EXO-NET™: A Novel, Rapid, Scalable Exosome Isolation Technology

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BACKGROUND

The EXO-NET™ technology represents an innovation in exosome capture for researchers and routine pathology laboratories. It consists of a covalently linked, multilayered three-dimensional matrix comprising several exosome-specific antibodies and spacer and linker molecules that interact to confer a characteristic topology to maximize specific binding and capture of exosomes from complex biofluids, in a reproducible manner.

The EXO-NET matrix is coated onto magnetic beads for rapid and highly specific exosome isolation from any liquid biopsy sample. EXO-NET has demonstrated compatibility with multiple downstream chemistries for analysis of lipid, protein and nucleic acids. The technology is highly scalable and is compatible with existing automated testing systems.

MATERIALS

SAMPLES
- Purified breast cancer cell line MCF-7 exosomes (SBI, Erivan Bio)
- Exosome-free FBS (SBI)
- Healthy and pancreatic cancer human plasma (BioIVT)
- Artificial human plasma (SeraCare)

ANTIBODIES
- Anti-CD9 antibody (exosome marker)
- Anti-CD63 antibody (exosome marker)
- Anti-calnexin antibody (Golgi marker)
- Anti-GPC1 antibody (Minomic Intl. Ltd.)

COMMERCIAL KITS
- miR21 RT-qPCR reagents (Thermo)
- RNA Isolation kits (Qiagen, Promega)
- ExoIP kit (Diagenode)
- Streptavidin-gold 700nm nanorods (Nanopartz)

METHODS & RESULTS

FIGURE 1. EXO-NET™ BEAD STRUCTURE AND FUNCTION

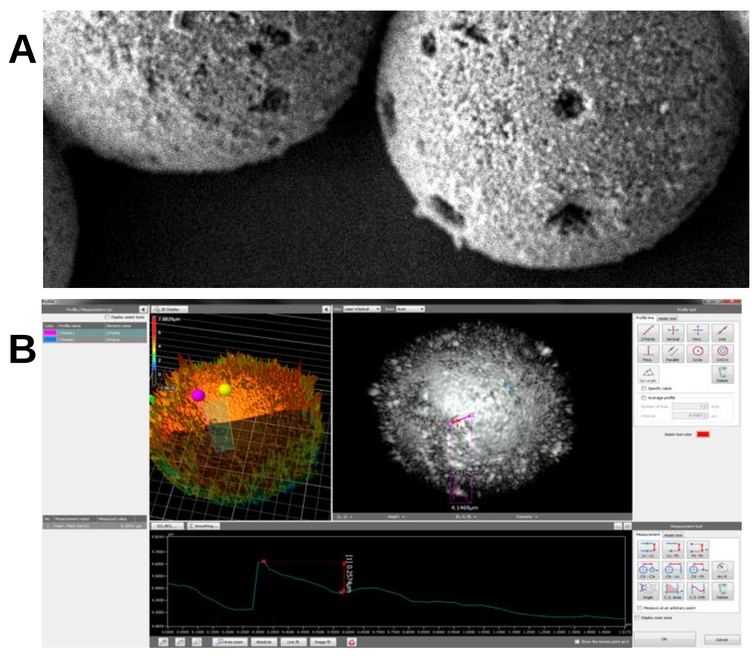

Figure 1. (A) Scanning electron micrograph showing ultra-structure of the EXO-NET™ matrix, including pores, coated on 2.5 um magnetic particles.

(B) 3D digital microscopic image (Keyence) of MCF-7-derived exosomes ranging in size from 40-250 nm bound to the surface of an EXO-NET™ matrix-coated magnetic bead. Yellow and pink pseudo-colored spheres are bound exosomes, and their spatial separation and thickness were measured using Keyence software.

(C) Western blot showing the contribution of affinity vs. porosity in binding and recovering CD9 protein from human exosomes captured by EXO-NET™ or by a NET matrix ("NET Structure") consisting of non-exosome specific antibodies but with similar pores. A 15 min incubation time is optimal for maximal exosome protein recovery. Total MCF-7 exosome lysate ("Total lysate") was used as the positive control.

