News Release

Title

Spatial exosome analysis using cellulose nanofiber sheets reveals the location heterogeneity of extracellular vesicles

Key Points

- Development of an innovative tool to capture and preserve exosomes from micro-volume body fluids.
- Potential of cellulose nanofibers, a new material derived from wood, for cancer treatment applications.
- The new method of directly attaching to organs to collect exosomes for in vivo spatial analysis.
- New characteristics of exosomes are revealed, leading to the development of therapeutic strategies.

Summary

Extracellular vesicles (EVs), including exosomes, are present in all human body fluids and have attracted attention as an essential tool for cell-to-cell communication. Although various methods have been developed worldwide to recover EVs, recovering them from tiny samples has been considered extremely difficult. We have developed an EV sheet with a nanopore structure suitable for EV capture using cellulose nanofiber, a new material derived from wood, and established a method to recover and preserve EVs from extremely small amounts of body fluids by taking advantage of its water absorbency and pore-closing property upon drying. The EV sheet can collect EVs from as little as ten microliters of body fluid, and the collected EVs can be used to analyze the bioactive molecules contained in the EVs. EVs can also be recovered from tiny volume of body fluids on the surface of moist organs by directly attaching EV sheets. We have validated the utility of the EV sheet in ovarian cancer. This malignant tumor is difficult to treat and has revealed new properties of EVs that had not been shown before. The EV sheet enables spatial and functional analysis of EVs in vivo, which was impossible to analyze before and is expected to be used for future medical applications. The EV sheet also enable spatial and functional analysis of EVs in vivo, which has just been possible now and is expected to have further medical applications.

Research Background

Ovarian cancer is a poor prognosis that affects about 13,000 people annually in Japan, and nearly half of them die. Because early screening is arduous, most

cases are diagnosed at an advanced stage, and the 5-year survival rate is said to be less than 45%. Our research group has been studying the function of extracellular vesicles (EVs), including exosomes released by ovarian cancer cells, to improve the prognosis of patients with ovarian cancer. In recent years, EVs have become increasingly valuable in medical biology as an indispensable tool for cell-to-cell communication. The heterogeneity of cells constituting cancer tumor tissue has been well-recognized and studied recently. Still, the diversity of EVs in body fluid has not been well understood due to the difficulty of EV analysis. However, the importance of understanding this phenomenon is internationally recognized. There have also been great expectations for applying EVs as biomarkers to assist in the presence or absence of disease and in selecting future treatments.

The development of EV isolation methods from body fluids is still underway worldwide. The trends are due to various barriers, such as the lack of an optimal method for EV recovery, the difficulty in recovering EVs with a high degree of accuracy, and the fact that a certain amount of sample is required for recovery. In particular, it is almost impossible to analyze EVs from body fluids with a volume of less than 100 microliters, and the development of a new method has been desired.

Research Results

In this study, we first developed EV sheets, a novel exosome trapping tool, using cellulose nanofibers, a new material derived from wood. Cellulose nanofibers are a sustainable biomass material derived primarily from wood cell walls. They have attracted significant attention due to their attractive properties, such as being lightweight and high strength. We have developed a cellulose nanofiber, an ”EV sheet,” with a nanopore structure suitable for EV capture by applying paper-making and solvent displacement technology. By taking advantage of its water absorbency and pore-closing properties upon drying, we have established a method to collect and store efficiently EVs from tiny amounts of body fluids, as little as ten microliters By taking advantage of its water absorbency and the ability to close its pores upon drying, we have established a method for efficiently recovering and preserving EVs from tiny amounts of body fluid, as little as ten microliters (Figure 1).

