ExoSense: a Microprobe Based Method for Single-step Isolation and Genetic Analysis of Exosomes

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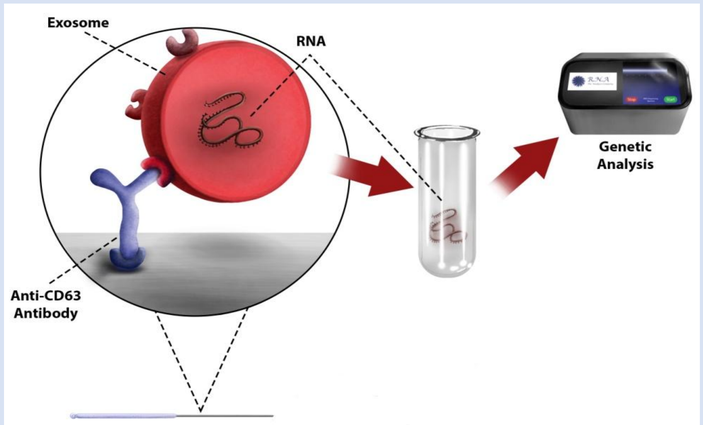
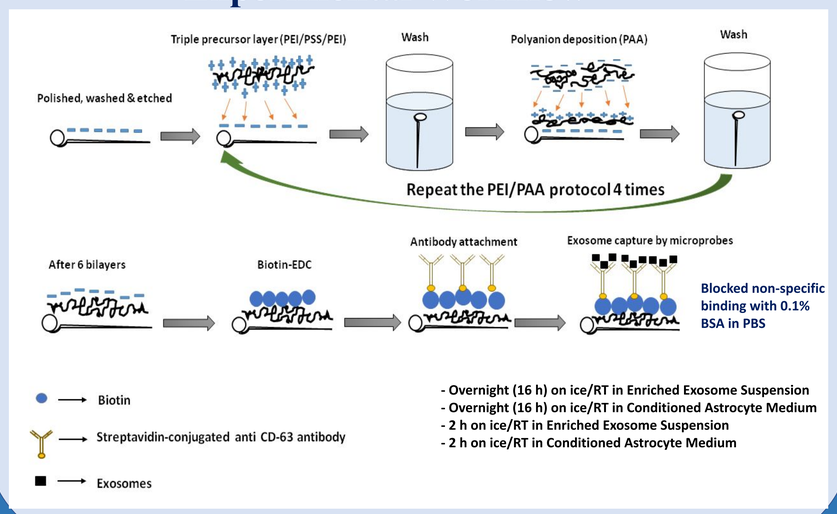
Overview and Significance

Detection and analysis of circulating exosomes is an emerging method for precise and non-invasive diagnosis and disease monitoring. However, their clinical utilization as biomarkers has not been fully realized due to technical challenges encountered in current liquid-phase methods for exosomes isolation.

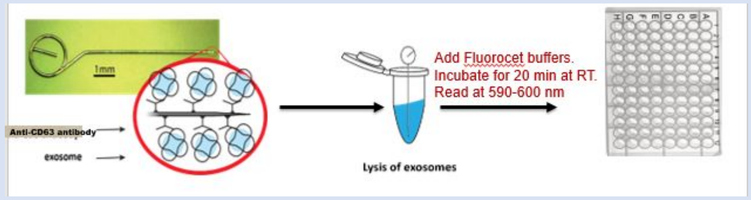
We successfully developed and characterized an immune-affinity protocol for selective purification and genetic analysis of extracellular vesicles that express the same surface marker. Stainless-steel microneedles (300µm×30mm were functionalized with exosome-specific anti-CD63 antibodies and the capture efficiency was assessed via Fluorocet assay.



Experimental Workflow



Fluorocet Assay



SEM/EDX Characterization

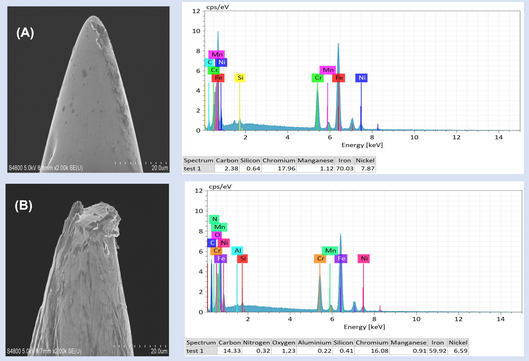


Figure 1: SEM micrographs (left panels) of (A.) Polished plain needles, (B.) Needles showing successful LBL coating of polyelectrolyte bilayers, and their corresponding EDX spectra (right panels).

