Exosomes characterization utilizing the immune system based on the interrupting currents by solid state nanopore

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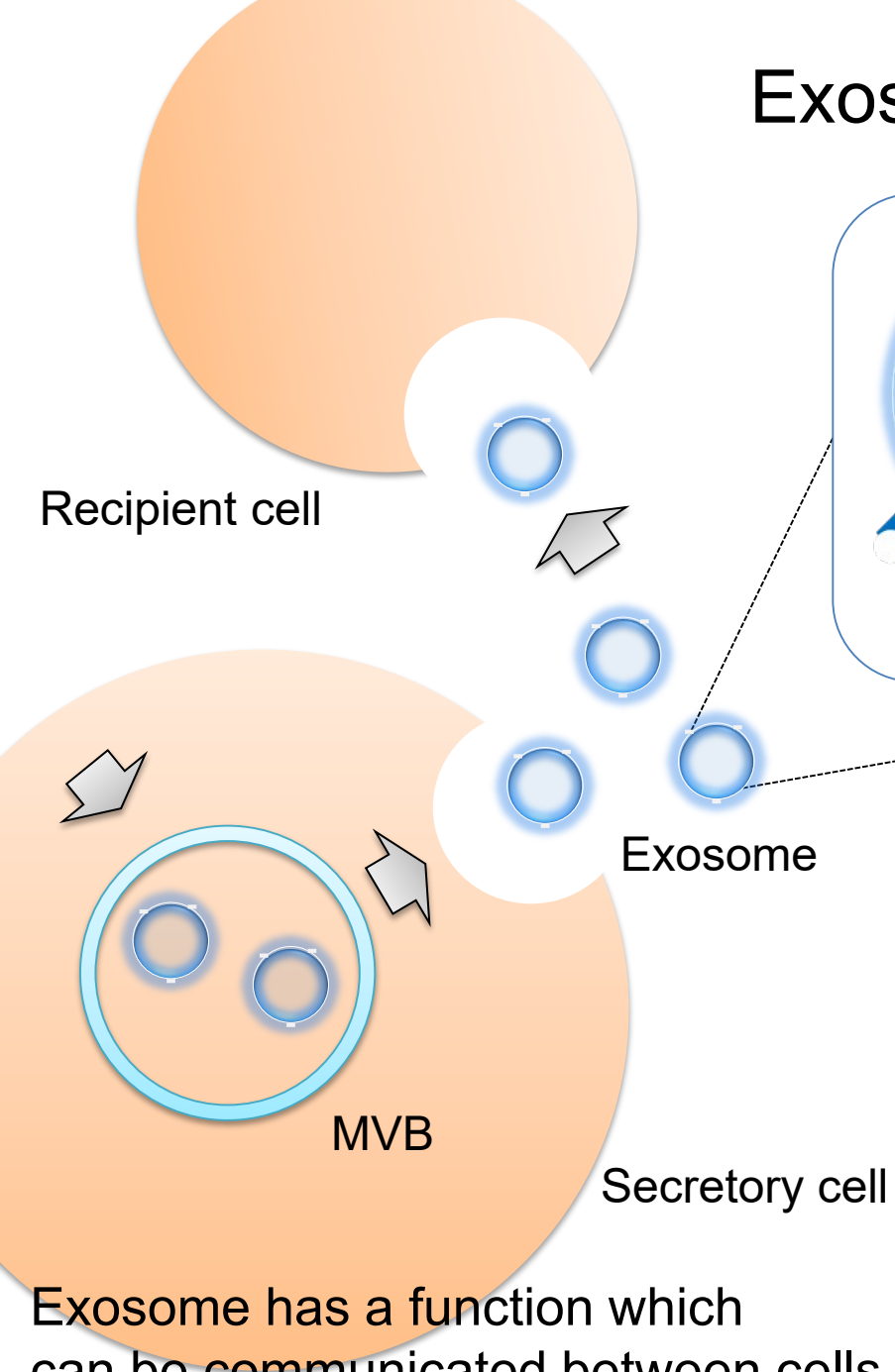
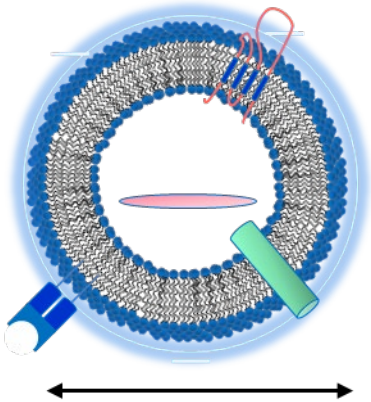
1, Tokyo University of Pharmacy and Lifesciences.

