Biocompare

The Buyer's Guide for Life Scientists

The Emerging Field of Exosome Research

Techniques and New Discoveries

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Your Tools for Exosome and EV Research

MagCapture™
Exosome Isolation Kit PS

PS Capture™ Exosome ELISA Kit
(Anti Mouse IgG POD)

PS Capture™ Exosome ELISA Kit
(Streptavidin HRP)

PS Capture™ Exosome Flow
Cytometry Kit

Wako

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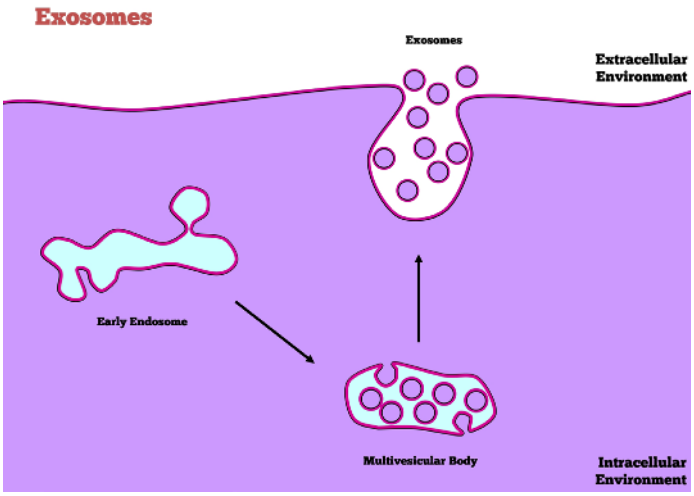
Exosomes: An Emerging Platform for Diagnostics and Therapeutics

Given these potentially high-value applications, researchers in fields like immunology, neuroscience, oncology, endocrinology, and cardiovascular research are looking at exosomes.

Research on extracellular vesicles (EVs) has been advancing rapidly. The number of scientific papers on EVs published in 2011 was approximately 200, which increased to more than 1,000 in 2016. During this time, several researchers have suggested the involvement of EVs in the cell's physiology and pathogenic mechanisms.

EVs are classified into at least two general categories: exosomes derived from endosomes, and microvesicles derived from plasma membrane. Separating these two classes of EV through standard differential centrifugation is difficult. In practice, EVs not settling at 10,000×g are called "small EVs," which are mainly composed of exosomes.[1]

Exosomes are small-membrane vesicles, approximately 30–100 nm in diameter, which are secreted by various cells and present in most body fluids and in many cell culture supernatants. Exosomes arise within intracellular vesicles, called "multi-vesicular endosomes," and are released into the extracellular space through the fusion of multi-vesicular endosomes with the cell membrane.



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Exosomes contain proteins from secretory cells, including those involved in intracellular transport, proteins originating from cell membranes, and RNAs. Exosomes also contain the cell membrane of secretory cells and lipids from the endosome membrane, for example, cholesterol and sphingomyelin.[2]

For years, scientists believed that exosomes were involved in the release of unimportant cell contents. However, exosomes are lately believed to mediate

cell-cell communication through the transportation of lipids, proteins, and RNAs. Exosomes have also attracted attention for possible clinical applications, including diagnostic biomarkers and possibly therapeutics.

Given these potentially high-value applications, exosome research now encompasses most biomedical research, including immunology, neuroscience, oncology, endocrinology, and cardiovascular research.

Exosome activity

For example, immune cell–derived exosomes contain antigen peptide/MHC complexes and various antigens, which suggests that exosomes might regulate activation or inactivation of immune cells and the exchange of antigenic information between such cells.[3] In the nervous system, exosomes are involved in regulating neural circuits[4] and in the extracellular release of proteins implicated in neurodegenerative diseases.[5]

Exosomes released by cancer cells contain biomolecules related to angiogenesis and immune evasion, suggesting that they might promote microenvironments optimal for cancer cell growth and progression.[6] Additionally, adhesion molecules on the surface of cancer cell exosomes may determine the destination of cancer metastasis.[7]