FIGURE 2. PLASMA AND CELL CULTURE-DERIVED EXOSOMES

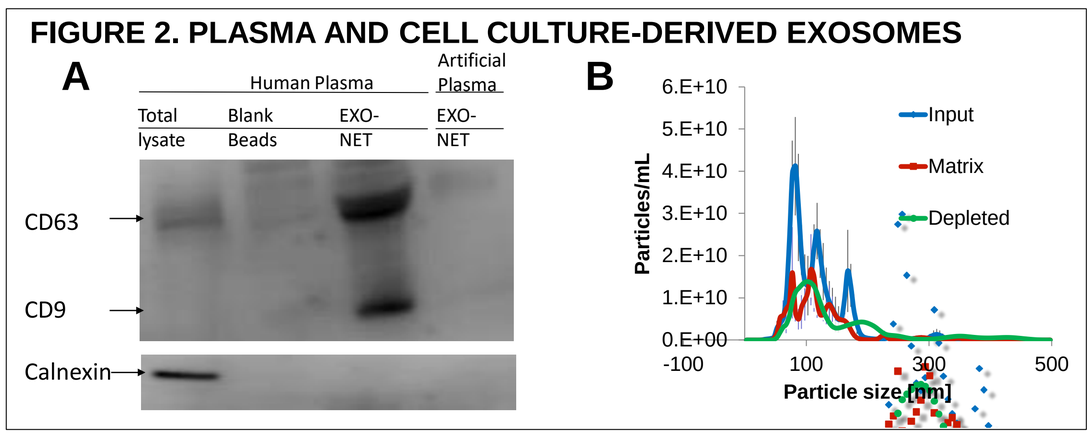

Figure 2. (A) Western blot of proteins extracted from control ("blank") beads or EXO-NET™-coated beads incubated with human or artificial plasma samples for 30 minutes.

(B) Particle size analysis (Nanoparticle Tracking Analysis (NanoSight)) of exosome-free FBS spiked with MCF-7 exosomes (~9E^10 - "Input"), exosome-free FBS ("Matrix") and the Input sample after incubation with EXO-NET™ beads for 30 mins ("Depleted"). The data shows almost complete depletion of the spiked exosomes by EXO-NET™.

FIGURE 3. SUPERIOR YIELD vs COMPETITORS

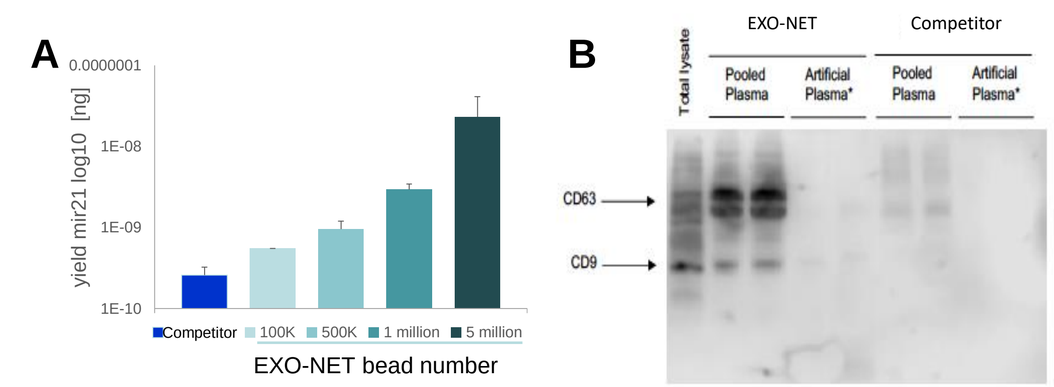

Figure 3. (A) Supernatants containing MCF-7 exosomes were treated using the Qiagen ExoRNeasy exosome RNA isolation kit or incubated with an increasing number of EXO-NET™ beads for 30 mins. Qiagen samples were eluted and PBS-washed EXO-NET™ beads were re-suspended in the same volume prior to analysis by miR21 RT-qPCR. The average concentrations of miR21 recovered from MCF-7 exosomes using the different methods are shown.

(B) Western blot of samples recovered from EXO-NET™ or an alternative (commercial) bead-based kit (ExoIP). Pooled human plasma (0.2mL) was incubated with EXO-NET™ beads for 15 min at RT or with ExoIP for 24h at 4°C, per manufacturer's instructions.

