Project 29: Optimization of a Menstrual Effluent Purification Pipeline for Exosomal Biomarker Testing

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Background

- Endometriosis is a chronic inflammatory condition
- Characterized by:
- Inflammation
- Chronic pelvic pain
- Infertility
- Current diagnostic methods relying on symptom evaluation and imaging can be misleading, and surgery is invasive.

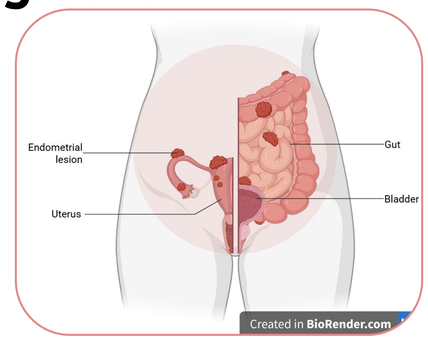

Figure 1: Diagram illustrating the effects of endometriosis on the body.

- Exosomes are extracellular vesicles (EVs) secreted by cells
- Contain molecular cargo such as:
- proteins
- mRNA
- nucleic acids

which can act as biomarkers!

- Exosomal biomarkers derived from menstrual effluent may provide insight into endometriosis-associated cellular activity.

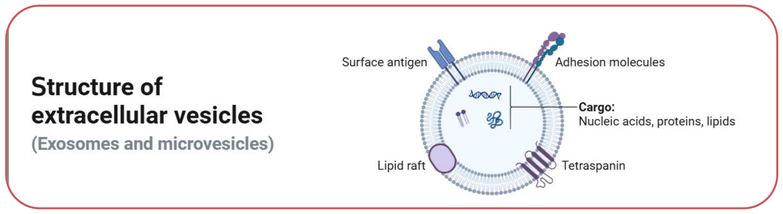

Figure 2: Structure of extracellular vesicles (EVs)

Objectives

Reproducibility: Allows for higher biomarker detection

Effective Isolation: Allows for higher purity and detection

Compatibility with Downstream Analysis: Allows for accessibility

Problem Statement

Given the want for less invasive diagnostic methods; A reproducible, standardized ME purification pipeline is needed to minimize variability.

Experimental Design

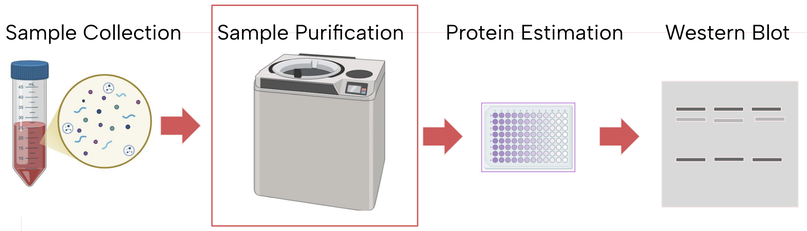

Figure 3: Overview of the menstrual effluent purification pipeline.

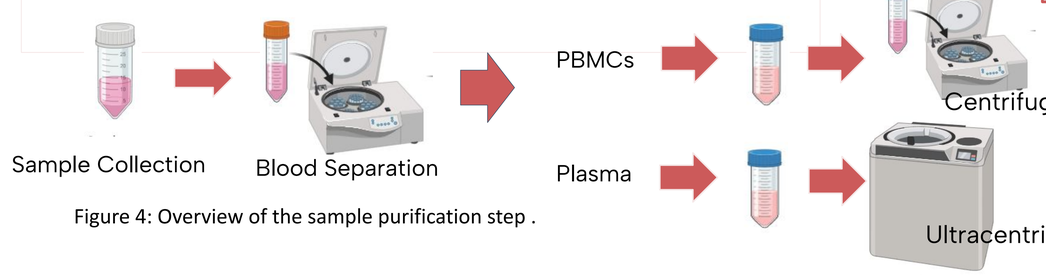

Figure 4: Overview of the sample purification step .

METHOD 1:
1 Spin x Fraction
3 Hour Spin (10k)
↓
1.5 Hour Spin (100k)

METHOD 2:
Plasma → 4℃
Overnight Storage of Plasma in 4℃
↓
Continue Processing Next Day

METHOD 3:
2 Spins for 100k
3 Hour Spin 10k)
↓
1.5 Hour Spin
↓
1.5 Hour Spin (100k)

Expected Outcomes

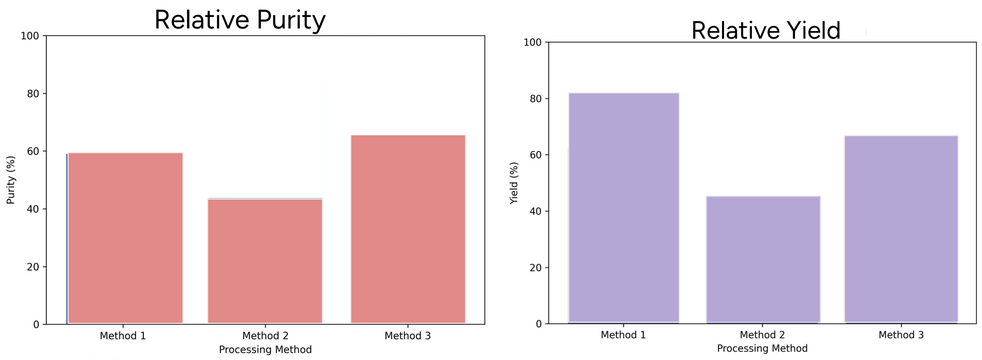

Figure 5: Relative purity and normalized yield of exosome samples processed using different purification methods. Method 1 demonstrated the highest relative yield, while Method 3 showed higher relative purity.

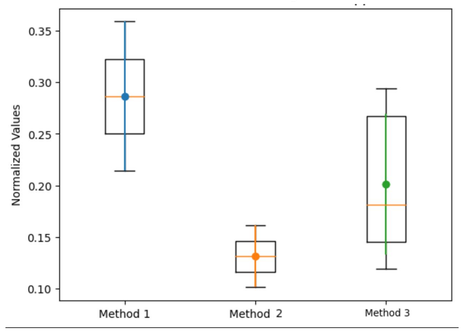

Figure 6: Normalized values protein counts of samples grouped by method, where Method 1 was found to have the most.

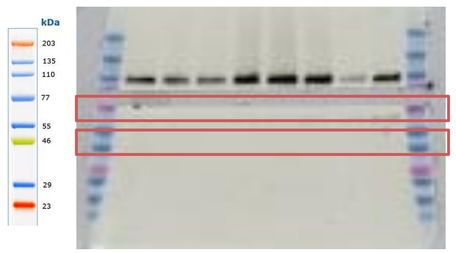

Figure 7: Normalized values protein counts of samples grouped by method, where Method 1 was found to have the most.

Conclusions

- Overall, Method 3 was selected as the optimal purification approach because it demonstrated the highest relative purity while maintaining a comparatively high normalized yield.
- Although Method 1 produced higher overall yield, it showed lower purity and greater potential contamination.
- These findings suggest that the additional ultracentrifugation step in Method 3 improved contaminant removal while preserving sufficient exosomal material for downstream analysis.
- **Limitations** : Sample heterogeneity, contamination risk, freeze-thaw degradation, and limited sample size may have affected reproducibility.
- **Future work:** improving sterilization, quality control, and sample-processing flexibility while maintaining high purity and yield.

Acknowledgements & References

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