2004; Thery et al., 2002). Intercellular transfer of mRNAs by exosomes that resulted in the translation of the transferred mRNA in the recipient cells has also been reported (Valadi et al., 2007). Fourthly, exosomes have an intrinsic ability to home to target tissues. Much circumstantial evidence suggests that exosomes have preferential homing targets depending on their cell source. For example, melanoma exosomes home preferentially to sentinel lymph nodes to promote tumor metastasis (Hood et al., 2011). Finally, exosomes are amenable to membrane modifications that enhance cell type-specific targeting. By overexpressing a fusion gene consisting of a neuron-specific RVG encoding sequence and LAMP2B encoding an exosomal membrane protein in dendritic cells, Woods and his co-worker demonstrated that these modified dendritic cells secrete exosomes with RVG peptide on their membranes. After loading these exosomes with siRNA, they further reported that these exosomes could cross the blood–brain barrier to knock down >60% of the siRNA-targeted gene in neurons, microglia, oligodendrocytes and their precursors (Alvarez-Erviti et al., 2011).

3. Production and purification of exosomes for drug delivery

The minimal requisites for using exosomes as drug delivery vehicles are a high-yielding cell source of exosomes and a reproducible scalable purification protocol to prepare a highly defined population of exosomes. Although most cell types produce exosomes, the amount of exosomes produced by each cell type is highly variable (Yeo et al., in press). Exosomes produced by cultured cells are normally isolated from the medium conditioned for one to 7 days by the cells using either serum-free medium or medium with serum that had been depleted of microvesicles. Currently, there are several commonly used protocols for exosome purification (Fig. 1).

The most commonly used isolation protocol for exosomes is differential centrifugation where an increasing centrifugal force from $200\times g$ to $100{,}000\times g$ is applied to first deplete the medium of larger particles and cell debris before finally sedimenting exosomes at $100{,}000\times g$ (Denzer et al., 2000; Lamparski et al., 2002; Zitvogel et al., 1998b). While this method significantly enriches for exosomes in conditioned medium, it is nevertheless a crude, non-specific purification method and the exosome preparation tends to be contaminated with protein aggregates and other particulates from the media and cell debris (Simpson and Mathivanan, 2012). In our hands, the resulting pellet often cannot be resuspended completely resulting in clumping of exosomes that can be visualized by electron microscopy (unpublished observation). We also observed a loss in biological activity of pelleted exosomes (unpublished observation). Additionally, this method is time-consuming, requires expensive specialized equipment and has poor scalability. To enhance the specificity of exosome purification by centrifugation, exosomes have been purified according to their flotation density using equilibrium density gradient centrifugation in sucrose density gradient or Optiprep. This greatly enhances the purity of the exosome preparation but further reduces the scalability of the centrifugation methodology.

Another common method for exosome purification uses immunoaffinity (Clayton et al., 2001; Mathivanan et al., 2010). Generally, a monoclonal antibody against exosome-associated antigen is conjugated to magnetic beads to extract exosomes directly from conditioned medium or other biological fluids. Although this is a highly scalable technique, most extracellular exosome-associated antigens are not exclusive to exosomes and immunoaffinity-based isolation protocols could potentially purify protein complexes and other particulates including non-exosome vesicles that carry the antigen, such as cell debris. Another drawback is the use of pH and salt concentration to release exosomes from the capturing matrix. In our hands, exosome function is affected by non-neutral pH or non-physiological salt concentration.

To circumvent the use of non-neutral pH or non-physiological salt concentration, ultrafiltration using membranes with different pore sizes such 100 Kd MWCO and gel filtration to eliminate smaller particles have been used. In our hands, these methods do enrich for exosomes without compromising the biological activity. However, these methods are still relatively crude and the size of the particles are highly heterogenous. Recently, our group used high performance size exclusion liquid chromatography (HPLC) to purify exosomes as a population of homogenously sized particles (Lai et al., 2010a, 2010b). Although the purity of the exosome preparation is relatively high, this method also requires expensive equipment such as HPLC and has poor scalability. The advantages of this purification method are that throughout the purification process, the exosomes remain in solution at a physiological pH and salt concentration and their biological activity is well preserved. To date, there is no ideal, scalable and cost effective method for the purification of exosomes.