2, Bio Research Center, Co., Ltd.

3, Elements SRL.

Exosome





- Nano-size cell vesicle
- Delivery material of cell information.
- Can regulates the biological activity of recipient cells.

Exosome has a function which can be communicated between cells

Role of exosome derived from tumor cells

Overexpression of miRNA / protein of tumor cells

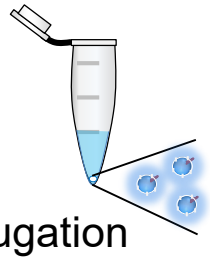
↓

Formation a cancer microenvironment / pre-metastatic niche for recipient cell.

Problems in detection of exosome from tumor cells

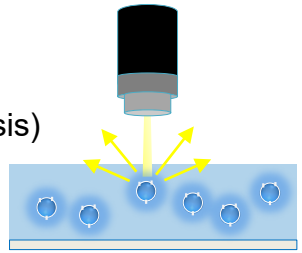
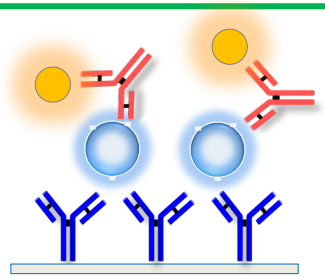
Exosome derived from tumor cells is less than 0.1% in blood

1, Purification

Method	Ultra-centrifugation 
Feature	Simple separation by ultracentrifugation
Problem	Collcet rate is less than 10%

2, Detection

*Separation from cultured cells

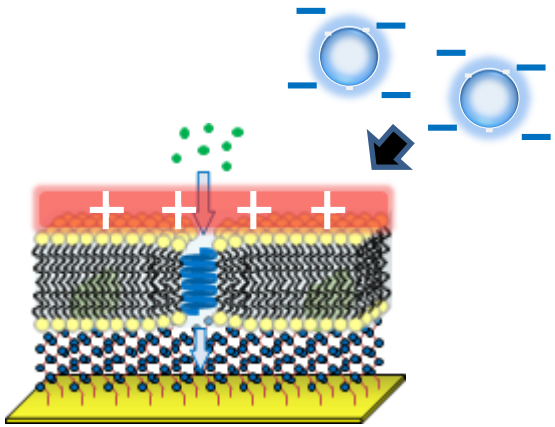
NTA (Nanoparticle Tracking Analysis) 	ELISA 
Light scattering by laser Non-label detection	Amplifying enzyme reaction and fluorescence
Low Sensitivity: 10^3~10^6/mL	Low Sensitivity: 10^3~10^6/mL

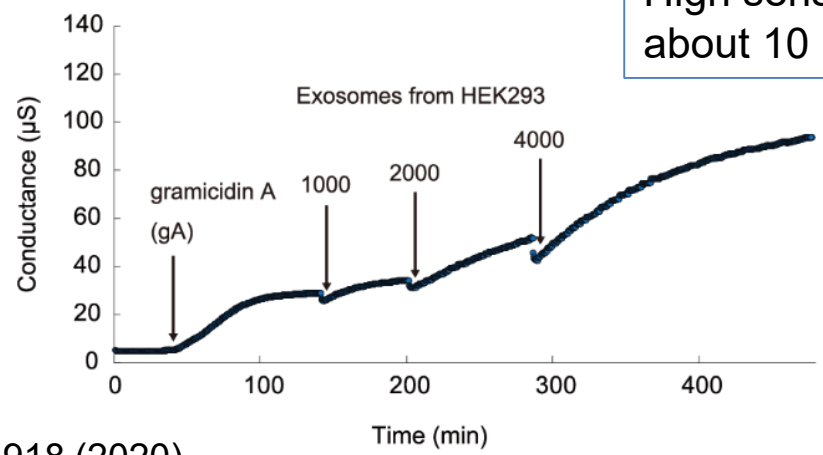
A separation / purification / assay are developing.

