Extracellular-Vesicle Catch-and-Release isolation System using a Net-charge Invertible Curvature-sensing peptide

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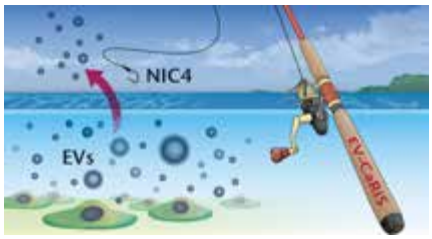
Abstract

Extracellular vesicles (EVs) carry various informative components, including signaling proteins, transcriptional regulators, lipids, and nucleic acids. EVs have shown great promise as pharmaceutical-targeting vesicles and have attracted the attention of researchers in the fields of biological and medical science because of their importance as diagnostic and prognostic markers. However, the isolation and purification of EVs from cell-cultured media remain challenging. Ultracentrifugation is the most widely used method, whereas it requires specialized and expensive equipment. A simple method in a short time using general experimental equipment found in ordinary laboratories is required for researchers to reproducibly isolate EVs.

We proposed a novel methodology to isolate EVs using a simple and convenient method, *i.e.*, an EV catch-and-release isolation system (EV-CaRiS) using a newly designed biosensor, net-charge invertible curvature-sensing peptide (NIC). Curvature-sensing peptides recognize vesicles by binding to lipid-packing defects on highly curved membranes, regardless of the expression levels of biomarkers. NIC has a net positive charge at weakly acidic pH to bind to negatively charged EVs through electrostatic interactions, whereas it has a net negative charge at weakly basic pH to dissociate from EVs by electrostatic repulsion. NIC allowed us to achieve reproducible EV isolation from three human cell lines and single-particle imaging of EVs containing the ubiquitous exosome markers CD63 and CD81 by total internal reflection fluorescence microscopy. Moreover, the EVs isolated by EV-CaRiS exhibited immune-stimulation-activities and anticancer effects.



Published paper
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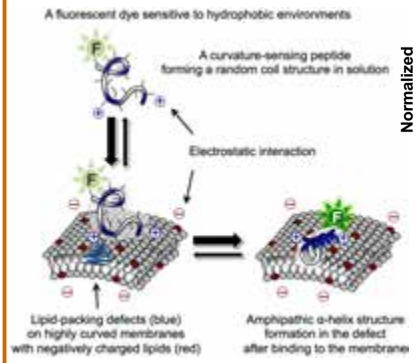
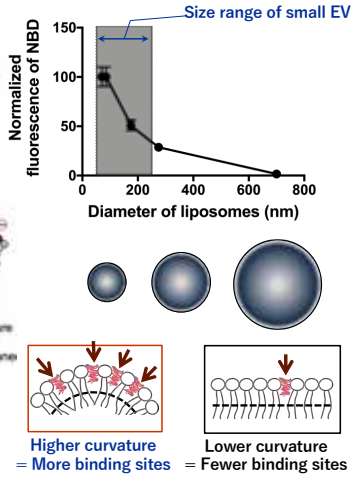
Background

Curvature-sensing peptide



Size-dependent binding manner



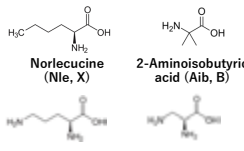
(1) Net-charge Invertible Curvature-sensing peptide (NIC)

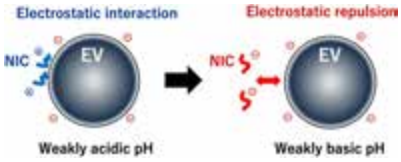
Peptide	Amino acid sequence	pH 6	pH 9
NIC4	EZBLLZXLNZBTEBLSZX-GSGSC-NH_2	+0.7	−2.0