4. Exosome loading

Although synthetic liposome-based drug delivery systems have been relatively successful, they have several limiting disadvantages such as the use of toxic or immunogenic synthetic lipids. Therefore natural lipid vesicles that mediate intercellular exchange of RNA, protein and lipid trafficking such as exosomes could circumvent some of

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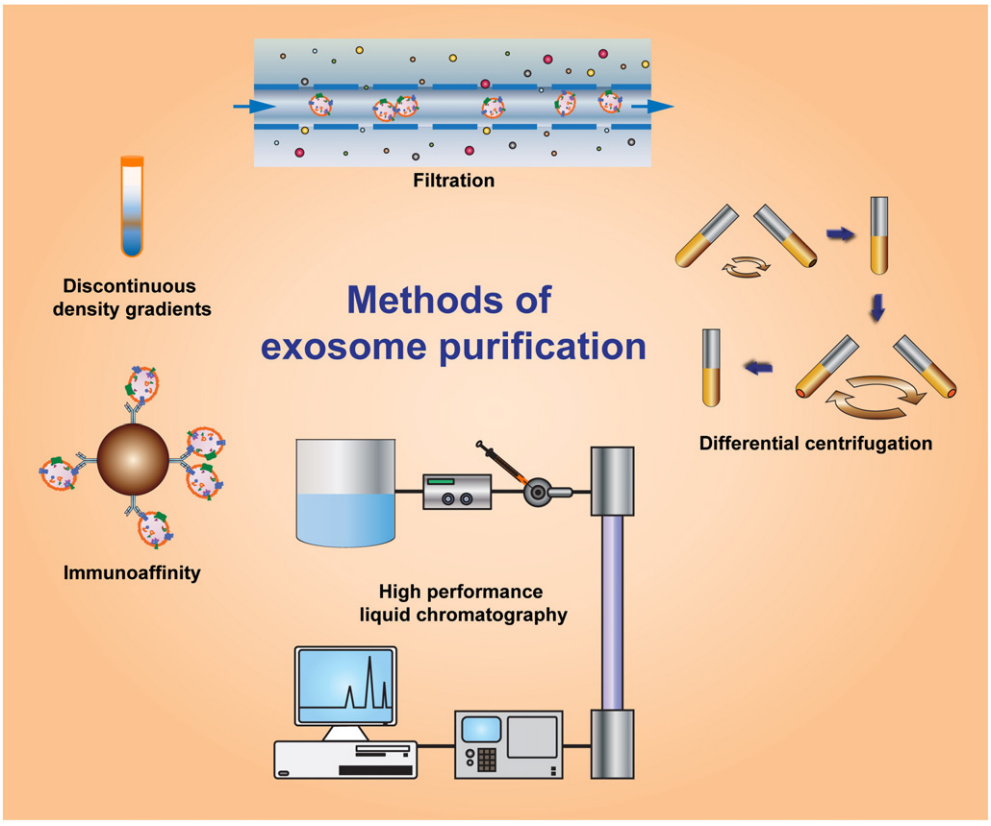

Fig. 1. Methods of exosome purification.

the limitations associated with synthetic liposomes. However, the development of exosomes as drug delivery vehicles will require drug loading strategies that are different from those used in loading liposomes. In principle, exosomes could be loaded either in vivo during their biogenesis or in vitro in purified exosomes (Fig. 2). However, the development and optimization of exosome loading strategies are currently limited by our superficial understanding of exosome biology, structure and biogenesis, and a lack of exosome-related research and development tools.