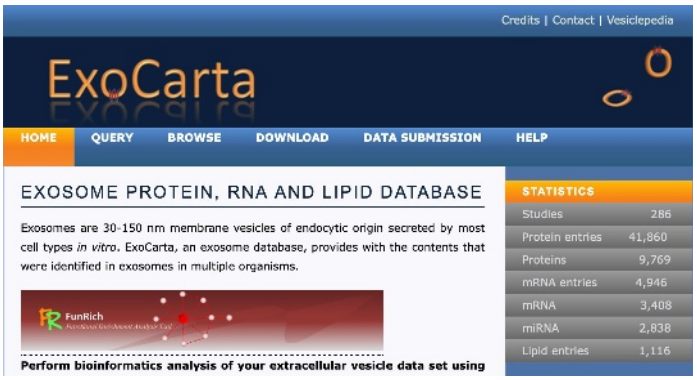
Recently, researchers found that exosomes released from adipocytes regulate hepatic gene expression.[8] Furthermore, since viruses leave cells through the same pathway as exosome production, bacteria and parasites infecting cells may regulate activities of pathogens infecting other cells via exosomes.[9,10]

Most exosome activity described herein is mediated by secretory cell-derived biomolecules contained in exosomes. Since secretory cell mRNAs and miRNAs have been identified in exosomes, the potential involvement of exosomes in horizontal transmission of gene expression between cells has attracted interest.[11] Since these RNAs are encapsulated within the lipid bilayer membrane, they are immune to RNase degradation and remain intact in blood or other body fluids long enough to be studied. When exosomes in target cells fuse with the endosome membrane, they release encapsulated RNAs into the cytosol of target cells, where they are translated into proteins while miRNAs suppress translation of target genes. Thus, exosomes regulate gene expression within target cells.

The fact that individual exosomes may carry several thousand mRNAs and miRNAs, tens of thousands of proteins, and a wide variety of lipids would alone justify the current interest in these vesicles. What makes exosomes relevant to modern biology is that the contents of exosomes reflects the biomolecular composition of the cells from which they originate. Most interestingly, exosome biomolecule composition reflects faithfully that which is found within the originator cell, suggesting a critical mechanism for loading exosome-specific biomolecules into exosomes from these parent entities.

These qualities make exosomes attractive as biomarkers and therapeutic targets. Furthermore, while exosome mRNAs incorporated into target cells induce expression of functional proteins, most exosomal miRNAs serve as precursors of functional miRNA through mechanisms that remain unclear but are the subject of intense investigation.

Hence the construction of ExoCarta, a curated database of exosome proteins, RNAs, and lipids. ExoCarta, which is currently undergoing classification by originating cell type, enables the current state of the art in exosome-based proteomics, transcriptomics, and systems biology. Research groups worldwide



employ FunRich, a non-commercial software tool, to identify biomolecules that are over-represented in exosomes compared with their levels within originating cells.

In the next article, we will describe the development of exosome-based therapeutics and diagnostics. As with many nascent, developing areas in the life sciences, the information yet to be discovered far outweighs our knowledge. As more research groups undertake exosome investigations, and with further elucidation of ExoCarta and related data-mining tools, we expect exosomes to become a rich source of information on how cells and larger systems operate. With that understanding, one can expect practical platforms to emerge for diagnosing and treating human disease.

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Exosome Assay Development

Methods for studying and characterizing exosomes are advancing rapidly.

At one time scientists believed that exosomes did little more than shuttle insignificant products of cellular metabolism from their locations of origin to other cellular compartments. Today, we know that exosomes mediate intercellular communication through the transportation of lipids, proteins, and RNAs. Their small size and unique lipid bilayer construction allows exosomes to interact with a wide variety of cells,[1] whose pathophysiology and protein expression they alter through the transfer of exosomal chemical messengers.

For these reasons, and also since exosomes originate from a wide variety of cells including tumor cells and immune system cells, they have been targeted for possible applications as diagnostics and therapeutics.[2]

Methods for studying and characterizing exosomes are under development, but many issues must be resolved, for example, exosome purification. Two standard techniques, ultracentrifugation and polymer precipitation, both of which are available as commercial methods, yield exosome preparations containing significant quantities of contaminants that interfere with subsequent experiments. Antibody-based affinity methods, while generating highly purified exosomes, do not yield intact exosomes, complicating the study of the vesicles' original physiological functions.