FIGURE 4. COMPATIBLE WITH DOWNSTREAM USES

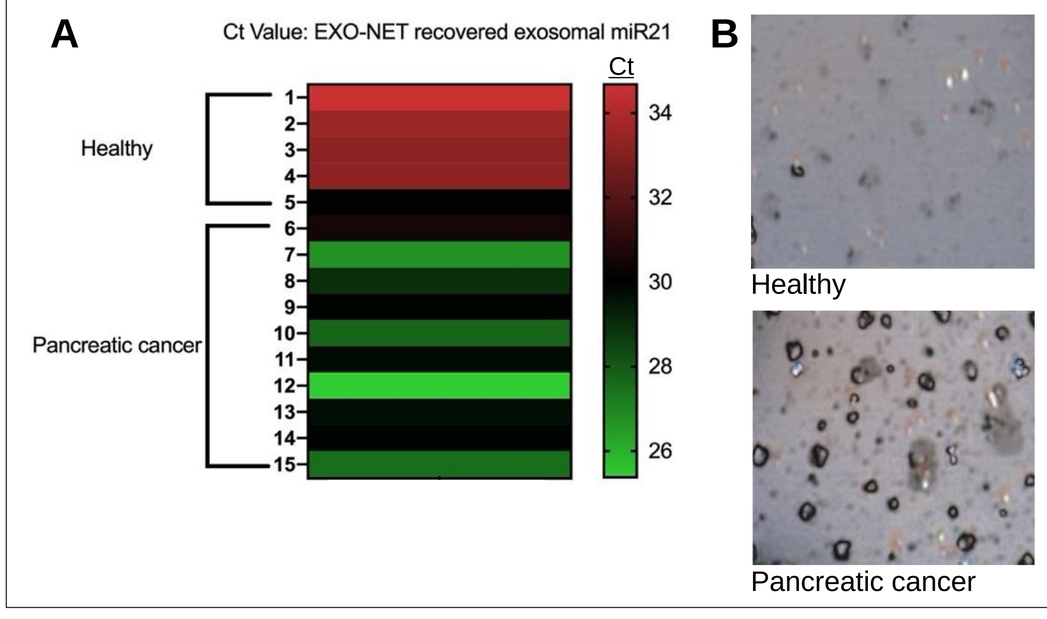

Figure 4. (A) Exosomes were recovered from 0.2mL pancreatic cancer and healthy human plasma samples (K2EDTA) using EXO-NET™ beads after a 15 minute incubation. Total RNA was extracted directly from exosome-bound EXO-NET™ using the Promega kit. RT and the miR21 qPCR assay was performed using a thermocycler (Applied Biosystems). Depicted is a heat map of miR21 Ct values obtained (GraphPad Software).

(B) EXO-NET™ beads were incubated for 15 min with 0.020mL healthy or pancreatic cancer plasma samples, washed and probed for 15 min using anti-glypican-1 (GPC-1) biotinylated monoclonal antibody (Minomic). Detection antibody-exosome-EXO-NET™ beads (EXO-NET™ "immune complex") were then washed and incubated with 2uL of a 1:500 dilution of streptavidin-gold nanorods for 5 min prior to washing and spotting 1uL on microscope slides. Spots were dried and were visualized by 3D digital microscopy (Keyence). Shown are 2 representative results obtained from healthy and pancreatic samples.

CONCLUSIONS

1. EXO-NET™ is a novel approach for rapid, pure and high yield capture of exosomes from complex samples.
2. Recovered exosome content is not contaminated with Golgi (Fig 2A), extraneous serum proteins, or free lipids (data not shown).
3. EXO-NET™ provides superior exosome-specific nucleic acid and protein marker results compared to market leading products and methods.
4. EXO-NET™ is compatible with small volume, high-throughput sample analysis using multi-omics, imaging or targeted approaches (MS/MS data not shown).
5. EXO-NET™ is a cost-effective tool for rapid isolation of highly pure exosomes for downstream analysis.

Reference: Stein EA, French PW. A novel molecular matrix for liquid biopsy biomarker capture utilises the Turing effect to produce enhanced biomarker capture. *Manuscript submitted.*