4.1. In vitro drug loading

In vitro drug loading refers to the loading of drugs into purified preparations of exosome. Since exosomes resemble liposomes in

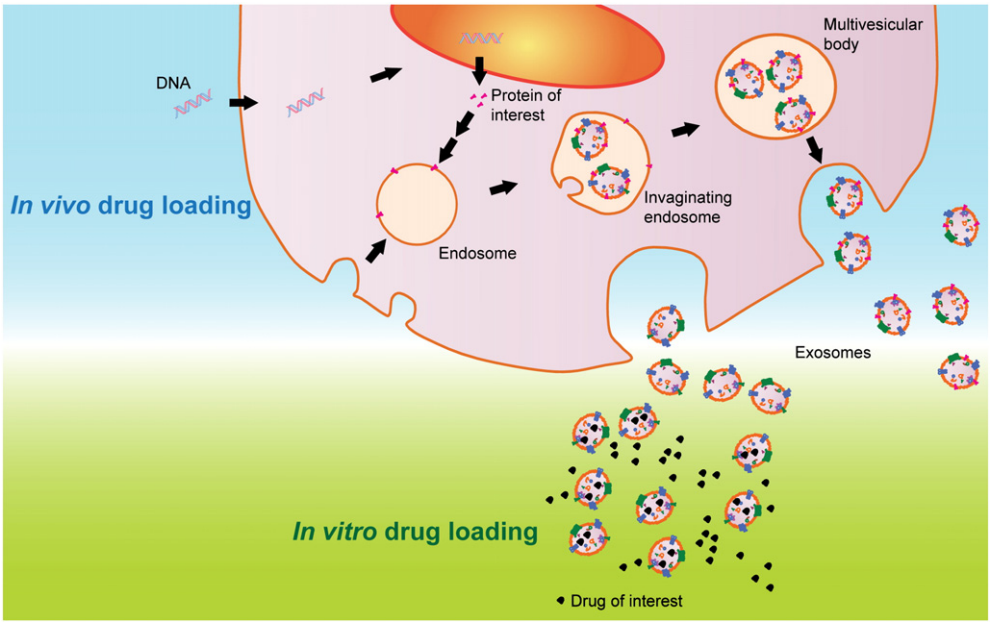

Fig. 2. Strategies to introduce drugs into exosomes. In vivo drug loading: The genes that encode for the RNA/protein of interest are introduced into exosome-secreting cells by standard gene transfection methods e.g. virus, lipofection, electroporation. To promote sorting into exosomes, the RNA/proteins are genetically tagged with exosome-localizing domains. In vitro drug loading: The drugs of interest are loaded into purified exosomes using standard membrane breaching techniques such as electroporation and lipofection.

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having a bilipid membrane and an aqueous core, they could potentially be loaded with both hydrophilic and lipophilic drugs. Zhang et al. (Sun et al., 2010) reported that curcumin, a hydrophobic polyphenol, complexes with exosomes purified from the EL-4 mouse lymphoma cell line at room temperature. The curcumin–exosome complex enhances the anti-inflammatory effects of curcumin and inhibits IL-6 and TNF-α secretion in activated myeloid cells as well as in a mouse model of lipopolysaccharide (LPS)-induced septic shock. This complex could also cross the blood–brain barrier into microglial cells to reduce LPS-induced brain inflammation (Zhuang et al., 2011).

For hydrophilic molecules such as nucleic acids that cannot incorporate spontaneously into the membrane of exosomes, the drugs will have to cross the exosome membrane into the aqueous core. For small molecules such as siRNA, loading could be achieved by a transient physical (e.g. electroporation) or chemical disruption (e.g. lipofection) of the exosome membrane for the uptake of the small molecules followed by the re-sealing of the membrane. Recently, Alvarez-Erviti et al. and Wahlgren et al. (Wahlgren et al., in press) electroporated siRNA into dendritic cell- and plasma-derived exosomes, respectively. These electroporated exosomes delivered the siRNA cargo into target cells resulting in specific gene knockdown. Alvarez-Erviti et al. further demonstrated that the electroporated exosomes could cross the blood–brain barrier.

4.2. In vivo drug loading

Another approach to exosome loading is to incorporate drugs into exosomes during their biogenesis. This is especially relevant for cargo that cannot be loaded onto purified exosomes such as cytosolic and transmembrane proteins or high MW RNA such as mRNA. However, the loading of exosomes during their biogenesis requires an in-depth understanding of the biogenesis and the mechanism for intracellular trafficking and localization of biomolecules to exosomes, particularly the cis-acting elements on proteins and nucleic acids that target them into exosomes.

