Tangential Flow Microfluidics for Size-Based Separation of Exosomes

Kilean Lucas[1], Henry Chung, Ph.D.[1], Alex Shestopolov, Ph.D.[2], Carla Beckham, MD-Ph.D.[3], James McGrath, Ph.D.[1]

[1]Department of Biomedical Engineering, University of Rochester, Rochester, NY

[2] Department of Chemical Engineering, University of Rochester, Rochester, NY

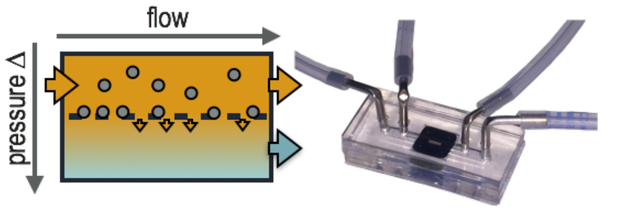
[3] Department of Urology, University of Rochester Medical Center, Rochester, NY

Background

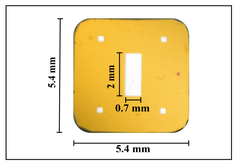
Introduction: There are a variety of isolation techniques available for obtaining populations of exosomes. The "gold-standard" method for obtaining a pure population of exosomes is ultracentrifugation, which is a lengthy process requiring large starting volumes, expensive equipment, and trained operators and there is a significant amount of residual contaminating protein after the purification. A commonly used alternative to ultracentrifugation involves a hydrogel "net" to collect exosomes by a centrifuge, but at significantly lower speeds. This method also leads to significant amounts of protein being captured with the exosomes. Thus, there is a need for a rapid, efficient and easy to use method of capturing exosomes that leaves them free of contaminating proteins. This research employs nanoporous silicon nitride nanomembranes in conjunction with microfluidics to satisfy this need.

Nanoporous silicon nanomembranes were discovered in the McGrath Lab in 2007 [1,2]. This material has the advantage of being very thin (the membrane is 50-100 nm thick) and having a very low resistance to transmembrane flow, allowing for numerous applications including separations, cell growth, and dialysis.These membranes offer the unique ability to capture and separate exosomes so that we can analyze them exosome-by-exosome, providing rapid, *in situ* characterization of disease.

Experimental Setup



The silicon nanomembrane is bonded in a PDMS microfluidic device. This device is set up to allow tangential flow, which removes contaminating materials (e.g. protein from plasma) and still provides enough transmembrane pressure (TMP) to retain exosomes on the membrane pores.

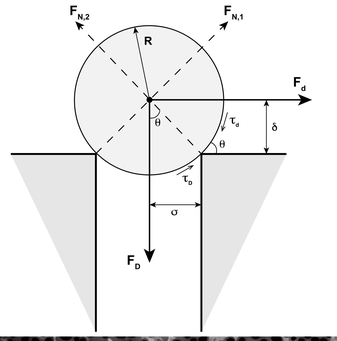


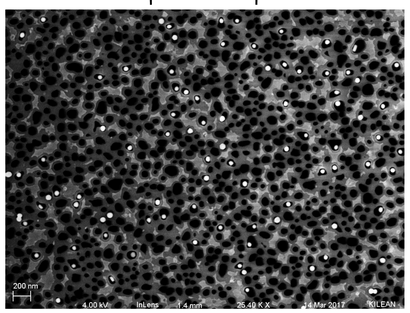
Analytical Model

An analytical model of the particle capture process is essential for understanding the appropriate conditions in which particles are retained on the pores while contaminants (proteins, etc.) are swept downstream and out of the system.

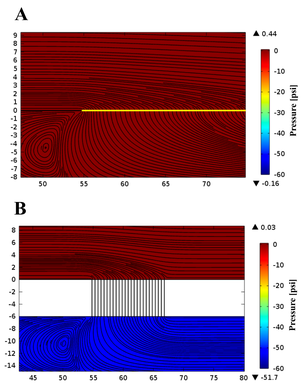
In order to retain the particles, the downstream drag force, F_d, must be balanced with the transmembrane pressure force, F_D.

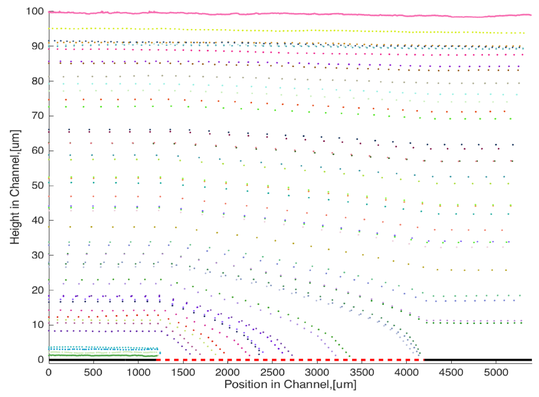
To experimentally test this model, we use gold nanoparticles as model exosomes and vary flow rates and pore diameters to achieve the best capture efficiency.