It needs a high sensitivity assay for intact exosome

Two approach for detecting an exosome by our study

1, Membrane fusion assay with an artificial cell membrane based on ion channel conductance.



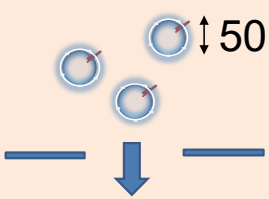


High sensitivity :
about 10 paricles/μL

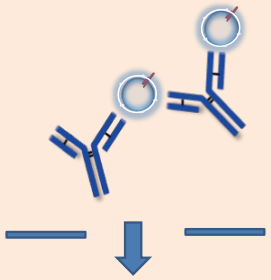
Nishio et al. *Biosens. Bioelectron.* **150**. 111918 (2020).

2, exosome detection by nanopore passage based on interrupting currents.

Exosome

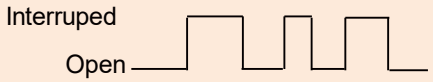


Pass through 300 nm nanopore



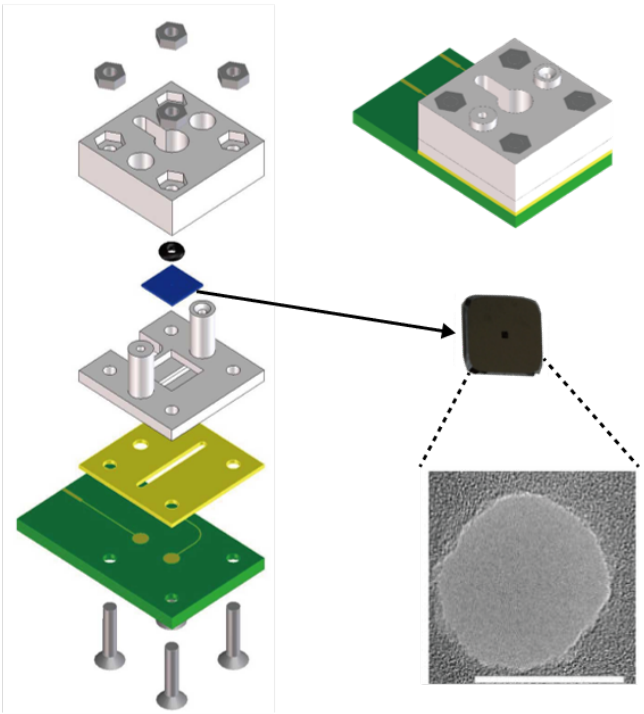
Antibody for targeting exosomal biomolecules

Record the interrupting currents



Nanopore Information

Nanopore Reader and SiN based nanopore chip from Elements SRL.



Feature of nanopore chip

- PCB (green part) with chlorinate electrodes
- 5 x 5 mm Nanopore chip secured with O-ring
- Low volume
  - 60 μL top solution
  - 30 μL bottom solution
- Rapidly change nanopore chip
- Load multiple flow cells for a series of experiments

