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Exosome Therapeutics: Academic Curiosity or Commercial Reality?

Cell and gene therapies have been called the emerging fourth pillar of healthcare systems. However, recent research suggests that the cell therapy pillar may, in fact, be underpinned by exosomes (also referred to as small extracellular vesicles; sEVs). It is now evident that exosomes are potent cell-to-cell communication vehicles and play essential roles in normal physiology and disease processes. This new knowledge has driven an explosion of academic research and growing interest in using exosomes for therapy (Figure 1). However, this raises two major questions: Can exosome manufacture be commercially viable; and will exosomes really work as therapeutics? Here we describe the key aspects of extracellular vesicle biology, opportunities for exploiting this biology, manufacturing challenges in the production of safe and effective exosome therapies, and regulatory considerations for this new class of biological medicines.

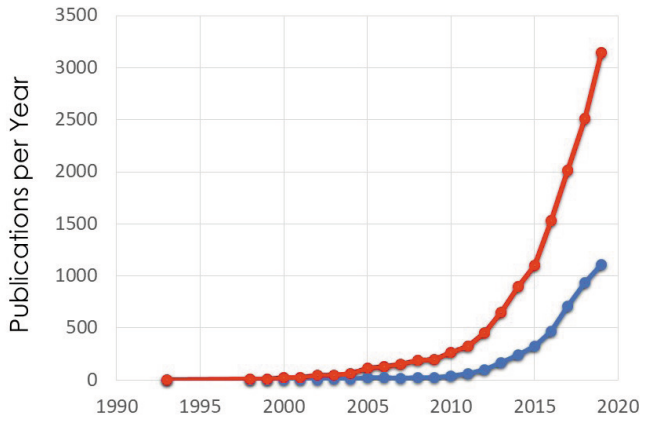


Figure 1. Publications listed in PubMed where the title contains "exosome" (red) or "exosome therapy" (blue). The number of exosome publications per year has risen from 266 in 2010 to 3150 in 2019.

Eukaryotic cells secrete a heterogeneous range of extracellular vesicles, including exosomes. Initially, exosomes were viewed as a form of "cellular waste". It is now clear that their functions go way beyond this. These vesicles of 50–150nm diameter are bound by a lipid bilayer and are laden with a mix of molecular cargo that includes protein, genetic material and lipids. Exosomes bind to recipient cells and release their cargo as a powerful mode of cell-to-cell communication. In doing so, they play important roles in normal physiological processes, in tissue response to injury and in disease processes such as cancer. Exosomes are a vehicle for cells to modify their environment by changing the phenotype of near or distant neighbours.

Exosome biogenesis is a controlled process involving the endosomal sorting complex required for transport. This process results in the incorporation of specific molecular cargo. A number of proteins are commonly found within the cargo or lipid bilayer of exosomes and are considered canonical exosome markers; these include the tumour susceptibility gene 101 protein (TSG101) and the lipid embedded tetraspanin proteins CD9, CD63 and CD81. The most attractive feature of exosomes from a therapeutic perspective is their diverse range of molecular cargo including mRNA, small RNAs (including mi-RNAs), proteins, lipids and peptides[1]. When delivered to cells, this cargo effects change in cell phenotype and function and underpins exosomes' therapeutic potential. Importantly, not all exosomes (as individual entities) contain the same mix or abundance of molecular species. Therefore, in contemplating a therapeutic exosome product, it is preferable to consider cargo from a population of exosomes, as this better reflects what would be manufactured and administered as a therapy. In addition, different cell types isolated from primary human tissues will each secrete exosomes with unique molecular cargo that is fit for its intended purpose.

This uniqueness of molecular cargo from different cell types and tissues is a key consideration for the manufacture of exosomes for therapeutic use. An understanding of the intrinsic properties of populations of exosomes isolated from different cell types and tissues informs how they could be applied therapeutically. Notably, it is widely acknowledged that the efficacy of mesenchymal stem/stromal cell (MSC) therapies for regenerative medicine can be attributed in large part to their secreted exosomes[2]. As a result, many companies developing exosome therapies are using bone marrow or adipose-derived MSC as their preferred cell type for exosome production. Moreover, if MSC-based therapy proves clinically useful, it is likely that exosomes derived from these cells will also be effective in the same clinical settings. As outlined below, the rate of exosome secretion and their molecular cargo can be enhanced or modulated by cell culture conditions. This provides an opportunity to further tune and tailor exosomes for their intended clinical application.

