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Exosome therapeutics for lung regenerative medicine

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ABSTRACT

Exosomes are 30 to 100 nm extracellular vesicles that are secreted by many cell types. Initially viewed as cellular garbage with no biological functions, exosomes are now recognized for their therapeutic potential and used in regenerative medicine. Cell-derived exosomes are released into almost all biological fluids, making them abundant and accessible vesicles for a variety of diseases. These naturally occurring nanoparticles have a wide range of applications including drug delivery and regenerative medicine. Exosomes sourced from a specific tissue have been proven to provide greater therapeutic effects to their native tissue, expanding exosome sources beyond traditional cell lines such as mesenchymal stem cells. However, standardizing production and passing regulations remain obstacles, due to variations in methods and quantification techniques across studies. Additionally, obtaining pure exosomes at sufficient quantities remains difficult due to the heterogeneity of exosomes. In this review, we will underline the uses of exosomes as a therapy and their roles in lung regenerative medicine, as well as current challenges in exosome therapies.

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Exosome; regenerative medicine; lung spheroid cell; stem cell

Introduction

Exosomes are a type of extracellular vesicle (EV) whose diameter ranges from 30 to 100 nm and are characterized by a cup-shaped morphology. They originate from multivesicular bodies (MVBs) and are released into the extracellular space following plasma membrane fusion. Exosomes carry cargo composed of proteins, lipids, and nucleic acids to recipient cells, which take up the cargo and initiate a response [1]. These EVs can target cells through ligand-receptor mediated interactions, membrane infusion, and endocytosis. Exosomes and their components have the ability to influence the tissue microenvironment to mediate cellular communication through local and distal alterations of extracellular matrix [2,3]. Exosomes can be classified through immunolabeling, by identifying exosomal surface proteins that include tetraspanins (CD9, CD63, and CD81), integrins (ITG), cell adhesion molecules (CAM), and growth factor receptors [4,5]. Variations in proteins and receptors on the exosome surface allow for targeting to recipient cells, enabling exosomes to be utilized as a tissue-specific delivery vehicle [6]. Although exosome characterization has been further elucidated, as scientific articles continue to be published regarding extracellular vesicles as a whole, the Minimal Information for Studies of Extracellular Vesicles (MISEV) provides guidelines to ensure standardized characterization, isolation, and purification of particular EVs [7]. With consideration of the MISEV, exosome production and characterization can be more replicable, resulting in more reproducible data across studies.

Exosomes as drug delivery vehicles

Drug systems that use exosome nanotechnology as a delivery mechanism are increasing in popularity since exosomes can be used to treat a wide range of diseases with increased therapeutic efficacy while remaining minimally invasive. A naturally occurring nanoparticle, exosomes are detected in the majority of organs and body fluids, making them a native delivery vesicle for targeted drug delivery with non-toxic and non-immunogenic characteristics [8]. The parent cell of the exosome contributes to the exosome's natural

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ability to target and carry out a tissue-specific response by controlling the RNAs, proteins, and lipids contained within the exosome [9]. To optimize targeting efficiency and retention, exosomes can be engineered by manipulation of specific cargo loading and surface decorating or "cloaking", such as in the fabrication of artificial chimeric exosomes [10–13]. Bio-engineered exosomes can allow for in vitro and in vivo traceability, cellular response initiation, and increased cellular uptake through surface charge modifications [13,14]. However, these manipulations reduce clinical translatability, by altering the exosome's natural state. Three main strategies have been developed for exosome drug delivery: isolating naïve exosomes and loading into drug-incorporated cells, loading drugs into parental cells which then release exosomes, and transfecting parental cells with DNA-encoding drugs which then release exosomes [15]. These strategies have clinical applications but remain difficult to scale up to human patients, due to time-intensive and costly production [15]. Currently, there are only two ongoing Phase I clinical trials using exosomes as drug delivery vehicles (Table 1). Under the M.D. Anderson Cancer Center, mesenchymal stromal cell-derived exosomes loaded with small interference RNA against KrasG12D, coined "iExosomes", are being used to treat participants with pancreatic cancer with a KrasG12D mutation (https://clinicaltrials.gov, NCT 03608631). Under the James Graham Brown Cancer Center, the effects of curcumin-conjugated plant exosomes are being evaluated in patients undergoing surgery for newly diagnosed colon cancer (https://clinicaltrials.gov, NCT 01294072). It is evident that exosomal cargo loading and surface decorating are effective therapeutic strategies with clinical applications. As exosome sourcing is refined, their targeting and retentive abilities will be optimized, making exosomes a more feasible nanotechnology.