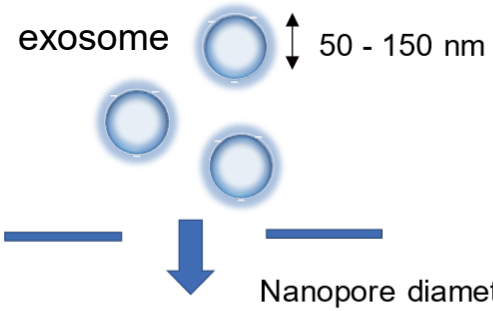
Experimental condition

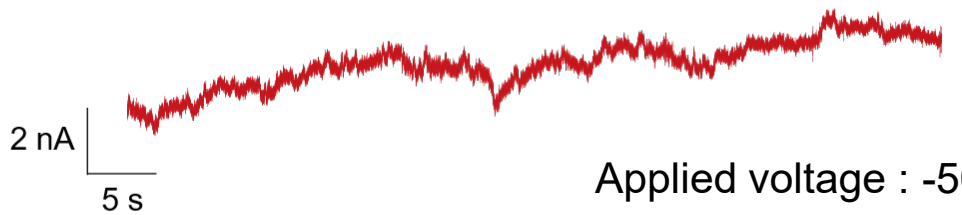
- Use nanopore with a diameter of 300 nm
- Applied voltage was -500 mV for passing through exosome
- All recording was carried out 100 kHz of sampling rate

elements
enabling technologies for Life Science

Interrupting currents by single exosome and antibody complex passages

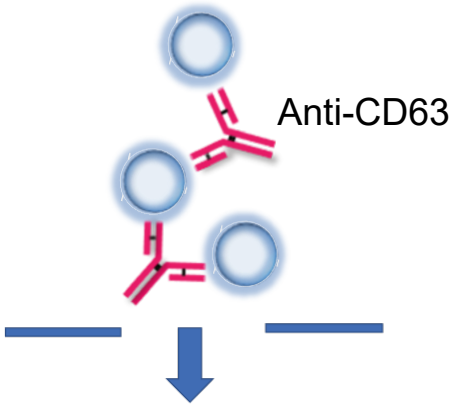
Only exosome derived from MCF-7 cells which is breast cancer