The current status of exosome therapy is reminiscent of the early days of cell-based therapy, where a limited understanding of effective cell dose and mode of delivery made it difficult to predict clinical outcomes, to develop manufacturing processes and to generate sufficient numbers of well characterised cells for clinical trials. However, the manufacturing technology and processes being considered for exosome manufacture are more closely based on those used for the manufacture of antibody and recombinant therapeutic proteins. Therapeutic exosome manufacture involves two linked steps: (a) upstream cell culture to generate the cell-substrate; and (b) downstream exosome concentration and purification. This is followed by a 'fill - finish' step to facilitate storage and transport. For detailed information on the approaches and challenges within the upstream and downstream steps of exosome manufacture, we refer the reader to recent comprehensive reviews by leading academic

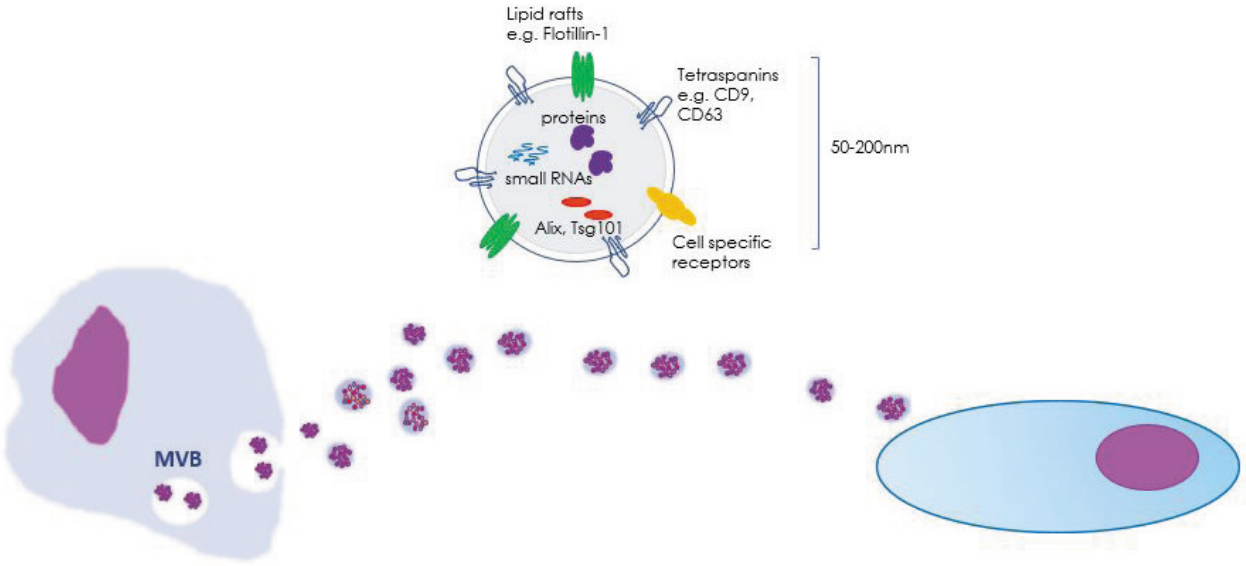


Figure 2. Cell to cell communication and impact on recipient cells as mediated by exosomes and their molecular cargo.

and commercial groups[3,4]. Herein we comment on the key considerations of:

1) upstream processing: cell selection and cell culture,
2) downstream processing: separation and concentration of exosomes,
3) product consistency and safety: in-process controls, exosome analytics,
4) regulatory requirements.

