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Microprobe-based platform for rapid immunocapture and genetic analysis of exosomes

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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 notably, contamination by non- exosomal proteins reported in high-speed ultra-centrifugation methods. To address this, we successfully developed and characterized an immune-affinity method for solid-phase exosomes purification using stainless-steel microneedles (140 µm diameter; 30 mm length) functionalized with an anti-CD63 antibody specific to a marker expressed on the surface of the exosomes. The capture efficiency of the microprobes was assessed via EXOCET colorimetric assay. The microprobes were incubated in astrocyte-derived exosome suspension enriched by a standard polymer precipitation kit, under different experimental conditions. Blocking experiments (3% BSA in PBS) were performed to eliminate non-specific binding. Our results indicated that the exosome loading capacity increased 10-fold when the needles were incubated overnight on ice (40×10^6 exosomes per needle) than those incubated for 2 hours at room temperature (3.5×10^6 exosomes per needle), suggesting that longer incubation times at lower temperatures favor exosome capture. We further investigated whether the surface area of the microprobes and the type of biological sample had an effect on the loading efficiency of the immunocapture technique. Our results confirm increased exosome loading capacity with increased microneedle dimensions (300 µm diameter × 30 mm length) as well as with direct incubation in cell culture medium collected from astrocytes derived cell media. The captured exosomes were lysed and the small RNA fraction was purified for subsequent RT-qPCR microRNA analysis. The RNA capture efficiency of an array of 10 probes was sufficient for successful miRNA amplification and detection. The antibody coated probes demonstrated excellent small RNA extraction performance with quantification cycle value for miR-21 and SNORD47 of 15.8 and 13.2 respectively. Future work will focus on the characterization of the proteomic cargo of captured exosomes and integration of the microprobe-based approach into a lab-on-a-chip platform.