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| **Comparative Isolation of Exosomes from Cell Culture Media Using Ultrafiltration, Sequential Ultrafiltration, Size Exclusion Chromatography and Ultracentrifugation** |
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| **Background:** Exosomes are ubiquitous throughout the body being produced by essentially all cell types each of which dictates the composition of the lipid bi-layer. Their role as cellular messengers is being harnessed to deliver therapeutic drugs to their target, reducing systemic distribution, lowering harmful side effects and increasing viability for chemo-resistant cancers. Therapeutic interest in exosomes has grown dramatically as their non-immunogenicity, biocompatibility and ability to cross the blood brain barrier are highly advantageous. In addition, a wide range of drugs can be encapsulated and ease of functionalisation can aid in specific targeting. However, efficient isolation and drug encapsulation has remained a problem. Lack of standardisation, low yields and low encapsulation efficiency have slowed their progress into clinical trials and personalized oncology. |
| **Methods:** The pancreatic cancer cell line BxPC-3 are incubated with serum free media and 45mL of the exosome containing cell culture media is clarified by differential ultracentrifugation to remove contaminants and larger extracellular vesicles following guidelines set out in MISEV 2018. Exosomes isolated by Ultrafiltration, sequential ultrafiltration, size exclusion and the gold standard ultracentrifugation are then analysed using DLS, zeta and NTA along with western blot for exosome specific protein markers and the Bradford assay for total protein quantification. |
| **Results:** DLS characterisation has shown polydisperse samples approximately 50nm and 275nm in diameter with the zeta potential at -12 mV. While exosomes are a heterologous mixture the larger population are potentially aggregations of the exosomes during isolation or larger extracellular vesicles. |
| **Conclusions:** So far, the data has shown exosomes isolation but with aggregation or potential contamination with large extracellular vesicles. Further experiments will use sonication or additives, such as trehalose, to reduce aggregation and allow for drug loading and aptamer functionalisation. |