The first key consideration for exosome manufacture, upstream processing, involves selection and culture of cells, at scale, to produce large volumes of cell culture supernatant as a starting substrate for downstream processing. As noted above, cells vary in their ability to produce exosomes and in the molecular cargo contained within their exosomes. Therefore, a prudent cell selection approach would involve screening a diverse range of cell types to identify those that not only have optimal growth properties but also secrete exosomes suitable for the intended clinical application. This requires a suite of analytical tools to quantify and characterise exosomes, annotate their molecular cargo and define their therapeutic potential using potency assays and relevant animal models. As an example of this approach and the associated challenges, we screened many of the MSC types currently used for regenerative medicine and found wide variation in exosome secretion and cargo (unpublished data). None of these MSC types were a suitable starting cell for manufacture of exosomes suitable for our target indication, peripheral arterial disease, where pro-angiogenic factors are likely to be critical for efficacy.

As mentioned above, large volumes of cell culture supernatant need to be produced for exosome therapy to be an effective modality. These volumes may be in the order of hundreds of litres. Fortunately, a number of well-established bioreactor platform technologies and Good Manufacturing Practice- (GMP-) compliant processes have already been used in industrial-scale pharmaceutical processes to produce recombinant proteins, viral vectors and cells for therapy. Optimisation or adaption of these mature technologies is an ideal strategy for exosome manufacture. However, there are several essential aspects of cell culture to consider in the application to exosome manufacture. This includes the cell culture medium: whether it supports long term cell proliferation, high cell viability and ongoing exosome secretion; and whether it contains "contaminating" vesicles and other components that interfere with downstream separation and concentration. Notably, medium supplements such as foetal bovine serum are rich in contaminating vesicles. A number of companies, recognising these issues, now manufacture customised media for exosome production and manufacture.

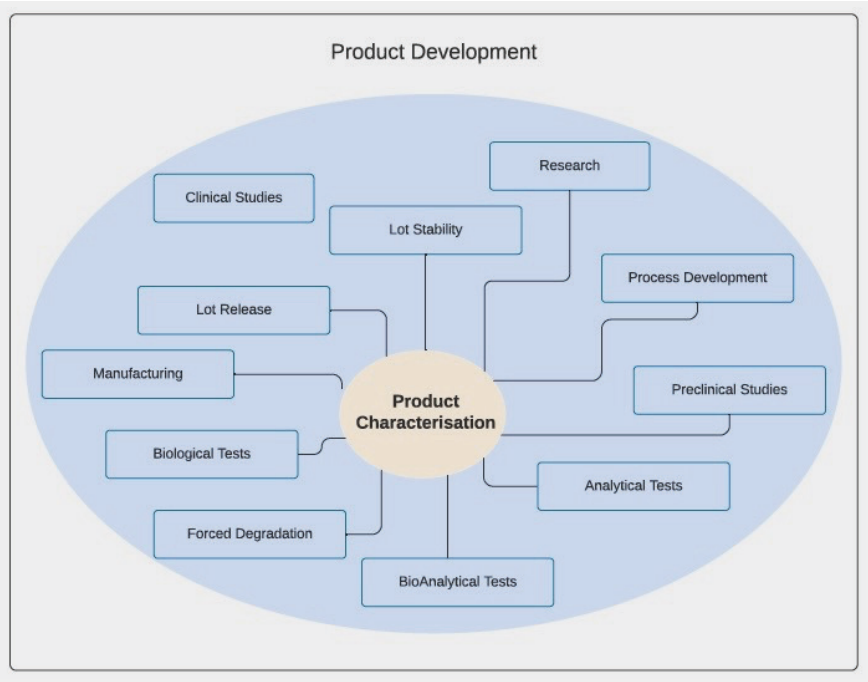


Figure 3. Product characterisation is core to the development of a complex biological product like exosomes.