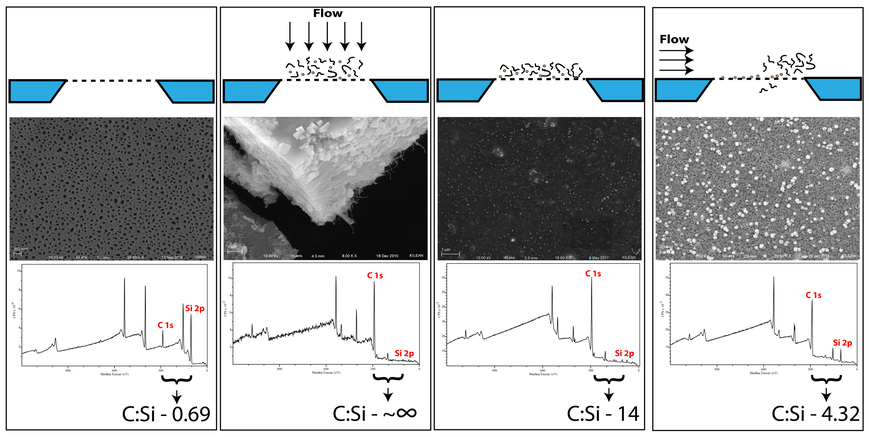
Computational Modeling





A computational model of the system was developed using COMSOL Multiphysics. This allowed for testing of pressures in the system and for the development of a particle tracking algorithm. The results of the model show that the nanoporous membrane has equal pressure on both sides of the membrane, while the track-etched (conventional) membrane had a pressure drop of over 50 psi which indicates that this system is high shear. Furthermore, the particle tracking algorithm allows for the visualization of what fraction of the flow will interact with the membrane, leading to higher capture from the total exosome population in the channel.

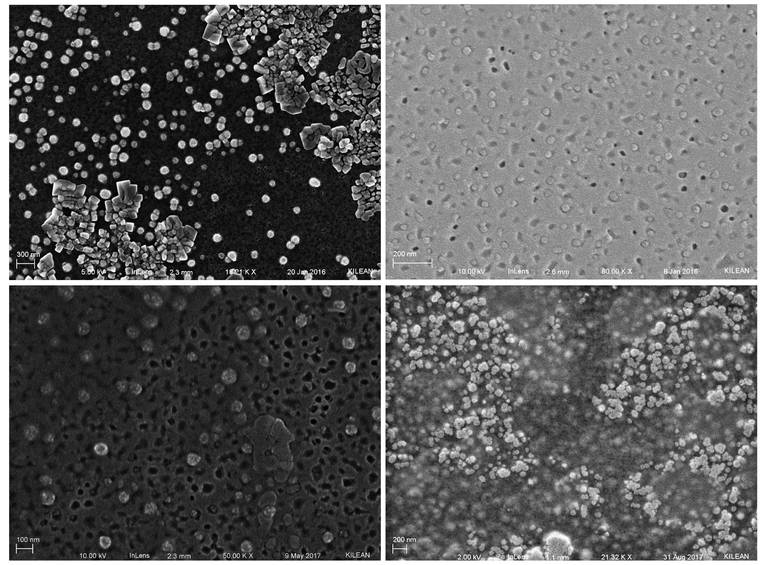
Exosome Purity



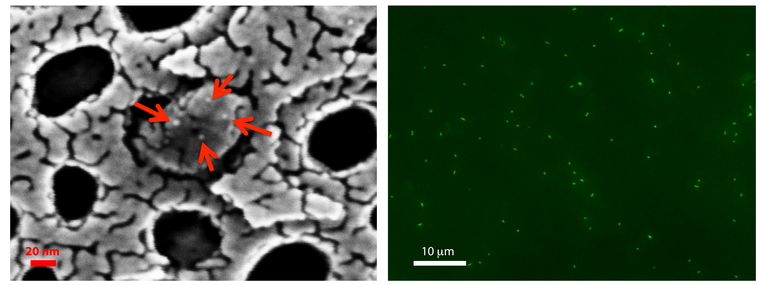
To compare the purity of the preparation to ultracentrifuge purified samples, the thickness of the carbon (protein) layer on the membrane surface was measured with X-ray photoelectron spectroscopy (XPS). XPS resolves only the first 10 nm of the surface, making it very sensitive for measuring contaminating layer thicknesses.

For this experiment, whole human plasma was flowed at the membrane in normal flow and in tangential flow while exosomes purified by ultracentrifugation from cell culture supernatant were deposited on the surface of a silicon nanomembrane. The normal flow experiment determined if simple centrifugation was a viable option for separation, but it led to a thick (~6 μm) layer of protein and other contaminants. The carbon to silicon peak ratio is a measure of the thickness of the contaminating layer (higher number means a thicker layer) that can be compared between spectra.

Exosome Capture



Exosome Identification



Exosomes captured on the membranes were labeled with a 10 nm immunogold secondary conjugated to an anti-CD63 primary as molecular identification for the exosomes. Additionally, SYTO RNAselect was used to fluorescently label the exosomal RNA for further identification.

Conclusion

We have shown that silicon nanomembranes in a tangential flow microfluidic device provide a rapid, efficient and contamination-free method for isolating exosomes from complex bodily fluids. Future work will be focused on refining the computational and analytical model of the system as well as developing a method for identifying exosomes of interest (cancer derived) *in situ* in an exosome-by-exosome manner.

References

1. Striemer, et al. (2007) Size and charge based separation of macromolecules using ultrathin silicon membranes, Nature 445:749-53
2. DesOrmeaux, J. P., et al. (2014) Nanoporous silicon nitride membranes fabricated from porous nanocrystalline silicon templates, Nanoscale. 6:10798-10805

Acknowledgements

The authors would like to thank Irena Nowack, Dr. Jonathan Flax, Alec Salminen, Dr. Richard Waugh, Tejas Khire, and Greg Madejski for their help and input in this work.