Isolation of Extracellular Vesicles from a Biofluid Using Ultrathin Microslit and Nanopore Silicon Membranes

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Precision Membrane Technologies

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Abstract

Urinary extracellular vesicles (UEVs) are emerging as a potentially powerful class of next-generation diagnostic analytes. Isolating UEVs is challenging, especially in the context of microfluidic systems. Here, we report on a new three-step UEV isolation procedure that has a total processing time of less than 1hr for 500µL human urine samples. The process relies on chip-format nanomembranes that can be integrated into microfluidics, including microslit silicon nitride (MSN) membranes for urine matrix factor removal and nanoporous silicon nitride (NPN) membranes for UEV capture.

Introduction

Urinary extracellular vesicles (UEVs) are 30–1,000 nm diameter, lipid bilayer vesicles shed by all cell types of the urinary tract and carry molecular cargo of potential diagnostic interest.

- Small-volume UEV isolation, especially in microfluidic devices, has remained problematic due to the sample preparation challenges of separating UEVs from urine matrix factors (e.g., cells and protein filaments).
- One possible solution for small-volume UEV isolation is use of silicon nanomembranes whose pore sizes have been optimized for sample preparation and capture/release of UEVs.

Materials & Methods

SepCon Devices

- SiMPore nanomembranes, including Nanoporous Nitride (NPN) and 0.5 micron Silicon Nitride (SiN) Microslits, were assembled into PP centrifugal spin columns and used for all experiments

Pre-filtration Experiments

- Healthy donor pooled human urine (Lee Biosciences) was used as received and filtered through 0.5 micron MS devices. After filtration Cell Counts were performed via hemocytometry, total protein was measured via OPA assay, and exosome yield measured by Nano-Tracking Analysis (NTA) and fluorescent RNA staining using a commercially sourced (Hansa) exosome reference standard

UEV Capture Experiments

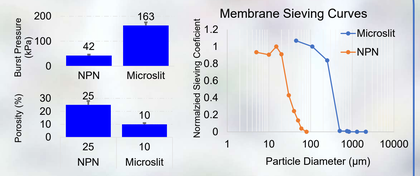
- 0.5 micron MS pre-cleared urine was filtered through 60 nm NPN devices, then characterized by immunoblotting (anti-CD63) and Fluorescent labeling of UEV RNA. Following capture, membrane-bound UEVs were labeled on the NPN membrane by total-protein stain (FITC) and imaged via fluorescent microscopy and Scanning Electron Microscopy (SEM).

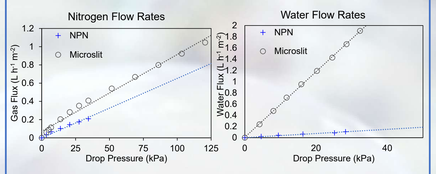
UEV Recovery

- Recovery of UEVs from NPN SepCons was characterized by NTA, fluorescent RNA staining, SEM, and Energy-Dispersive X-Ray Spectroscopy (EDX)