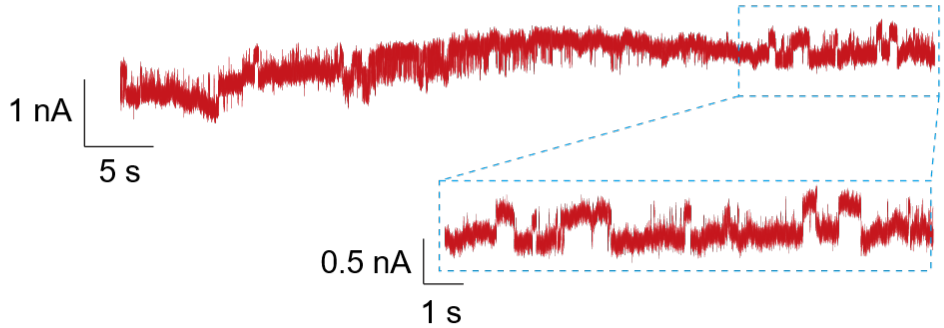




Applied voltage : -500mV

Formation of exosome - antibody complex

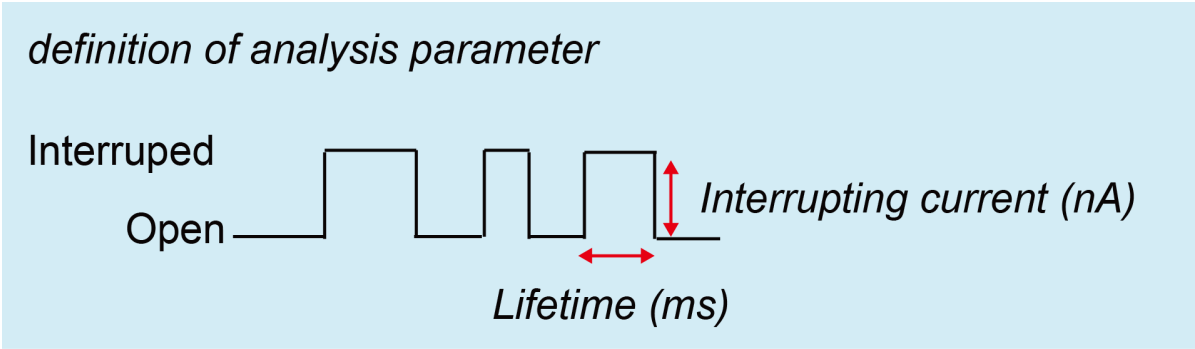




Applied voltage : -500mV

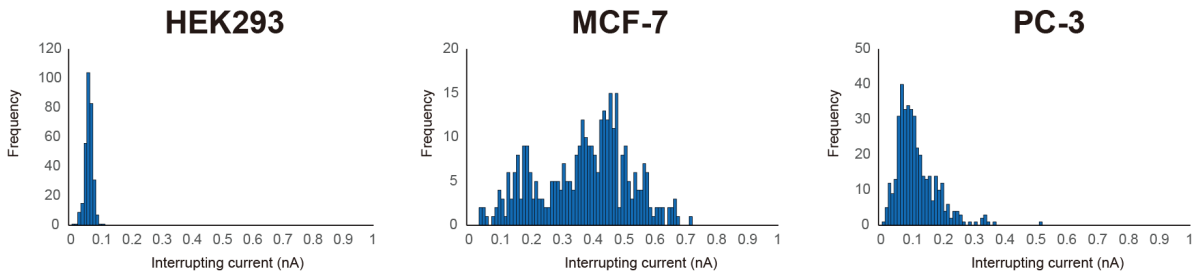
We aimed to observe the interfered current by formation of antibody complex

Interrupting currents by single exosome and antibody complex passages



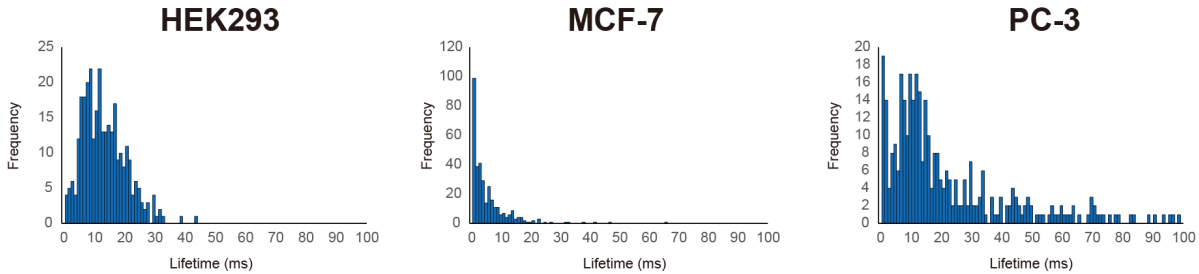
1,histgram analysis of Interrupting current amplitude

Interrupting currents (nA)



2,histgram analysis of Interrupting current time

Lifetime (ms)



*Interrupting currents were analyzed from 400 events by exosome passage.

Please come to our poster of **B587**!

Presentation data : 18th, Wednesday

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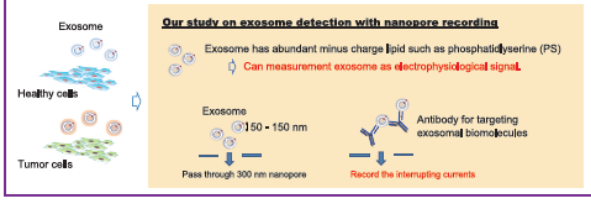
[1]Tokyo University of Pharmacy and LifeSciences., Department of Pharmacy.
[2]Bio Research Center, Co., Ltd. [3]Elements SRL.