Exosomes for regenerative medicine

Exosomes are already a feasible therapy for tissue regeneration across all major organs. Selectivity based on parent cell type and exosomal cargo loading increases therapeutic outcomes and specificity while minimizing adverse effects. Exosomes have demonstrated regenerative properties by reducing inflammation and apoptosis while promoting proliferation and angiogenesis. Mesenchymal stem cells (MSCs) and MSC-derived exosomes are popular in regenerative medicine, due to their multipotency and self-renewing properties. This multipotency provides therapeutic benefits to many organ and tissue types. For example, exosomes are able to combat cardiovascular disease, promote bone remodelling, and regress liver fibrosis, despite vast differences across disease models [16–18]. Exosomes have also shown promising regenerative effects in a wide variety of models, such as myocardial infarction, kidney injury, and neurological injury. Injection of MSC-exosomes has been shown to reduce infarct size in a rat myocardial infarction model, while restoring long-term cardiac function [19]. Human cardiosphere-derived exosomes have shown greater global heart function than media controls in an acute MI mouse model [20]. Hypoxic cardiac progenitor cell-derived exosomes improve cardiac function, fibrosis, angiogenesis, and hypertrophy in a rat model of ischaemia reperfusion [21,22]. Exosomal microRNA miR-21-5p helps regulate apoptosis and angiogenesis and improves heart function in a mouse model of myocardial infarction [23]. Exosome-mediated delivery of Sonic hedgehog protein improves therapeutic efficacy of human CD34+ stem cells in a mouse model of acute myocardial infarction [24]. Engineered hydrogel patches loaded with induced pluripotent stem cell-derived cardiomyocyte EVs offer a slowly released cell-free therapeutic that reduces infarct size and cell hypertrophy in a rat model of acute myocardial infarction [25]. Delivery of MSC-exosomes overexpressing miR-let7 c targets and reverses kidney fibrosis in a murine unilateral ureteral obstruction model [B. 26]. Exosomes secreted from hypoxic rat renal proximal tubular cells are protective against renal tubular cell injury [27]. Additionally, human kidney tubular cells and their secreted exosomes prevent renal injury in a rat model of bilateral renal ischaemia [28]. Adipose-derived mesenchymal stem cells with overexpressed miR-181-5p provide targeted therapy to hepatic stellate cells and attenuate liver injury in the mouse model of liver fibrosis [29]. Modified exosomes can effectively deliver miR-124, bypassing the blood-brain barrier and inducing neurogenesis following ischaemic injury in a murine photothrombosis model [30,31]. Human neural stem cell-derived EVs have a neuroprotective effect, leading to significant improvements at the tissue and function level of both mouse models of thromboembolic stroke and pig models of ischaemic stroke [32,33]. It is evident that exosome-mediated tissue regeneration has potential, as exosomes and their secreted factors have both shown improvement in tissue regeneration. MSC-secreted bioactive molecules have also been shown to exert paracrine effects on surrounding cells in the tissue microenvironment, providing therapeutic benefits [34,35]. This indicates that both MSC-exosomes and their secreted factors contribute to tissue regeneration. Exosome secretome can be manipulated to improve its therapeutic effects through cellular pre-conditioning [35]. Identification of the optimal combination of cargo-loading and culture components will

Table 1. Clinical applications of exosomes in drug delivery and regenerative medicine.