Stephen J. Gould and his team showed that using cross-linking antibodies that oligomerized plasma membrane proteins or cytoplasmic proteins anchored to the membrane with an N-terminal acylation tag would localize proteins in exosomes (Fang et al., 2007). These observations were further corroborated using different plasma membrane anchors and oligomeric proteins (Shen et al., 2011). Therefore, target proteins could be shuttled into exosomes by genetically engineering the proteins to carry plasma membrane anchors and oligomerization domains. However, such engineering may affect protein structure and function resulting in a loss of therapeutic potential.

Unlike proteins, the search for similar cis-acting elements that target RNA to exosomes has, to date, been limited to computational prediction. Generally, mRNAs are under-represented while non-coding RNAs are over-represented in exosomes. By comparing the sequences of exosome-enriched RNAs against cytoplasmic RNAs, Batagov et al. (2011) identified several candidate motifs at the 3′-ends of exosome-enriched RNAs.

5. Mesenchymal stem cell as an ideal source of exosomes for drug delivery

Another important consideration in using exosomes for drug delivery is the cell source of exosomes. As mentioned earlier, dendritic cell exosomes have commonly been utilized for both experimental and clinical purposes. For example, DC exosomes have been purified and loaded with antigenic peptides to stimulate T cell proliferation (Hsu et al., 2003; Qazi et al., 2009), and its potential as a vaccine against cancer or infectious diseases is currently being tested clinically. However, DC exosomes are immunogenic and their use as drug delivery vehicles would require the use of immune-compatible exosomes. Therefore, an ideal cell source of exosome for drug delivery would be one that produces an abundance of non-immunogenic exosomes. We have previously described human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs) as an ideal cell source of exosomes for this purpose (Yeo et al., in press).

5.1. MSCs

MSCs (also referred to as multipotent stromal cells or simply adult stem cells) are multipotent fibroblast-like cells that could be readily isolated from many different tissues such as bone marrow (Kern et al., 2006; Lee et al., 2004), adipose tissue (Banas et al., 2007; Lee et al., 2004), liver (Gotherstrom et al., 2003; in 't Anker et al., 2003), muscle (Jackson et al., 2009; Young et al., 2001), amniotic fluid (Roubelakis et al., 2007; Tsai et al., 2004), placenta (Fukuchi et al., 2004; Miao et al., 2006), umbilical cord blood (Erices et al., 2003; Kern et al., 2006), dental pulp (Huang et al., 2009; Perry et al., 2008). They also have a large capacity for *ex vivo* expansion (Giordano et al., 2007). Although most MSCs differentiate primarily into adipocytes, osteocytes and chondrocytes (Bruder et al., 1998; Dennis et al., 1999; Haynesworth et al., 1992; Johnstone et al., 1998; Pittenger et al., 1999; Yoo et al., 1998), they have been reported to be capable of endothelial and cardiovascular differentiation (Gojo et al., 2003), neurogenic differentiation (Kohyama et al., 2001; Sanchez-Ramos et al., 2000; Woodbury et al., 2000), and neovascular differentiation (Kobayashi et al., 2000; Sato et al., 2011; Tomita et al., 1999). MSCs are also known to have immunosuppressive properties (Le Blanc and Pittenger, 2005) and therefore could be used in allogeneic transplantation. Together, these features have made MSCs the stem cells of choice for regenerative therapy. As such, they are presently the most extensively studied stem cells with more than 1000 articles cited in Scopus each year since 2005. Most remarkably, they have demonstrated reparative effects in various animal models of human diseases and clinical trials. MSCs have been evaluated in clinical trials for efficacy in treating diseases such as cardiovascular diseases (Hare et al., 2009), graft verse host disease (GvHD) (Le Blanc et al., 2008), bone/cartilage defect, Crohn's disease (Duijvestein et al., 2010), acute kidney injury, spinal cord injury (Wright et al., 2011) and diabetes (Bhansali et al., 2009).

