Simplified visualization of extracellular vesicle preparations by silicon nanomembrane-enabled microfluidic devices

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Abstract

Introduction
Developing and characterizing methods for separation and concentration of extracellular vesicles (EVs) remains an area of significant interest. EV applications increasingly require isolated EVs of high purity and yield to perform basic biological, diagnostic, and especially therapeutic analyses. An ability to characterize EVs by simple and rapid means before, during, and after isolation steps would benefit understanding of both currently used methods and development of new methods. However, assessing EV preparations for EV size, concentration, and presence/absence of molecular cargo currently requires multiple dedicated instruments and practiced knowledge of these systems' procedures and pitfalls. We aim to bridge this gap by developing a user-friendly EV visualization procedure for determining concentration and detection of molecular markers within EV preparations.
Methods
Here, we report on our assessment of a microfluidic device capable of rapid visualization of EVs using simple pipet-driven loading and on-membrane EV capture and imaging. This device, named the µSiM-EV, was assessed for its ability to catch and visualize EVs by a number of microscopy techniques. On-membrane immunofluorescence was used to detect EV surface proteins, while comparisons to nanoparticle tracking analysis were used for assessing EV concentration.
Results
We assess nanoporous silicon nitride membranes, which are incorporated into the µSiM-EV device, for their ability to capture small- and medium-sized EVs. We showcase the ability to visualize EVs when captured on-membrane by optical and electron microscopy. We further compare nanoparticle tracking analysis to the µSiM-EV for particle concentration determination.
Summary/Conclusion
The µSiM-EV offers ease of use for rapid EV visualization during separation and concentration procedures. Future work will focus on expanding the variety and number of EV preparations from different sources that are analyzed via the µSiM-EV procedure.

EV Visualization Concept

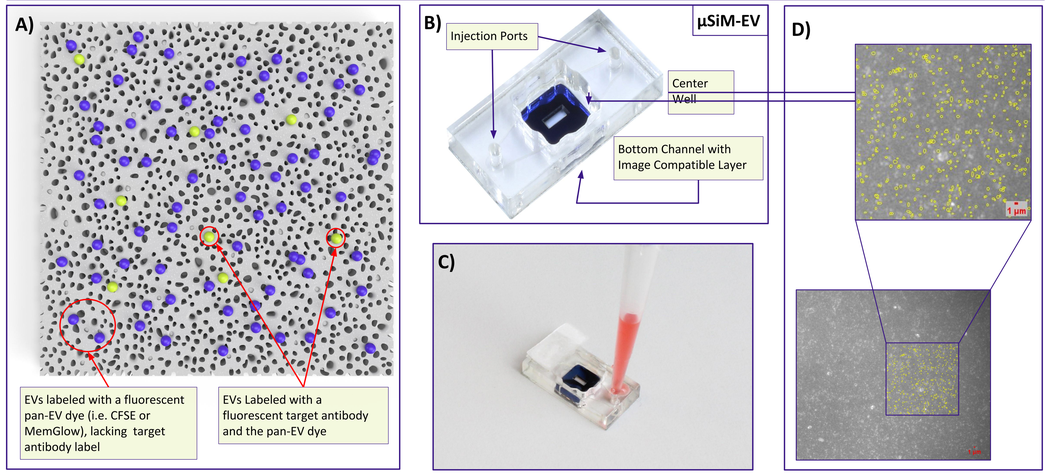

Figure 1: The EV Visualization Concept. A) Representative nanomembrane with EV-sized pores that capture individual fluorescent affinity-tagged EVs; fluorescent (green) color indicates EVs positively labeled with affinity tags. B) Microfluidic device enabled by a silicon membrane for EV visualization ("the µSiM-EV"), with two injection ports, center well, and bottom channel with imaging-compatible layer. C) EV samples can be easily loaded into the µSiM-EV by pipet, where EVs are captured on an optically transparent membrane and unwanted species pass into the center well. D) Labeled EVs can be imaged with epifluorescent microscopy and Image J used to count particles.