	Drug delivery	Regenerative medicine
Definitions	In which exosomal cargo is modified to contain a specific compound, including but not limited to a particular protein, miRNA, or pharmaceutical drug, for treatment	In which exosomes are used as naturally produced for treatment
Number of Papers	360	338
Number of Clinical Trials	2	13
Pros	Highly specific organotropic behaviour [6,15] Biocompatible [6] Can be engineered to increase drug load or improve stability and targeting ability [6,12,15] Stable during cryopreservation [16] Naturally crosses cell membranes and the BBB [30,54] Avoid macrophage uptake, resulting in long blood circulation [54] Large surface area to volume ratio allows exosomes to carry a significant amount of surface cargo [4] Can carry hydrophobic and hydrophilic drugs [15]	Highly specific organotrophic behaviour [40,48,54, B. 18,26] Nonimmunogenic [54] Can be selected to carry specific proteins, receptors, or nucleic acids for gene therapy via cell culture microenvironment [9,16,35,64, B. 26] Stable during cryopreservation [16] Naturally crosses cell membranes and the BBB [63] Regulate tissue homoeostasis [36,54] Initiates tissue repair and regeneration [2,17,34,36,40,48] Released by most cell types, enabling tissue-specific signalling [48,54] Long-lasting paracrine effects [39,40]
Cons	Difficult to produce enough yield to be effective in humans [6] All isolation techniques have cons, including time, monetary cost, forming aggregates, introducing contaminants [6,54] Systemic administration has not reflected targeting potential [6]	Difficult to produce enough yield to be effective in humans [10,64] All isolation techniques have cons, including time, monetary cost, forming aggregates, introducing contaminants [16,63,66] No single way to manufacture exosomes of a specific potency [10,16]
Representative Companies	Anjarium Biosciences ArunA Biomedical Codiak Biosciences Evox Therapeutics Exogenus Therapeutics	Aegle Therapeutics BreStem Therapeutics Capricor Therapeutics Kimera Exosomes ReNeuron Tavec Pharma Xollent Biotech

allow for more specialized and effective exosome targeting and regeneration, increasing clinical translatability. Currently, there are numerous clinical trials using exosomes for regenerative medicine, ranging from cutaneous wound healing to macular hole regeneration (Table 1). Under Kumamoto University, serum-derived exosomes are being used to accelerate cutaneous wound healing in patients with intractable cutaneous ulcers (https://clinicaltrials.gov, NCT 02565264). Under Tianjin Medical University, mesenchymal stem cell-derived exosomes are being used to promote healing of large and refractory macular holes (MHs) in patients diagnosed with large and long-standing idiopathic MHs (https://clinicaltrials.gov, NCT 03437759). Exosome use for regenerative medicine is a feasible therapeutic, offering practical applications for a wide range of diseases.

Lung regenerative medicine

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are among the most popular stem cell types in research, due to their differential potential and ability to maintain tissue homoeostasis [36]. MSCs can be obtained from multiple sources, such as bone marrow, adipose tissue, peripheral blood, placenta, and umbilical cord. The use of MSCs in clinical trials continues to expand each year, with over 900 studies listed in ClinicalTrails.gov and over 50 for the use of MSCs for lung diseases specifically (https://www.clinicaltrials.gov). The trials focus on a range of lung diseases including acute lung injury, idiopathic pulmonary fibrosis, acute respiratory distress syndrome, and bronchopulmonary dysplasia, demonstrating that MSCs can be used to treat a wide spectrum of diseases, even within a particular organ. Yi et al. found that overexpressing miR-30b-3p in MSC-derived exosomes helped relieve inflammation and repair alveolar epithelial cells in a mouse model of acute lung injury [37]. Systemic administration of MSCs to two patients with acute respiratory distress syndrome alleviated respiratory, haemodynamic, and multiorgan failure [38]. Intranasal delivery of MSCs helped attenuate lung injury in a neonatal rat model of bronchopulmonary dysplasia [39]. MSCs and MSC-derived exosomes are clearly effective biological therapeutics for lung regenerative medicine. Small-scale clinical studies show promising results for MSCs in their early stages, giving hope for future acellular exosome clinical applications. Transitioning from stem cell therapies to their acellular exosomes will help overcome current obstacles of low cellular engraftment and stability in the lung, making MSC-derived exosomes an attractive alternative [40]. Despite these

advances over the past several years, challenges such as batch consistency and purity remain when transitioning from rodent models of lung disease to large-scale clinical trials [41]. Nevertheless, MSC-exosomes offer an alternative to cellular therapies that may provide similar regenerative abilities, devoid of adverse effects following cellular administration.

MSC-exosomes carry a unique and diverse group of nucleic acids, proteins, and lipids that have yet to be identified to reside in one or multiple types of exosomes (Figure 2) [36]. This array of cargo elicits a diverse range of cellular responses to provide therapeutic benefits for various diseases but may require higher specificity to provide superior tissue regeneration and homoeostasis for organs like the lung [36]. Although MSCs and MSC-exosomes are popular biological agents in lung regenerative medicine, elucidating their cargo and developing consistent batches would help unveil key targets for specific lung diseases.