Although many MSC clinical trials were predicated on the hypothesis that transplanted MSCs home and engraft in injured tissues and then differentiate into cells to replace damaged cells, it is becoming apparent that engraftment and differentiation at the injury site is unlikely to account for the therapeutic effects of MSC transplantation (Dai et al., 2005; Iso et al., 2007; Meirelles Lda et al., 2009; Noiseux et al., 2006; Phinney and Prockop, 2007). Studies have shown that less than 1% of transplanted MSCs migrated to the injury site to replace damaged cells, with most of them trapped in the liver, spleen and lungs (Phinney and Prockop, 2007). Treatment response was also not correlated with engraftment efficiency (von Bahr et al., 2012). There is increasing evidence that therapeutic efficacy is mediated through soluble factors (Caplan and Dennis, 2006; Chen et al., 2008; Hung et al., 2007; Kinnaird et al., 2004; Li et al., 2009; Lin et al., 2011; Togel et al., 2007; van Poll et al., 2008). Our group demonstrated that the secretion of hESC-MSCs was cardioprotective in pig and mouse models of myocardial ischemia/reperfusion injury (Timmers et al., 2008). We subsequently demonstrated that this secretion-based effect was mediated by exosomes (Lai et al., 2010a).

In summary, the animal models and clinical trials demonstrate that MSC transplantations are generally safe and could be used in allogenic recipients, and that MSCs secrete potentially therapeutic exosomes. We therefore extrapolate from these observations to propose that MSC exosomes are likely to be benign and would not elicit an intrinsic adverse effect or immune rejection response when used as drug delivery vehicles.

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5.2. Using MSC for sustainable production of exosomes

A requisite of a cell source to be used for producing exosomes as drug delivery vehicles is an infinite capacity for reproducible production of exosomes. Currently, all MSCs used in clinical trials are primary cells with a large but finite ex vivo expansion capacity. Therefore, the use of primary MSCs to produce exosomes for drug delivery would require repeated isolation of cells and this would inevitably introduce batch-to-batch variation and incur recurring cost for testing and validation. To circumvent the finite expansion capacity of primary MSCs, we have immortalized hESC-MSC with a c-myc transgene (Chen et al., 2011). Although immortalization reduced their differentiation potential, it did not compromise the quantity and therapeutic quality of the exosomes. Notably, over-expression of the myc protein in the immortalized hESC-MSCs did not lead to a detectable presence of myc protein in the exosomes. This is not unexpected as transcription factors are under-represented in the proteome of exosomes (Sze et al., 2007).

5.3. Immune tolerance of MSC exosomes

MSCs have been reported to be able to exert suppressive and regulatory effects on both adaptive and innate immune cells in an autologous and allogeneic manner (Marigo and Dazzi, 2011). For example, MSCs were reported to inhibit proliferation of mitogen-activated T cells (Bartholomew et al., 2002; Di Nicola et al., 2002; Le Blanc et al., 2003; Potian et al., 2003; Tse et al., 2003), induce an anti-inflammatory tolerant phenotype in dendritic cells (DCs), naive and effector T cells and natural killer (NK) cells (Aggarwal and Pittenger, 2005) and inhibit B cell proliferation (Corcione et al., 2006). Clinical trials to test the therapeutic efficacy of MSCs in modulating the innate and adaptive immune system are underway. For example, Osiris's Prochymal, an intravenously administered formulation of MSC has been approved by Health Canada for treatment of acute GvHD and is being evaluated in clinical trials for other immune diseases such as Crohn's disease and type 1 diabetes (http://www.osiris.com/clinical.php). Since the cells are well tolerated in allogeneic hosts, it is likely that their secretion including exosomes will also be well tolerated. Consistent with this hypothesis, we observed in mouse and pig animal studies that intravenous injections of hESC-MSC secretion or exosome was therapeutically efficacious in immune-competent animals that were not treated with immunosuppressive drugs (Lai et al., 2010a, 2010b; Timmers et al., 2008, 2011). Our preliminary data suggest that some of the immunomodulatory properties of MSC were transferred to their exosomes. For example, hESC-MSC exosomes, like MSCs inhibited proliferation of Concanavalin A-activated lymphocytes (Yeo et al., in press). This ability to exert suppressive and regulatory effects in an allogeneic or autologous manner could enhance the longevity of MSC exosome-derived drug delivery vehicle and bioavailability of its drug cargo. Therefore it is conceivable that intravenously infused MSC-exosomes would be well-tolerated, facilitating more accurate dosing of its drug cargo.