Results

Membrane & Device Properties

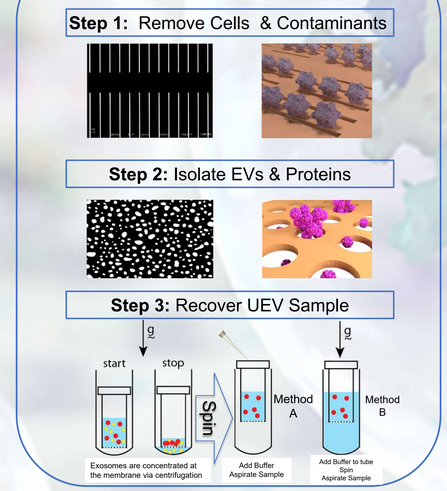




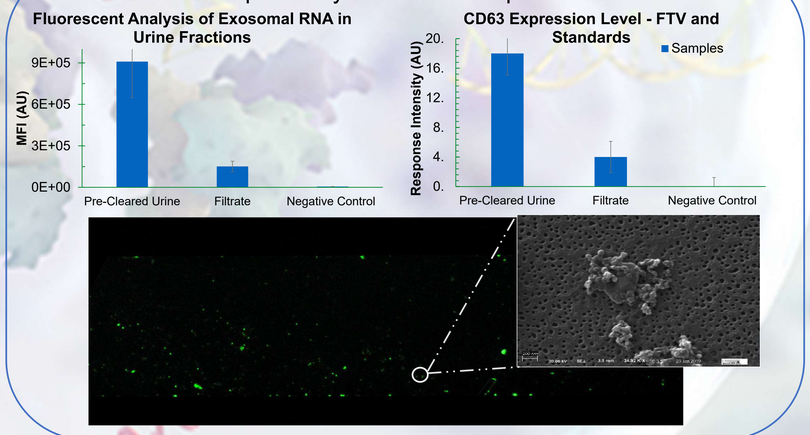
SepCon™ Device Parameters

Sample Volume:	20 – 500 µL
Maximum Centrifugal Force:	1200 x g
Recommended Starting Concentration:	<0.5% solids
Tube & Vial Material:	HDPE, Polypropylene
Gasket Material:	Platinum-cured Silicone, USP VI

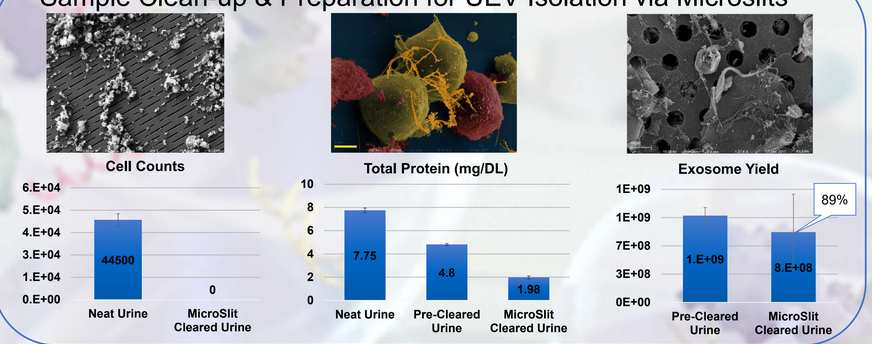
Sample Processing Workflow



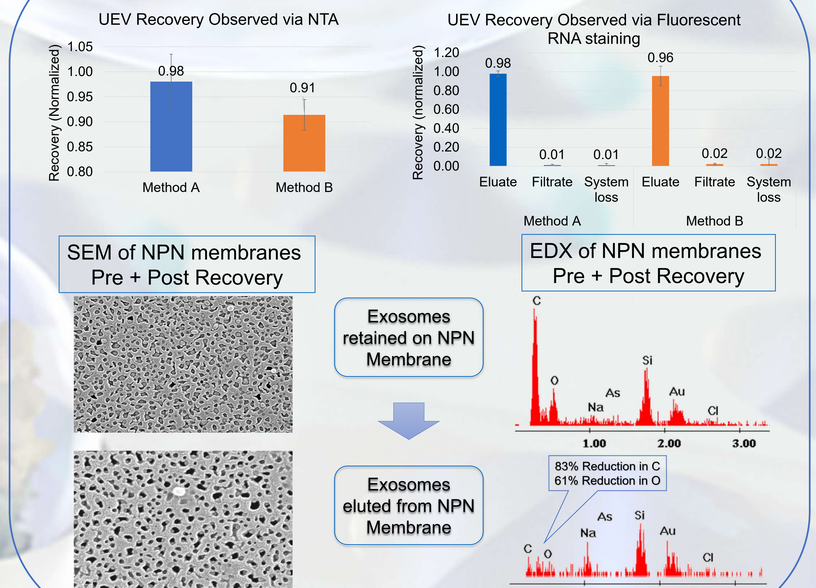
UEVs are Captured by SiMPore Nanoporous Membranes



Sample Clean-up & Preparation for UEV Isolation via Microslits



UEVs are easily recovered from SiMPore Nanoporous Membranes



Conclusions

- Microslit and nanoporous silicon nanomembranes offer unique capabilities for UEV isolation, enabling a three-step workflow with practical total processing time and sample volume capacity.
- Microslit silicon nitride membranes offer high-flow rates, urine matrix factor removal and nearly quantitative filtrate yield, fulfilling the need for effective UEV sample preparation.
- Nanoporous silicon nitride membranes offer nearly quantitative UEV capture and release, yielding partially purified UEVs that are ready for use in downstream applications.
- Chip-formatted silicon nanomembranes should find utility in a variety of small-volume UEV isolation systems.

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