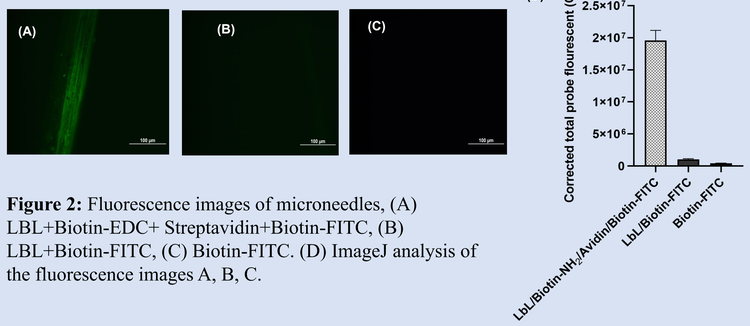


Figure 2: Fluorescence images of microneedles, (A) LBL+Biotin-EDC+ Streptavidin+Biotin-FITC, (B) LBL+Biotin-FITC, (C) Biotin-FITC. (D) ImageJ analysis of the fluorescence images A, B, C.

Microprobes Capture Capacity

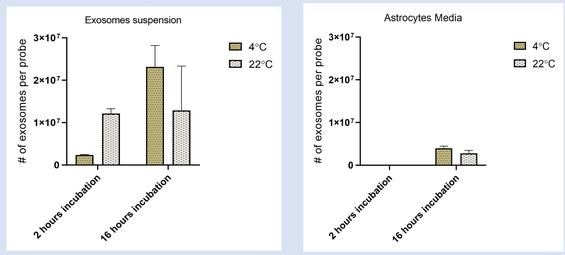


Figure 3: Exosome capture from enriched exosome suspension and astrocyte medium

Genomic and Proteomic analysis of Isolated Exosomes

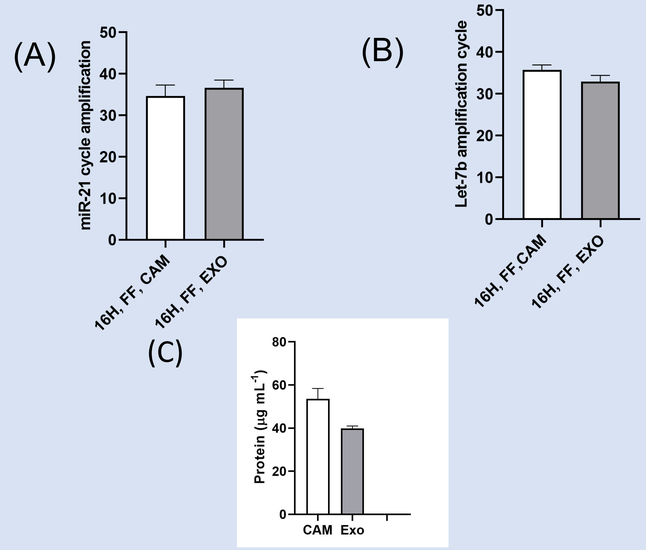


Figure 6: RT-qPCR amplification cycles of (A) An oxidative stress marker, miR-21 (FF-CAM 36.2; FF-EXO 38.7), (B) A tumor suppressor gene, miR-let-7b (FF-CAM 34.4; FF-EXO 33.0) from an array of 20 probes, (C) Protein quantification from needles in CAM and EXO

Potential Applications of the Technology

- Precise and non-invasive method for liquid biopsy
- Automated exosomal RNA isolation with high throughput analyses
- Facilitate the studies of exosomes in development and progression of biological disorders.

Experimental Checklist

- The LBL immobilization protocol provided an efficient and stable precursor layer for subsequent immobilization of biotin and streptavidin-conjugated CD63 antibody
- Exosomes were isolated from different types of fluid media (CAM & EXO) at cold and room temperatures, and within a 2-hour incubation.
- The probes demonstrated excellent RNA and protein extraction performance for both fluid media
- CD63-expressing exosome subpopulation was captured; further analyses are required to demonstrate **specificity** of the technology.

Future works will also focus on integration of this microprobe-based technology into a lab-on-a-chip platform as a step towards process automation.