Net charge

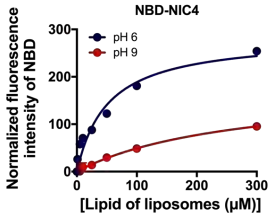
Weakly acidic Weakly basic

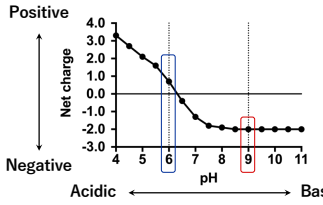
Unusual amino acids



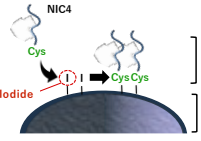


pH dependency of the net charge



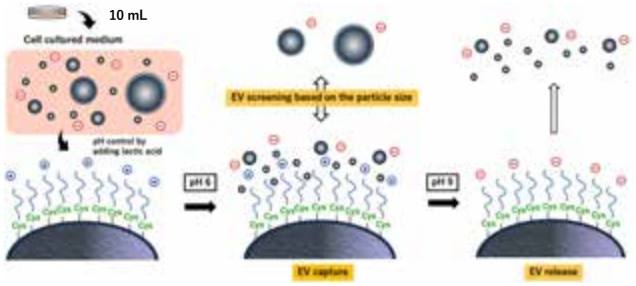


POPC : POPS : Cholesterol = 75 : 10 : 15 mol% (diamter : 80 nm)

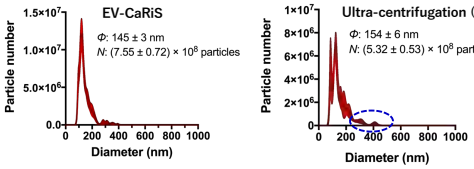


A schematic diagram of NIC4 coupling onto the SulfoLink™ resin

(2) EV Catch-and-Release isolation System (EV-CaRiS)

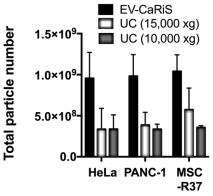


Nanoparticle tracking analysis (NTA)

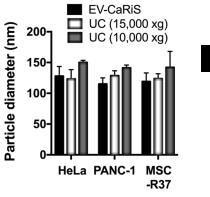


<Cell lines>
HeLa: human cervical cancer cell
PANC-1: human pancreatic carcinoma cell
MSC-R37: human bone marrow mesenchymal stem cell

The number of particles



The size of particles

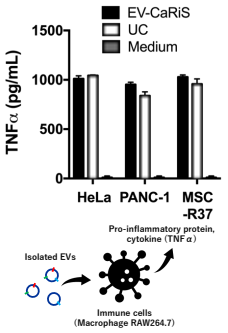


The time required

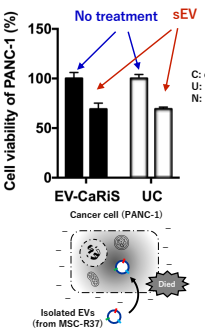
EV-CaRiS : 1.5 h

Conventional UC : 4–6 h

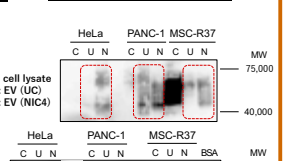
Immune activities



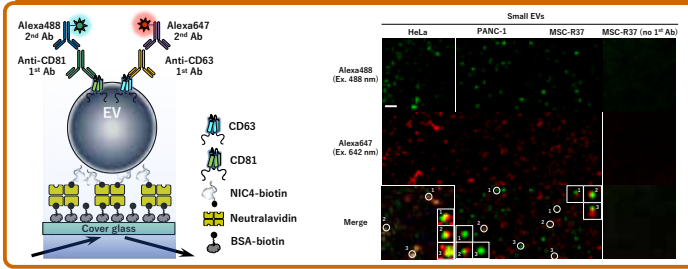
Anti-cancer activities



Western blotting



(3) Fluorescent Immunostaining (Single-particle Imaging)



Conclusion

EV-CaRiS does not require any specialized experimental equipment and can isolate sEVs with high purity from a small amount of cell-cultured media (10 mL) in a relatively short time (~1.5 h). EV-CaRiS was clearly demonstrated as a simple and convenient methodology for sEV isolation.