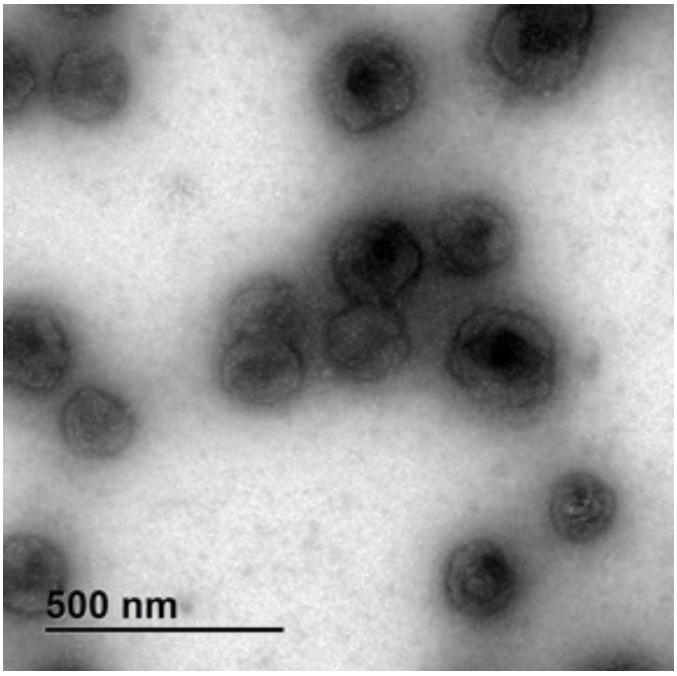
Another important aspect of cell culture is how changes in culture conditions impact on exosome biogenesis. These conditions include pH, oxygen tension, hydrodynamic shear stress, and culture surface topology and chemistry. There is significant opportunity to optimise cell culture conditions to increase exosome secretion and tailor molecular cargo, thereby increasing product yield and clinical efficacy. This underscores an essential aspect of exosome manufacture: the need to assess and monitor the impact of the process on the "product". Upstream processes for cell culture need to be supported by real-time assessment of cell viability, exosome number and exosome characteristics.

The second key consideration for exosome manufacture, the downstream processing, involves separation and concentration of exosomes from cell culture supernatant. This step represents both the major challenge and the major opportunity for the sector. The aim is to yield a highly purified exosome fraction that is free of contaminating biological material which could modulate the biological properties of the exosomes or result in adverse effects. The challenge is to remove proteins, cellular debris, other microvesicles and host cell DNA, while concentrating the exosomes. Although ultracentrifugation and density gradient separation are very useful techniques for the concentration of EVs for research purposes, they are not easily scaled. They can also damage the exosome structure and hence reduce function. As a result, these methods are unsuitable for the manufacture of therapeutic exosomes.

Although there are multiple technologies available for the upstream and downstream steps of exosome manufacture, selection of preferred options should consider the commercial and clinical demands and limit technological and financial risks. Navigation through this maze of considerations is critical for companies. The recent development of a decision support tool and costing model that identifies the cost of consumables, labour and hardware is significant for the sector[5]. Application of this tool identifies large-scale culture and exosome harvesting technologies as the most important considerations in manufacture of sufficient exosomes to meet clinical demand.

The third key consideration facing manufacturers of therapeutic exosomes is ensuring product consistency and safety. This is impossible without a comprehensive toolkit of analytics for both in-process control and final product characterisation. A comprehensive process control strategy is key to achieving process consistency and product quality.

In today's regulated bioprocessing environment, this type of control strategy is known as continuous process verification (CPV). It is the third phase in the Food and Drug Administrations (FDA)'s lifecycle approach to process validation; the first being process design using quality by design principles and the second being process performance qualification. A process control strategy starts with a well characterised product and an understanding of how each product attribute impacts its safety and functionality. While the strategy will evolve throughout product development, it must start early so that the exosomes are sufficiently well characterised with regard to their identity, safety, purity and biological activity prior to a preclinical and first-in-human study. To be effective, a process control strategy requires reliable analytics to evaluate product quality attributes



and extensive online monitoring of process parameters. All aspects of product development are interrelated, with product characterisation being central to understanding of the product. Figure 2 shows the elements necessary for successful product development.

