Characterization of Human Plasma-Derived Exosomes in Sarcoidosis

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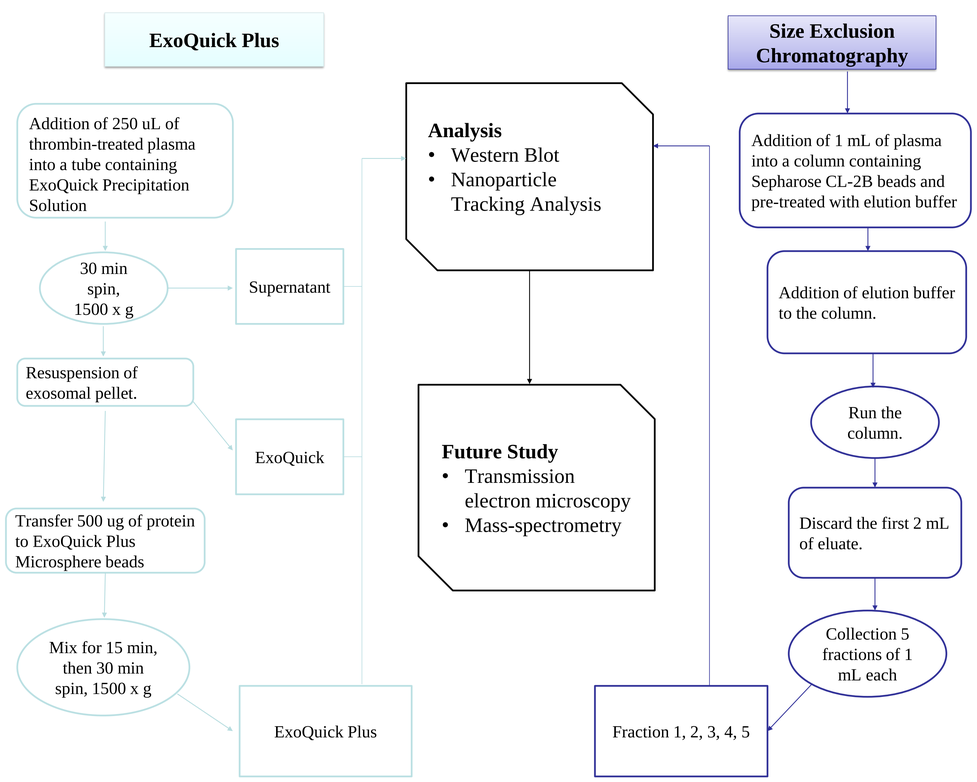
Introduction

- Sarcoidosis is a systemic granulomatous disease of unknown cause, with highly variable manifestation and disease course.[1]
- Currently, no specific and sensitive biomarkers for diagnosis and prognosis in sarcoidosis are available for routine clinical use.[2]
- Exosomes are extracellular vesicles ranged from 30-100 nm in size and involved in cell-to-cell communication through the delivery of cargo that may contain potential disease biomarkers.[3]
- We hypothesized that characterizing the exosomal cargo will provide novel insights into the disease mechanism and biomarkers in sarcoidosis.

Objectives

- Our long-term goal is to identify organ-specific sarcoidosis-related pathways for precise therapy.
- For this UROP project, we developed methods to obtain and characterize exosomes from human plasma with the following aims:
  1) Compare the efficiency of size exclusion chromatography (SEC) and ExoQuick Plus in isolating exosomes
  2) Use Western blot and nanoparticle tracking analysis (NTA) to assess the quality of the isolated exosomes.

Methods



Results

Figure 1: **Characterization of the isolated exosomes by Western Blot.** 11ug of proteins were loaded for all samples. HSP70, TSG101, and CD9 were used as exosomal markers, while albumin was used as a contamination marker.

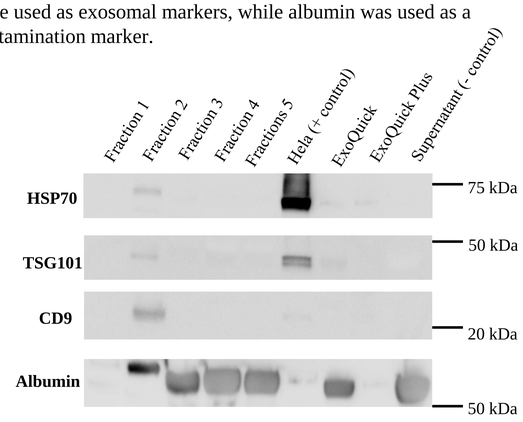
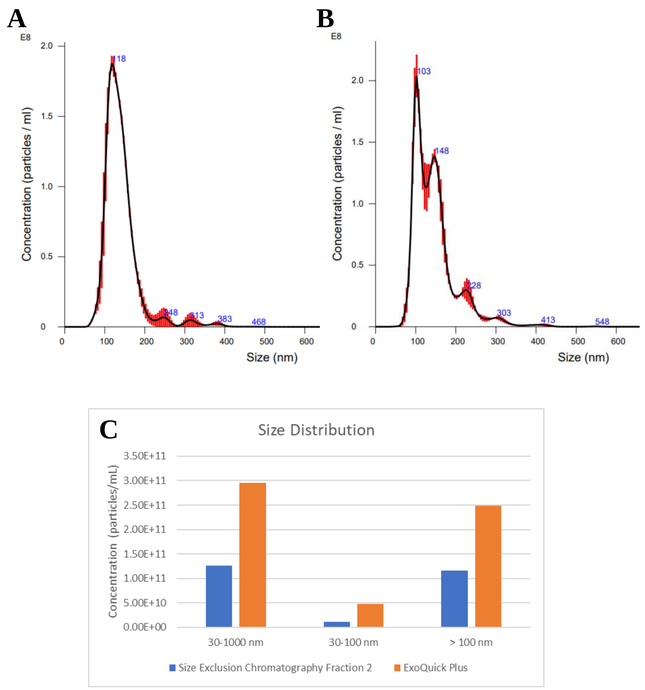


Figure 2: **Characterization of the isolated exosomes by nanoparticle tracking analysis (NTA).** Size exclusion chromatography yielded a modal size of 118 nm (A), while ExoQuick Plus yielded a modal size of 103 nm (B), with size distribution of $1.27x10^{11}$ particles/mL and $2.96x10^{11}$ particles/mL, respectively, for all particles ranged from 30-1000 nm (C).



Discussion

- Detection of HSP70, TSG101, and CD9 for size exclusion chromatography (SEC) sample indicates the presence of exosomes.
- Although ExoQuick Plus removed more albumins than SEC, only a weak signal of exosomal marker HSP70 was observed for ExoQuick Plus sample.
- A greater number of particles within the size range of exosome was observed for ExoQuick Plus than that of SEC. However, the lack of signals for TSG101 and CD9 for ExoQuick Plus may indicate the presence of co-isolated lipoproteins, which share similar size range with exosomes.[4]
- Since NTA does not distinguish exosomes from other particles of similar size, transmission electron microscopy is recommended to confirm the NTA results and the presence of lipoproteins.[4,5]

Conclusion

- SEC enriched exosomes with all three exosomal markers found to be positive, but was contaminated with albumins.
- ExoQuick Plus removed highly-abundant protein albumins.
- We will use 100 kDa centrifugal filter device to remove albumins from SEC samples for future experiments and further characterize exosomes using transmission electron microcopy and mass-spectrometry.

References

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