Lung progenitor and spheroid cells

Lung progenitor cells (LPCs) prove to be popular cell types for lineage tracing and differentiation studies, regulatory studies in cancer models, and lung regenerative medicine [42–44]. As opposed to MSCs, lung progenitor cells are a more intrinsic stem cell type and better facilitate lung cell regeneration after injury, offering greater potential targeting and retention abilities [45]. Our lab has developed a heterogeneous lung cell line from healthy human whole-lung and transbronchial biopsies, that consists of a lung progenitor cell core supported by a surrounding layer of stromal cells [40]. The lung progenitor cell core, positive for alveolar type II cells (ProSPC+) and secretory cells (CCSP+), is formed when cells outgrow from lung tissue explants and aggregate into three-dimensional lung spheroids when seeded onto ultra-low-attachment flasks [45]. Supported by stromal-like cells, positive for CD90 and CD105, the progenitor core significantly increases stemness when assuming a 3D structure [40]. This three-dimensional culturing technique may also enhance stemness by more closely mimicking an in vivo stem cell niche. These spheroids can reactivate the plasticity of differentiated lung cells upon disease or injury and have greater therapeutic targeting to the lung than traditional adherent mesenchymal stem cells [45–47]. When plated on an adherent surface, lung progenitor cells can dissociate from their three-dimensional spheroid shape to a monolayer of cells we termed "lung spheroid cells" (LSCs) (Figure 1). LSCs maintain a similar phenotype to lung progenitor cells but may differentiate into mature lung cells, indicated by an increase in Epcam+ expression [40]. LPC and LSC expansion and conditioning can provide novel exosomes and exosome-secretome that may elicit a greater regenerative effect to the lungs than traditionally used MSCs. Such intrinsically sourced cell therapy can elucidate therapeutic targets and pathways that current cell models cannot achieve.

The effect of adult human stem cells, such as our LSCs, is superior to multipotent stem cells for the treatment of lung-specific diseases by providing a more specialized tissue microenvironment [40]. Although a heterogeneous lung cell population, allogeneic LSCs can alleviate inflammation, minimize immunological response, and slow fibrotic development through paracrine mechanisms and direction regeneration in both mouse and rat models of bleomycin-induced pulmonary fibrosis, in a superior fashion to MSCs (Figure 2) [40,48]. LSCs are retained for several weeks in the lungs of mouse and rat models of pulmonary fibrosis, providing prolonged cellular therapy to target diseased tissues [40,48]. Additionally, major

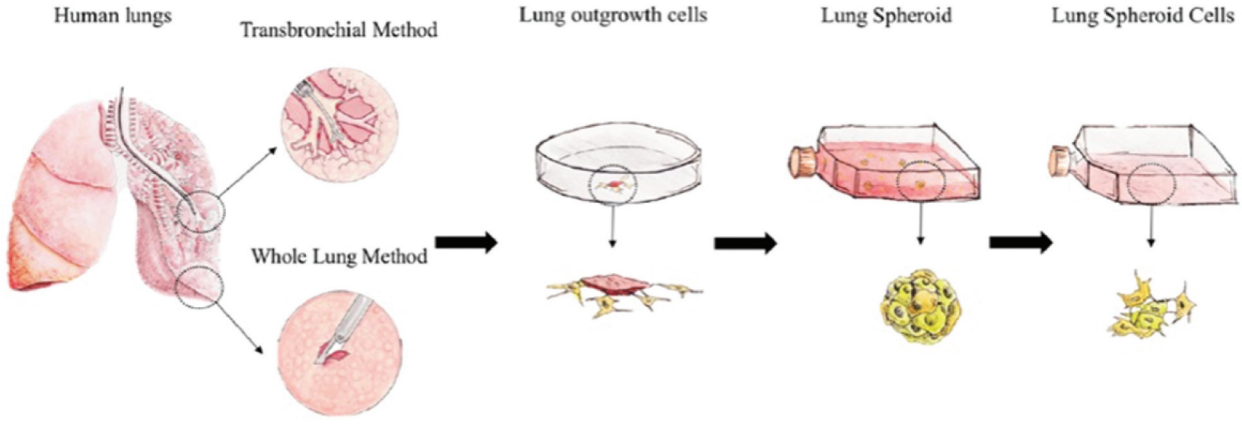

Figure 1. Schematic of the derivation of lung spheroid cells (LSCs) from whole lung and minimally invasive transbronchial lung biopsies [From Respiratory Research 40].