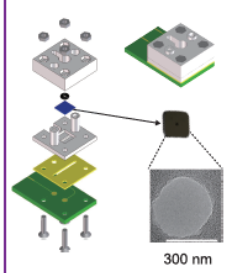
Introduction

Exosomes, which releases from all living cells, are nano-sized vesicles. They are well known to distribute between the cell communication because they circulate through a living organism as a carrier containing information derived from secretory and are incorporated into recipient cells. Exosomes derivative tumor cells are the main topic for medical therapy and diagnosis of cancer disease, they play an essential role for metastasis as a trigger because of containing its cell-specific intercellular protein and μRNA. Furthermore, cellular uptake of exosomes from tumor cells indicates that tumor cells are more accessible uptake into recipient cells depends on membrane protein and lipid composition than healthy cells. In addition, some researcher has reported that exosomes uptake changes preferentially by pH conditions and possessed extracellular matrix proteins. Therefore, the measurements of exosomes derived from tumor cells are expected for early checkup and diagnosis contribution. However, the fraction of exosomes derived from tumor cells in the blood exists at a very low level of several percent, so more sensitivity method needs to be established for exosomes discrimination. This study demonstrates by measuring the current as electrophysiological recording of exosomes passing thorough a nanopore, because exosomes are at the nano scale size and contains many negatively charged lipid such as phosphatidylserine (PS) in the membrane composition. It is possible to inducethe passage of exosomes through nanopores by applying a voltage, and detect their passage by observing the interrupting currents. Furthermore, for analyzing specific exosomes separation, we observed the interrupting currents after forming immune aggregates using antibody to react the antigen which is specific proteins.



Experiment

Nanopore Reader and SiN based nanopore chip from Elements SRL.



Feature of nanopore chip

- PCB (green part) with chlorinate electrodes
- 5 x 5 mm Nanopore chip secured with O-ring
- Low volume
  - 60 μL top solution
  - 30 μL bottom solution
- Rapidly change nanopore chip
- Load multiple flow cells for a series of experiments

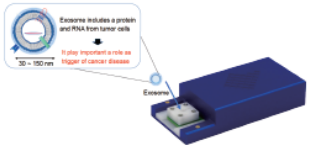
Experimental condition

- Use nanopore with a diameter of 300 nm
- Applied voltage was -500 mV for passing through exosome
- All recording was carried out 100 kHz of sampling rate

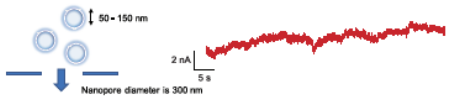
exosome signal was obtained as electrophysiological into nanopore passing through

Results

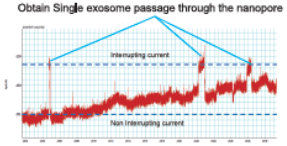
1. Typical trace of interrupting currents into nanopore passing through exosome and exosome - antibody complex



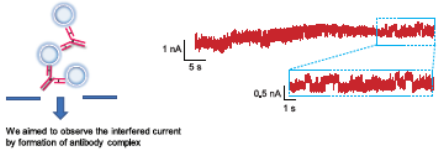
Only exosome derived from MCF-7 cells which is breast cancer



Obtain Single exosome passage through the nanopore

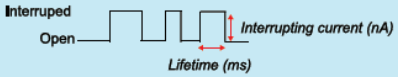


Formation of exosome - antibody complex

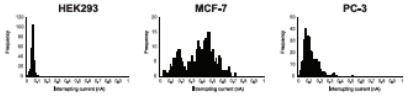


2. Interrupting currents analysis by exosome passage into nanopore

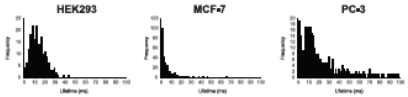
definition of analysis parameter



1.histgram analysis of Interrupting current amplitude



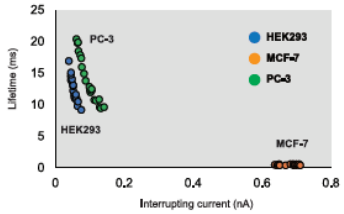
2.histgram analysis of Interrupting current time



*Interrupting currents were analyzed from 400 events by exosome passage.

3. Cluster analysis for exosome characterization

The cluster analysis was calculated from integrated current of single exosome passage event. Recorded events only were used the median value of 20.



Conclusion

The nanopore assay was helpful to detect valuable exosome. These results could analyzed by interrupting current and it is possiblity to identify exosome from normal and tumor cells. Because between normal and tumor cell were different of membrane composition and protein, a interrupting current parameter was changed. This method has an advantage of measurement by using non-label exosome and use exosome characterization.