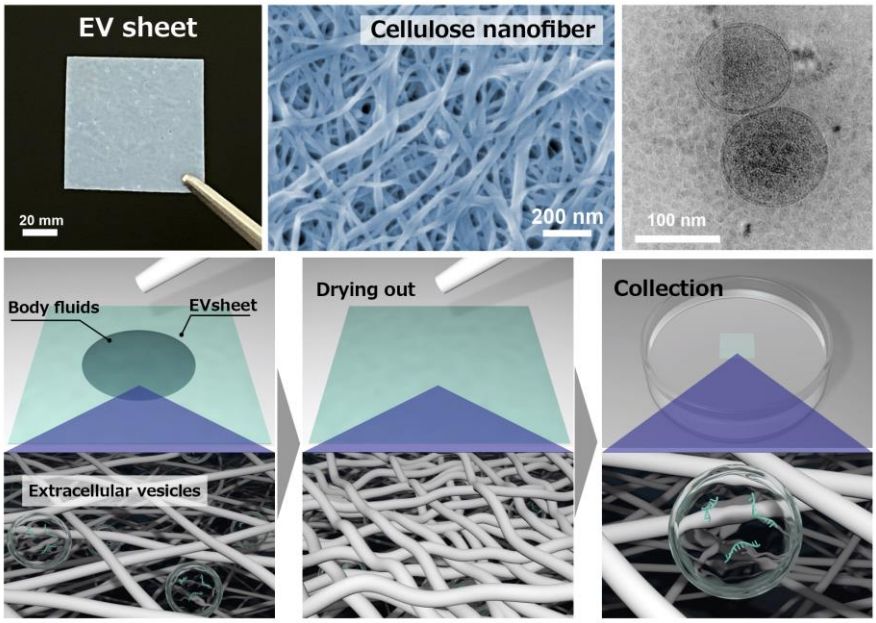

Fig.1 EV collection by EV sheets

Furthermore, by taking advantage of the EV Sheet's ability to collect EVs from minute amounts of body fluid, we have devised a method to collect EVs directly from the surface of moist organs (Fig. 2). When the EV sheet is attached to the organ for a few seconds, body fluid is absorbed into the EV sheet by capillary force, and the EVs in the fluid are selectively trapped in the nanopore structure of the EV sheet by subsequent drying treatment.

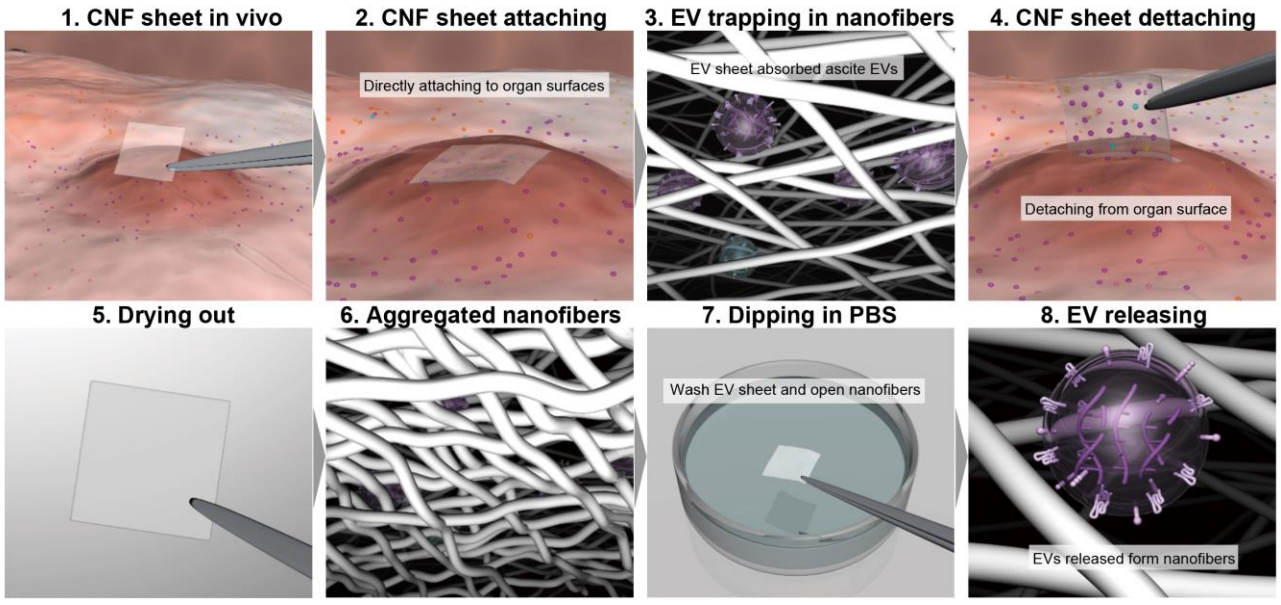

Fig.2 The EV sheet attachment method

The recovered EVs can be analyzed for bioactive molecules such as nucleic acids and proteins. In this study, RNA was extracted from the EVs recovered by