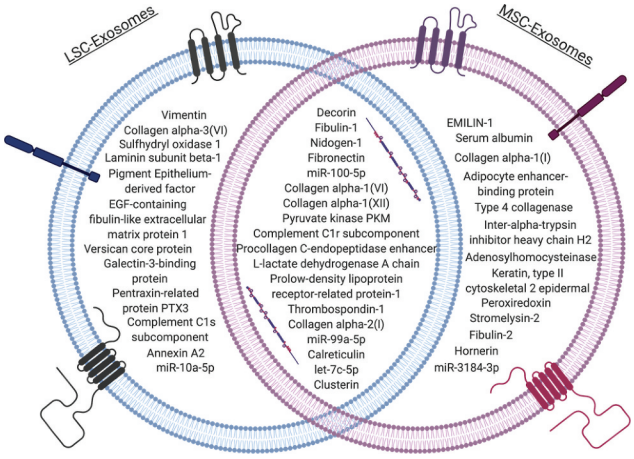

Figure 2. Comparison of exosomal cargo between lung spheroid cell- and mesenchymal cell-derived exosomes [49].

organs including the lung heart, liver, kidneys, and spleen are devoid of tumour formation in both mouse and rat models [40,48]. Mice who received LSCs had less fibrotic thickening and tissue infiltration than mice who received adipose-derived MSCs [45]. Additionally, administration of LSC-derived exosomes yields therapeutic effects in a pulmonary fibrosis mouse model, providing a cell-free alternative [49]. LSC-derived exosomes decrease fibrosis and attenuate the alveolar epithelium and vasculature, making exosomes and their unique array of cargo a potential target for cell-free therapy [49]. This intrinsic source of stem cells has the ability to treat a variety of pulmonary diseases and is not limited to fibrosis models, making LSCs and LSC-derived exosomes noteworthy cellular therapies in regenerative medicine. Similar strategies using cardiosphere derivation and cardiosphere-derived exosomes are also implemented in models of ischaemic heart failure and myocardial infarction. Allogeneic cardiosphere-derived cells (CDCs) extracted and isolated from human myocardium have promising preclinical and clinical results, triggering native cardiomyocyte proliferation and recruiting endogenous progenitor cells [50]. CDCs have been utilized in several clinical trials, including the CADUCEUS and DYNAMIC trials [51,52]. A decrease in intramyocardial scarring and minimization of adverse remodelling has also been demonstrated in a pig model of acute and convalescent myocardial infarction treated with CDC-exosomes, demonstrating clinical potential for a cell-free alternative [53].

3D culturing of intrinsically sourced stem cells into spheroids is a promising cellular therapy for a wide variety of diseases beyond the heart and lungs. Studies should expand beyond traditional mesenchymal cell sources, to elicit greater therapeutic effects and enhance cellular targeting and retention.

Challenges in exosome therapy

Exosome production, expansion, and delivery remain prominent challenges in regenerative medicine. As purification and production continue to improve, exosomes in regenerative medicine will become more targeted and tissue-specific. However, further research needs to be conducted to fully understand the complete contents of exosomes, methods of tissue targeting, impact on targeted tissues, and long-term safety effects [54]. Overall, standardizing the production of exosomes will improve consistency, dosage, and potency in the translational medicine community.

Isolation and purification of exosomes

Isolation and purification of exosomes remains imperfect and difficult to produce in large scales for clinical applications. Although considered the current optimal method for exosome isolation, ultracentrifugation-based isolation techniques suffer from contamination and exosome loss due to the heterogeneous nature of exosomes [55]. Despite these drawbacks, ultracentrifugation remains a popular isolation technique because it is easy and relatively cheap. Two common types of ultracentrifugation are differential and density gradient ultracentrifugation. In differential ultracentrifugation, a series of centrifugation cycles isolate sample components by size, with forces ranging around ~100,000 x g. These cycles are used to separate exosomes from unwanted components, such as apoptotic cells and cell debris. In density gradient ultracentrifugation, samples are separated through centrifugation in a density gradient medium. This creates defined solute zones of specific densities [55]. However, due to the heterogeneous nature of exosomes, these isolation techniques cannot perfectly differentiate exosomes from all other unwanted components; overlaps in cell size and density across exosome samples hinders current isolation techniques. Immunoaffinity capture-based techniques have also been developed to isolate exosomes using exosome-specific surface markers, such as CD63 [10]. This technique provides highly specific and pure isolation, but is costly and is hindered by the lack of exosome-specific surface markers. A possible solution to improve isolation and purification of exosomes is to combine ultracentrifugation with immunoaffinity capture techniques, using surface markers that specifically target exosomes from the sample undergoing such isolation and purification. An additional solution involves implementing higher resolution density gradients when undergoing ultracentrifugation. Higher resolution