More importantly, a process control strategy requires detailed analysis, acquired through carefully controlled experiments, to understand the impact of process parameters and material attributes on the quality and function of the exosomes. The ideal approach is to incorporate real-time analytics as an integral component of the manufacturing process. However, one of the obstacles exosome manufacturers face is the absence of an integrated control system where real-time product test data allows for detection and adaption to process changes. This highlights the importance of online (ideally) and offline process monitoring assays and data, specific to exosomes, that comprise part of the Chemistry, Manufacturing and Contol (CMC) section of the regulatory submission for product approval, including approval of release criteria.

For complex products like exosomes, it is necessary to evaluate several assays that can measure the product attributes related to the mechanism of action (biological activity). During the product development phase, several assays will be explored with the hope that one or more will be robust enough to be validated as a potency assay for lot release. However, it is more likely that one assay will not be sufficient, and that an assay matrix approach will be required. Assays should include critical measures of process reliability and consistency: the amount (content) of exosomes in the biomass (upstream) and the purified drug substance (downstream), and the identity and amount of miRNA and/or protein species in the cargo. Exosomes, mi-RNA and protein cargos must meet defined release specifications to be suitable for clinical use. Regardless of the purpose and the type, assays used in the manufacture of exosomes for clinical or commercial supply must be well controlled. This control is achieved through the use of standard protocols for sample collection, processing, analytical methodology, and

data analysis/interpretation. Assays must be validated to the standards of ICH and relevant US Pharmacopeia.

To assure product safety, multiple issues need to be addressed, including cell bank qualification and product purity. Cell bank safety testing and characterisation are essential steps toward obtaining a uniform final product with lot-to-lot consistency as well as to demonstrate that cell lines are free from adventitious agents and endogenous viruses. Product purity is critical; contaminants such as host cell DNA, extraneous soluble proteins and viruses will lead to adverse side-effects and increase the risk of immunogenic responses.

The fourth consideration concerns regulation of exosome therapeutics. Although exosomes have been used clinically, no exosome-based therapeutic has been approved by a regulatory agency. For exosomes to reach the clinic, and eventually market, numerous regulatory considerations need to be addressed. The use of allogeneic exosomes requires submission and approval of a new drug application (NDA). Several countries offer accelerated approval pathways, which may be influenced by the indication (e.g., orphan, rare disease, unmet need). Despite the possibilities for accelerated approval, exosome-based therapeutics may be defined differently in different countries. However, as they can be considered a subset of cell therapies, exosomes are likely to be regarded as biologicals. Regulators will also need to be assured that exosome manufacturing processes are controlled (i.e., GMP compliant) and all components used in manufacture appropriately qualified to ensure the biological activity of the exosomes. Here a comprehensive characterisation of the cell source is imperative. In addition, donor eligibility criteria must be carefully selected and applied in accordance with the appropriate ethical and regulatory requirements. Donor screening should include a comprehensive medical record review, physical assessment, medical history interview and screening for infectious disease, in compliance with the appropriate regulatory framework.

Finally, manufacturers need to address measures of exosome product potency. Regulators define potency as the products' specific ability or capacity to affect a given result. With no gold standard technique for quantification of exosome potency, assessment of potency for the intended clinical use helps overcome inconsistent preparations and lot-to-lot variation and guides clinical use. Standardisation of exosome preparations remains a challenge for the field. The International Society for Extracellular Vesicles (ISEV) has produced a set of guidelines (MISEV2018) which ascribe current best practice methodologies and parameters for the accurate reporting of EV experiments[6].

Current and future regulatory requirements will drive developers of exosome-based therapeutic products to incorporate robust quality attributes early in the design phase. This will be instrumental to ensure a focus on patient safety by means of a high degree of process understanding. Early and regular dialogue with the regulators through the development programme is strongly encouraged. Exosome therapeutics are a next-generation therapeutic modality that has the potential to treat a diverse number of diseases. The widespread clinical use of and commercial success of exosomes depends on the development of large-scale GMP-compliant processes to deliver quality products of known composition. This requires the resolution of several technological issues and a holistic approach to manufacturing process control. A cautious and strategic approach between regulators and industry is required to ensure patients are treated with safe and effective exosome products.

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