the EV sheet, and information on the microRNAs (miRNAs) contained in the EVs was analyzed using a next-generation sequencer. MicroRNAs are small RNAs about 22 nucleotides long. They are contained in cells and body fluids such as blood, saliva, and urine. Recent studies have revealed that the types and amounts of microRNAs vary in the blood of patients with cancer and other diseases, and they are expected to become an entirely new diagnostic marker. Ovarian cancer causes ascites fluid accumulation, and cancer cells and EVs circulate in the fluid, resulting in metastasis, etc. By deciphering the miRNA information carried by EVs in minute amounts of ascites fluid collected by EV sheets, it was found that EVs in ascites fluid, which have been analyzed centrally as ascites fluid, show changes based on the location of collection (Figure 3). (Fig. 3). Also, miRNAs different from those carried in the EVs of ascites fluid were present in the EVs on the tumor surface. Some of these miRNAs are decreased after tumor resection, indicating that these miRNAs may be new biomarkers contributing to diagnosis and therapeutic drug selection.

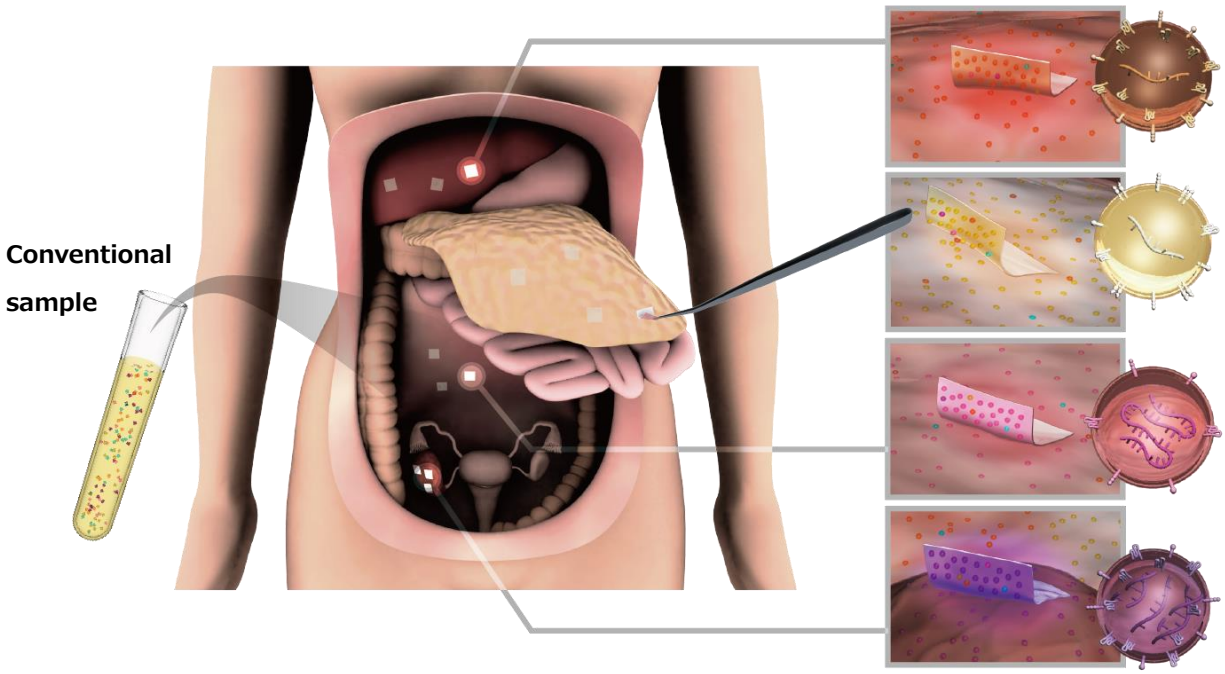

Fig.3 Spatial exosome analysis in patient body

Research Summary and Future Perspective

These results indicate that EV sheets can be used for spatial and functional analysis of EVs in vivo, which has not been possible before. EV sheets have various advantages. Recently, the practical application of cellulose nanofibers, a new material that originated in Japan and derived from wood, has been progressing, and EV sheets open up entirely new functions and applications for

cellulose nanofibers. In the future, we will promote medical applications of EV sheets for various diseases.

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