Dynamic Range of the µSiM-EV

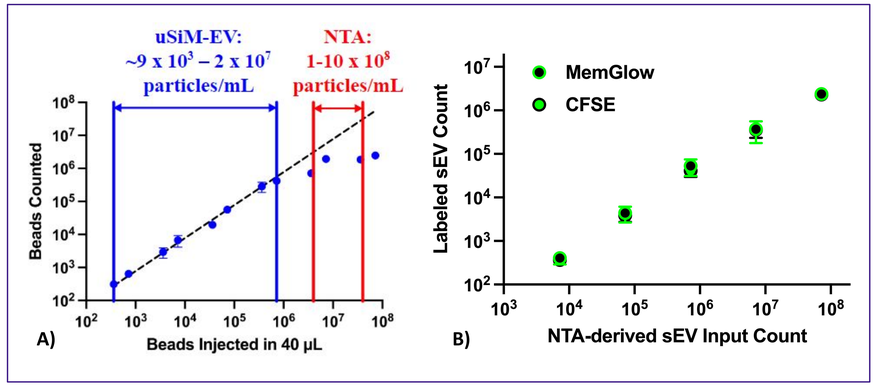

Figure 2: Dynamic Range of µSiM-EV Vs. Nanoparticle Tracking Analysis (NTA). A) EVs were collected by ultracentrifugation from culture media conditioned by primary bladder epithelial cells. Recovered particles were counted by NTA, serially diluted in PBS, and were subsequently labeled with either a lipophilic dye (MemGlow 488) or an amine-reactive dye (CFSE). B) Samples were then processed via the µSiM-EV and images analyzed via NIH ImageJ and ComDet. Data are plotted as mean +/- SEM (n = 3). Note greater dynamic range for µSiM-EV vs. NTA and good agreement between two labeling methods in this preparation. Data shared by Samuel Walker and Dr. James McGrath, University of Rochester.

Human Plasma DMC Fraction 6 Antibody Staining

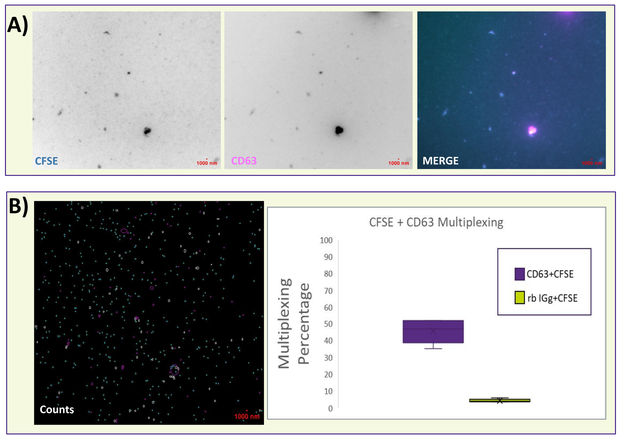

Figure 3: Immunolabeling Analysis by µSiM-EV. A) EVs from 0.5 mL of human plasma were passed through a dual mode chromatography column (size-exclusion + ion-exchange) to separate particles based on size and charge and remove positively charged particles. Eluate was collected in 1 mL fractions and fraction 6 was stained with CFSE and anti CD63, and loaded into a µSiM-EV containing an NPSN membrane with 50 nm pores. CFSE (cyan) and anti-CD63 (magenta) fluorescent micrographs are shown from representative fields-of-view from across the µSiM-EV's membrane. B) Particle counts are rendered from ImageJ ComDet using 5 px max separation, 3 px/500 nm max particle size, and 3 intensity threshold.