5.4. MSC as a cost effective producer of exosomes for drug delivery

A major consideration in the selection of a cell line to produce exosomes for drug delivery would be the exosome yield per cell which will impact the final production cost. We have recently surveyed several cell lines that include skeletal myocyte line, insulin-producing cell line, HEK cells, small airway epithelial cell line and THP1 cell line. hESC-MSCs were determined to be the most prolific producers of exosomes as defined by the presence of CD81. They produced at least 10 times more CD81 + vesicles than any other cell lines (Yeo et al., in press).

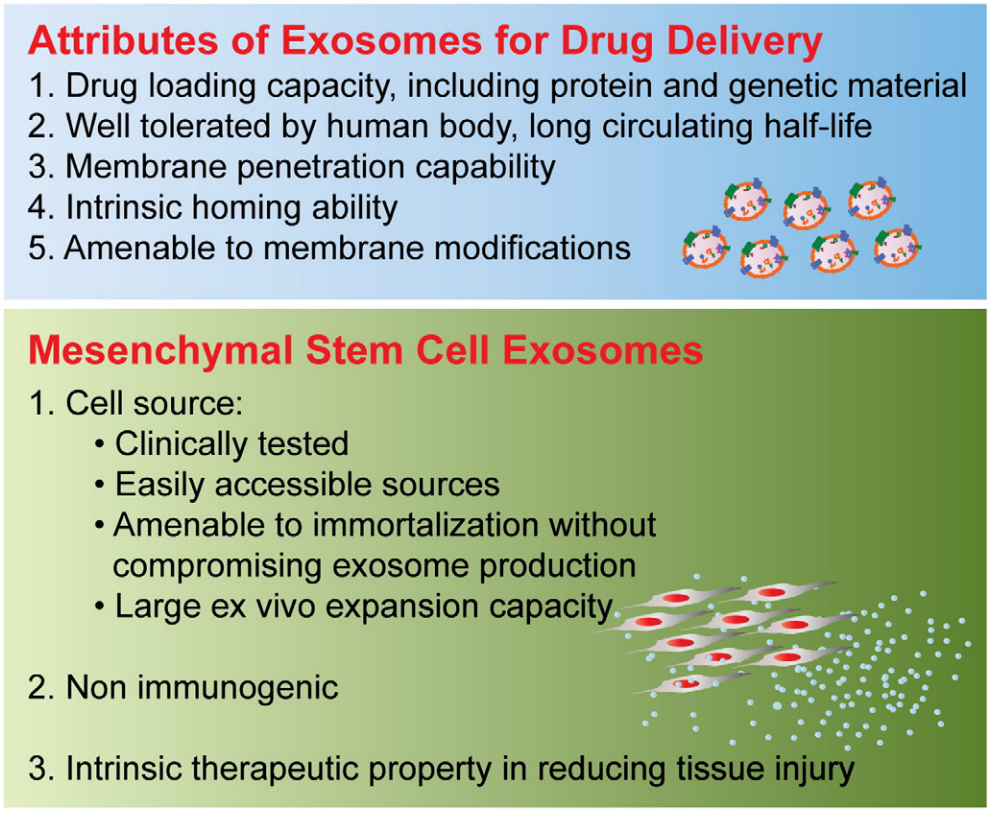

Fig. 3. Attributes of exosomes for drug delivery.

6. Conclusion

In conclusion, exosomes represent the most promising candidate for a natural drug delivery vehicle (Fig. 3). It carries many of the desirable attributes of a synthetic liposome vehicle such as the capacity to carry hydrophobic drugs in its bi-lipid membrane and hydrophilic drugs in its aqueous core. It could be modulated to deliver drugs to target cells and into cells via membrane fusion or endocytosis. Unlike liposomes, exosomes are generally well tolerated by our bodies and do not have inherent toxicity. However, the utility of exosomes as a delivery vehicle has been largely unexplored and untested. The major challenges in developing exosome-based drug delivery vehicles are the technology for loading of exosomes without compromising their biological properties and the scalable reproducible production of the appropriate type of exosome which ideally should be biologically benign.

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