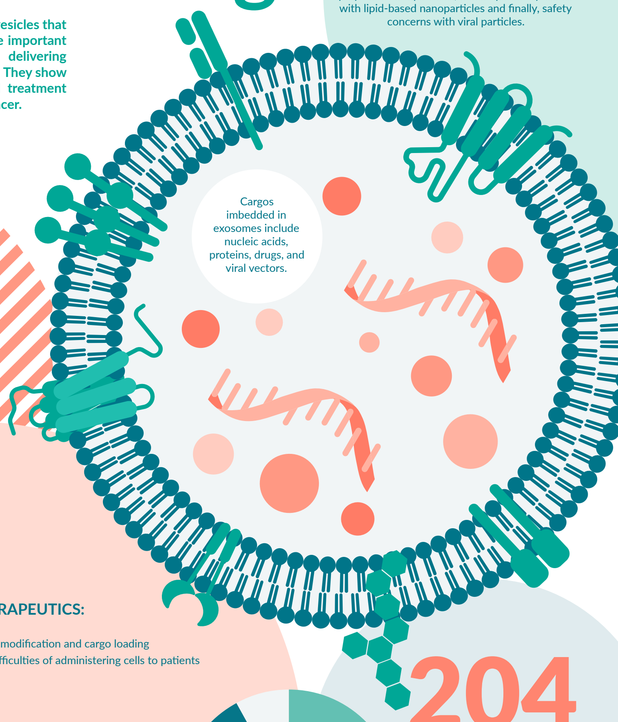
Exosome manufacturing

Exosomes are small (30-150 nm) extracellular vesicles that are released by exocytosis from cells. They are important mediators of cell-to-cell communication, delivering genetic and bioactive molecules to target cells. They show significant promise in novel diagnostic and treatment strategies for a range of diseases, including cancer.

Exosomes can offer an alternative to nanoparticles and viral particles because they circumvent some of the issues associated with them. Metallic toxicity is associated with metallic nanoparticles, low yield and biodegradability associated with polymeric nanoparticles, biocompatability issues with lipid-based nanoparticles and finally, safety concerns with viral particles.



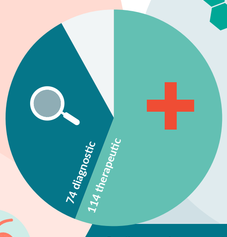
Cargos imbedded in exosomes include nucleic acids, proteins, drugs, and viral vectors.

THERAPEUTIC USES OF EXOSOMES

- Biomarkers for diagnostics
- Cell-free therapies
- Drug-delivery systems:
  - Direct method: exosomes are loaded with therapeutic agents
  - Indirect method: cells are genetically engineered or cocultured with therapeutic agents to produce artificial exosomes
- Cancer vaccines
- Personalized medicine

ADVANTAGES OF EXOSOMES AS THERAPEUTICS:

- Ability to cross the blood brain barrier
- Ability to engineer them including labelling, targeted modification and cargo loading
- As a cell-free therapeutic, they avoid the risks and difficulties of administering cells to patients
- High blood circulation clearance
- High cellular uptake
- Stability in the body
- Biocompatability
- Ubiquity



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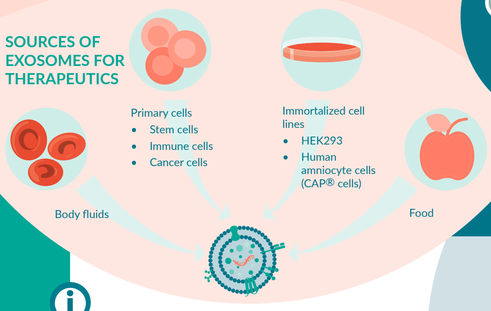
clinical trials using exosomes

For clinical trials, exosomes are required to comply with CGMP. CGMP manufacturing comprises

- Cell type and origin
- Culture method and medium
- Purification
- Characterization and identification method

Quality, consistency and functionality of exosomes is greatly impacted by the type, quality and heterogeneity of the source material. Therefore, in order to minimize exosome heterogeneity, tight control over the heterogeneity of the source material is essential.
Assessment of therapeutic indexes of source materials such as age should be considered.

SOURCES OF EXOSOMES FOR THERAPEUTICS



MANUFACTURING CHALLENGES

- Lack of standardization in isolation methods
- Primary cells usually grown in serum-containing media. Serum needs to be exosome free and removed prior administration.
- Achieving high yields coupled with high purity
- Exosomes derived from different sources have different features and therefore require different manufacturing, characterization, and purification protocols.

EXOSOME MANUFACTURING

CELL LINE CULTURE AND EXPANSION

- Rounds of division and expansion to generate a sufficient number of exosome-producing primary cells
- Expansion of suspension cells in controlled bioreactors when manufacturing at scale
- Challenge: scale up of adherent primary cell culture method

EXOSOME ISOLATION

- Normal flow filtration (NFF) to separate exosomes from extracellular vesicles (EVs)

CONCENTRATION AND PURIFICATION

- Traditionally ultra centrifugation but it can disrupt exosome integrity and does not remove macromolecule contaminants. Therefore it has a lower yield and purity.
- NFF or tangential flow filtration (TFF) - higher yield and better batch-to-batch consistency
- Size exclusion chromatography
- Affinity column chromatography
- Anion-exchange chromatography
- Magnetic beads

Purification challenges:
The size overlap between exosomes and other extracellular vesicles such as microvesicles make them difficult to separate.

ANALYTICS: CHARACTERIZATION AND IDENTITY TESTING

Exosome characteristics must be analyzed in order to determine the quality of the produced exosomes.

CHARACTERIZATION

Exosome particle number per volume
- Nanoparticle tracking analysis (NTA)

Presence of positive exosome markers
- Immunoblotting

Presence of negative exosome markers
- Immunoblotting, qPCR, MS, ELISA

Size and structure of the lipid bilayer
- Cryo-electron microscopy

Total protein
- BCA protein assay

IDENTITY

Assays detecting originator cell-specific markers or common exosome markers, such as proteins, lipids, or nucleic acids, can be used to analyze exosome identity

Exosome-specific proteins
- Western blotting, fluorescence activated cell sorting (FACS) with a specific antibody

Cargo loading/active pharmaceutical agent
- Immunoblotting, nano-flow cytometry, multiple reaction monitoring, ELISA, PCR

Protein identification
- Liquid chromatography, LC-MS, MS, Western blotting, flow cytometry

Surface marker profiling
- Flow cytometry, Western Blot, ELISA

Lipid identification
- LC-MS, MS

Transcriptomics
- Sequencing

There is still a lack of quality control guidelines over exosome stability, safety, potency, and quality requirements.

PURITY

Purity
- SE-HPLC

Host cell protein
- ELISA

Host cell RNA
- HPLC or gel electrophoresis

Host cell DNA
- Residual DNA quantitative assay

SAFETY

Mycoplasma
- Microbiological culture method, qPCR

Bioburden
- Membrance filtration

Adventitious virus
- *In vitro* assay, qPCR

Sterility
- Direct innoculation

Endotoxin
- Gel clot, photometry

***In vitro* potency assays – cell based biological assays**

References

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9095511
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9473149
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8766409
- https://www.frontiersin.org/articles/10.3389/fimmu.2022.865245/full
- https://www.frontiersin.org/articles/10.3389/fbioe.2021.811971/full

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