DMC Analysis

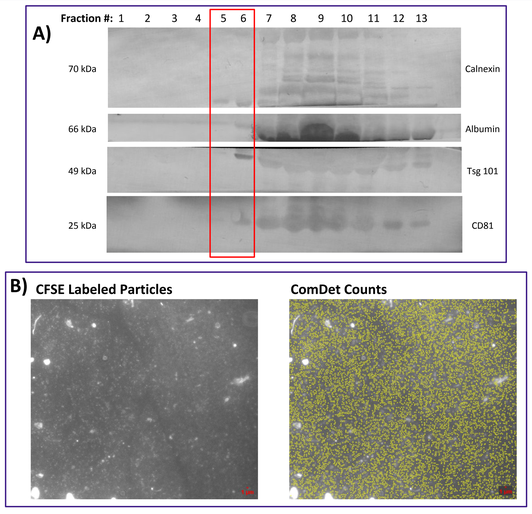

Figure 4: µSiM-EV Analysis of EVs Prepared by Dual Mode Chromatography. A) Human plasma (0.5 mL) was applied to a column comprising 10 mL of Sepharose CL-4B (size-exclusion) and 2 mL FractoGel-sulfate (ion-exchange; settled resin volumes with CL-4B on top). Fractions were collected and analyzed by Western blotting for EV markers (CD81, TSg101), matrix factor (serum albumin), and intracellular proteins (calnexin). EV containing fractions delineated with red. B) Column fractions were further analyzed by NTA or by CFSE labeling and µSiM-EV loading for total particle counts. Fraction 6 from the column was loaded onto a µSiM-EV with a 50 nm cut-off membrane after fluorescent labeling with CFSE (left). Imaged particles were counted with ComDet Plugin in ImageJ (right). Each image shows 1% of the membrane, ~5300 total particles counted.

SEM Analysis of Captured Particles

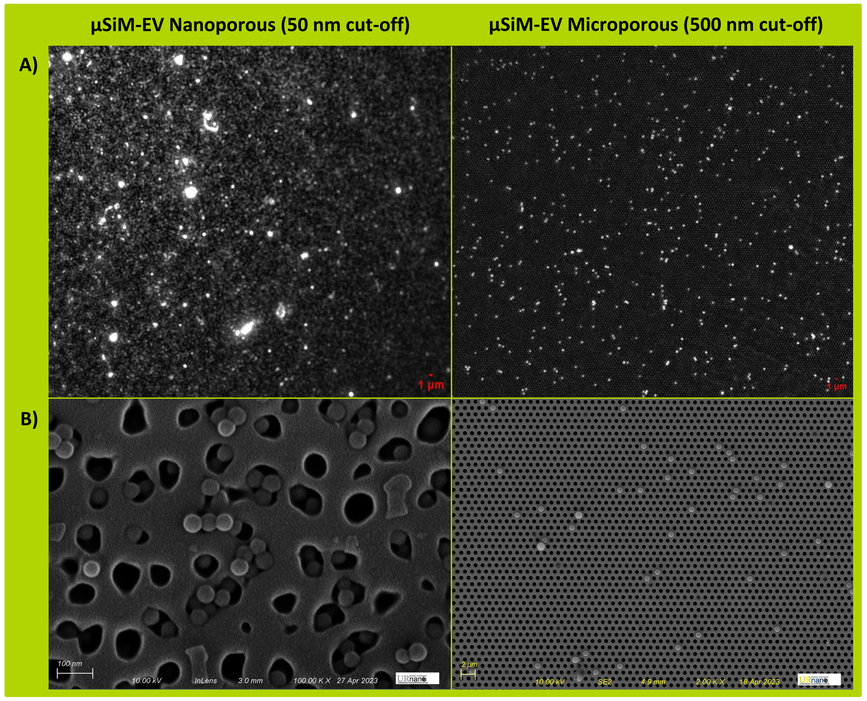

Figure 5: Scanning Electron Microscopy Analysis of µSiM-EV-Captured Particles. A) Particles were injected into the µSiM-EV and imaged via fluorescence microscopy. B) The same µSiM-EV units as in A) were prepared for scanning electron microscopy (SEM) by removal of the bottom layer and deposition of ~7 nm Au, then imaged via SEM. For µSiM-EV with nanoporous membranes (50 nm cut-off, left), 45 nm diameter polystyrene beads stained with fluorescent Nile Red were injected at a concentration of ~$1x10^{11}$ beads/mL (40 µL injected ≈ $4x10^{9}$ beads on the membrane. For µSiM-EV with microporous membranes (500 nm cut-off, right), 830 nm diameter polystyrene beads stained with fluorescent Jade Green were injected at a concentration of ~$3x10^{6}$ beads/mL (40 µL injected ≈ $1x10^{5}$ beads on the membrane).

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NTA data was collected using the NanoSight system in Thomas Gaborski's laboratory at the Rochester Institute of Technology

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