density gradients are necessary to produce more pure exosome fractions and gain a better understanding of exosome composition [56]. Jeppesen et al. employed a combination isolation technique by using density gradient fractionation to separate extracellular vesicles from non-vesicular material, along with direct immunoaffinity capture to specifically isolate exosomes [56]. This combination successfully separated exosomes for other heterogenous sample matter, allowing for more accurate and specific identification and analysis of exosomal properties. Chemical-based isolation techniques isolate highly purified exosome populations and are available as commercial kits, but risk composition alteration and chemical retention in exosome samples [57]. Commercial kits for polymer precipitation have also been developed to overcome these disadvantages, by using solutions of superhydrophilic polymers for isolation. However, these kits are expensive and not suitable for large sample sizes. Additionally, EV type cannot be differentiated, making biomarker elucidation difficult [57]. Microfluidic devices have gained popularity for EV isolation, by trapping vesicles of defined diameter into nanofilters [58]. Although this allows for isolation customization, complex fabrication of the device along with low recovery rates makes microfluidic devices hard to translate to clinical settings [58]. Because of the heterogeneous nature of exosomes, optimal isolation strategies vary with each sample. Although many exosomes types contain similar surface markers (CD9, CD81, and CD63) that can be targeted for affinity-based purification, chemical alterations and low yields may outweigh the benefits of such pure isolation. A combination of isolation techniques optimized for particular exosome samples will allow for proper sample selection while maintaining clinically relevant yields. Overall, as the characteristics and surface phenotypes of tissue-specific exosomes become more defined, isolation and purification techniques will improve and become more clinically translatable.

Large-scale production and immortalization

Translation to large-scale production of exosomes for clinical trials remains difficult, as traditional cell culture is inefficient in terms of time and cost. Production will be optimized once the ideal exosomal microenvironment is identified in an in vitro system [59]. Currently, 2D culture techniques remain common means of production, but they are labour-intensive and result in low exosome yield. Transitioning to 3D culturing techniques increases efficiency, but still does not meet the demands for clinical application, which requires significantly more cells for treatment than traditional animal models. Bioreactors and microcarriers provide large-scale expansion methods, by maximizing surface area for stem cell and exosome growth [60]. Exosome yields can be increased ~40-fold through bioreactor culture, in comparison to conventional cell culture [61]. Although requiring more media and more frequent passaging, these larger-scale methods maximize surface area and allow for efficient cell expansion, all without comprising cellular function. However, exosome isolation in large-scale applications remains impractical, with structural damage and batch inconsistencies hindering clinical translation. Although solutions such as higher resolution density gradients would help produce purer batches, they are not a feasible approach for large-scale production and GMP standards, due to discrepancies in successful isolation and retained quality [62]. Systems such as tangential flow filtration (TFF) have been developed to combat such limitations, assuring more efficient exosome yields and decreased vesicular damage [63]. By directing liquid suspension flow tangentially, as opposed to dead-end, filter clogging by larger particles is minimized and particles are separated more efficiently. This redirection yields higher concentrations of isolated exosomes from the same sample, making TFF desirable for large-scale production. Cell line immortalization can also produce higher yields and ensure infinite supply. Because some stem cell lines, such as our LSCs, have only a finite number of cell divisions before they senesce, discovery of immortalization agents for these specific cell types will allow infinite cell division and safer expansion. Chen et al. implemented this strategy by immortalizing MSCs through transfection of lentivirus carrying the MYC gene [64]. This transfection was a practical strategy that not only ensured an infinite supply of cells and exosomes but also increased proliferative rates [64]. Although immortalization generates large genomic and proteomic outputs, gene expression is manipulated and may no longer represent its native stem characteristics [65]. Stem cell marker expression must be validated upon each passage, to ensure cell characteristics and functions do not deviate among generations. Large-scale exosome production will be optimized through a combination of advanced culturing and isolation techniques. Because of the heterogeneous nature of cell types and their secreted exosomes, no one combination of large-scale production and exosome isolation is optimal for all cell types. An individual evaluation of the risk factors associated with the translation to large-scale production and cellular immortalization must be taken before achieving a clinically relevant product.

Dosage and potency

Standardization of exosome dosage and potency needs to be implemented but is difficult due to the heterogeneous nature of exosomes across various progenitor cells, variations in animal models, and differences in cellular characteristics and evaluation. Therefore, extrapolating exosome dosage from animal models to humans also requires careful consideration. Inconsistencies in dosage methods and potency quantifications make it difficult to reference and exchange data across studies and clinical trials [66]. Since exosome characteristics also vary among cell types, standards may be more effectively formed when considering the tissue being targeted and the mechanisms by which the exosomes reach the target tissue. An in-depth evaluation of the pharmacokinetics and pharmacodynamics of exosomes across various tissue sources will help elucidate optimal dosage and catalyse regulatory approval. The biodistribution and cellular uptake of exosomes need to be identified, to further investigate exosomal half-life, off-target organ accumulation, and surface modification to minimize non-target cell interactions [67]. Standardization will allow for more comparable results and will increase consistency across batches [68].

Regulatory hurdles

FDA approval processes pose as obstacles in translating exosomes to clinical trials. Many commercial companies have emerged, producing products and therapies that utilize exosomes, with Aegle Therapeutics being a noteworthy company to receive IND clearance from the FDA (Table 2). However, the heterogenetic nature of exosomes between batches, cell sources, and purification techniques complicates the approval of such biological products by impeding reproducibility. With the FDA's definition of biological products continuing to be revised, it is difficult to interpret and fit within these standards. In addition, extensive testing on exosome stability, sterility, quality, and potency must be measured and maintained during processing and shipping conditions. The effects of such processing are not well known and may result in exosome content or composition variations during each processing step. As guidelines for exosome therapeutics are developed specifically to aid in manufacturing and validation during each processing step, exosomes will become easier biological products to approve.

Exosome-based biomarker development

Currently, there are FDA-approved diagnostic tests that utilize exosome collections from liquid biopsies, such as blood and urine [69]. In such minimally invasive diagnostic tests, unique exosomal surface antigens and cargo are targeted and serve as precise biomarkers for disease diagnosis [70]. Disease evolution can also be evaluated through exosome concentration, proving exosomal-based liquid biopsy to be a powerful tool for cancer metastasis or disease progression [70,71]. However, improvements need to be made to increase sensitivity and diagnostic accuracy in other disease models to expand FDA approval outward from liquid biopsies [72]. From a clinical standpoint, Exosome Diagnostics offers cutting-edge technology that utilizes patient-derived exosomes for biomarker discovery, RNA analysis, mutation detection, protein detection, and signal enhancement through their approved productions: Exolution RNA™, Exolution Plus™, and EDDE™ (Table 2). From a research standpoint, atherosclerosis diagnosis through circulating microRNAs in blood is a promising diagnostic tool, but still targets eight microRNAs, whose expression levels vary as atherosclerosis manifests [73]. Although early detection would significantly improve patient outcome, since clinical signs may take years to develop, identifying a single or combination of microRNAs that are sensitive and precise at the right concentration would be crucial for FDA approval [73]. Translating exosomes to a clinical setting is feasible, but refining biomarker selection for diagnosis is imperative for FDA approval.

Exosome therapeutics

Exosomes pose as a cell-free alternative to cellular therapies but continue to face challenges when translating to clinical studies. Although the immunogenicity, safety, and efficacy of exosomes from xenogeneic and allogenic sources have not been fully characterized, numerous preclinical studies have made such evaluations in cellular therapies. With studies showing disease improvement in both allogeneic and autologous treatments, it is promising that pre-clinical studies using exosomes from such cellular sources would better overcome regulatory hurdles when translated to a clinical setting [48,74,75]. Since some exosome studies have shown unaffected differentially expressed genes related to inflammation or toxicity, as well as no visual signs of toxicity after repeated administration, exosome therapies show promise [76,77]. However, the efficacy of such treatments is more difficult to validate, as disease outcomes following exosome therapeutics may vary between patients due to product variations. Federal regulation of exosome therapies will help improve consistency by implementing standardized data collection, product-tracing information, dosage levels, and potency quantification. These regulations would help facilitate data exchange across studies and disease models. Exosome therapy has

Table 2. List of commercial companies that use exosomes for therapies.

Company name	Product/therapy description	URL
Aegle Therapeutics	Phase 1/2a clinical trial of MSC-derived exosomes to treat severe dermatological disorders.	https://www.aegletherapeutics.com/index.html
Anjarium Biosciences	Hybridosome™ platform for vesicle modification to provide additional specificity and biodistribution.	http://www.anjarium.com
ArunA Biomedical	Neural exosomes to treat various central nervous system and neurodegenerative disorders.	https://arunabio.com
BioVision Incorporated	Production of exosome quantification, isolation, and DNA/RNA extraction kits. Production of disease-specific exosomal marker antibodies.	https://www.biovision.com
Capricor Therapeutics	Exosomal technology for the treatment of severe, rare, and inflammatory disorders at a clinical level. Exosomes isolated from chromosphere-derived cells.	http://capricor.com
Codiak Biosciences	Engineered exosomes for precise targeting. Industrial quality and scale of exosomes for clinical applications.	http://www.codiakbio.com
Evox Therapeutics	Targeting technology, manufacturing, and purification methods for transformational therapeutics. Molecular engineering, drug loading, and targeting strategies.	https://www.evoxtherapeutics.com
Exogenus	Exosome-based drug development for the treatment of skin and autoimmune diseases.	http://www.exogenus-t.com
Exopharm	Development of Ligand-based Exosome Affinity Purification (LEAP) technology to isolate exosomes using affinity chromatography.	https://exopharm.com
Exosome Diagnostics	Detection, diagnosis, treatment, and monitoring of cancer and other diseases. Serum/plasma and urine kits for biomarker discovery, liquid biopsy, clinical diagnostic development, and targeted therapies.	http://www.exosomedx.com
Exosome Sciences	Exosome-based biomarkers to diagnose Alzheimer's, Chronic Traumatic Encephalopathy, and other neurological disorders. Development of the TauSome™ biomarker used in blood testing to identify Chronic Traumatic Encephalopathy.	https://www.exosomesciences.com
Exosomics	Biofluid tests for cancer screening and liquid biopsy using tumour exosomes. Development of tumour-derived DNA/RNA from exosomes and cancer-derived exosome standards.	https://www.exosomics.it
Kimera Labs	Development of purified placental MSC exosomes for research and therapeutic purposes, including wound healing and immunological manipulation.	https://kimeralabs.com
ReNeuron	Pre-clinical development of permanent stem cell line, CTX, -derived exosomes.	http://www.reneuron.com
Tavec Pharma	Pre-clinical development of injectable miRNA-loaded exosomes to reduce cholangiocarcinoma tumours.	http://tavecpharma.com

great potential and FDA approval will catalyse clinical trials for translational medicine.

Conclusion

Exosomes have great potential to treat a wide variety of diseases. Secreted by many cell types and into almost all biological fluids, exosomes harness the great potential for biological therapies. Drug delivery and regenerative medicine are just some of the many applications. Through bioengineering techniques such as cargo loading and surface modifications, exosomes can be enhanced to deliver superior therapeutic components at greater concentrations to targeted cell types. Exosomes in regenerative medicine continue to improve across all major organs and tissues, as parent cell selection enhances cargo specificity, void of adverse immune responses or rejection seen in cellular therapies. Intrinsic sources of exosomes further facilitate these enhancements, expanding sourcing from traditional multipotent cell lines to cells derived from the target tissue. Nevertheless, new manufacturing techniques need to be developed and regulated to perfect exosome production, expansion, and delivery. Isolation and purification will be improved as separation is perfected through an optimal combination of techniques. Large-scale production of exosomes will meet demands as culturing techniques transition from standard 2D practices, to 3D culturing techniques and bioreactors. Immortalization of stem cell lines will also ensure infinite expansion, but at the cost of cell manipulation. Standardization of exosome dosage and potency across studies and trials will improve data exchange and consistency with help through federal approval and regulation. Even with production optimizing, exosomes will continue to face challenges in clinical translation, with federal guidelines and requirements continuing to change and become more stringent. As the heterogeneous nature of exosomes is further elucidated, exosomes will become more reproducible and easier to translate. Exosomes currently harness the great potential for biological therapies and will continue to improve as current challenges are met.

Disclosure of interest

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