Similarly, western blotting and ELISA, which are also widely used for exosome detection, require relatively large quantities of exosomes so are unsuitable for studying molecules with low expression levels.

To overcome these deficiencies, FujiFilm scientists have developed a method for exosome purification employing the Tim4 protein, which binds to the phosphatidylserine (PS) on the surface of exosomes. Because the binding is Ca^{2+}-dependent, intact extracellular vesicles release from Tim4 by the simple addition of calcium chelating agents. The PS-Affinity Method yields exosomes of higher purity than those obtained through conventional purification methods. ELISA analysis based on PS-Affinity Method is more sensitive than both western blot and ELISA kits based on antibody affinity method.

PS-Affinity Method

The PS-Affinity Method begins with typical samples, for example, cell culture supernatant, serum or urine, and the MagCapture™ Exosome Isolation Kit PS from FujiFilm. The kit consists of biotinylated Tim4, streptavidin-immobilized magnetic beads, exosome capture immobilizing buffer, exosome binding enhancer, washing buffer, elution buffer, and reaction tubes. When the kit reagents combine and are mixed with sample, the Tim4 moiety binds

strongly to phosphatidylserine on the vesicle membrane. After collecting the bead-Tim4-exosome complexes, the exosomes are eluted by the addition of the Ca^{2+} chelator EDTA at neutral pH.

Among existing exosome purification methods, MagCapture Exosome Isolation Kit PS is the only technique that provides intact vesicles of high purity and high yield with easy, stable operability. Purification typically takes 3.5 hours.

FujiFilm scientists and academic collaborators investigated the performance of the MagCapture Exosome kit on detecting exosomes from the supernatant of a COLO201 cell culture.[3] Compared with standard western blotting, PS Capture™ Exosome ELISA Kit was between 50 and 1,000 times more sensitive. The kit shows excellent dilution linearity in handling vesicles derived from serum and plasma, which indicates the technique is suitable for quantifying exosomes from those sources. Figure 1 illustrates the method's linearity under dilution.

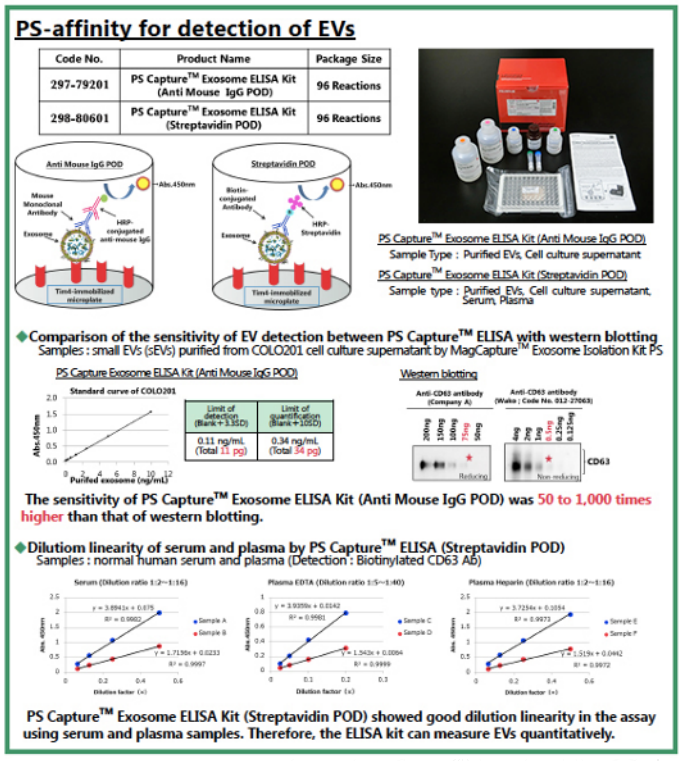

Figure 1. The FujiFilm affinity-based ELISA method

However, exosomes isolated by ultracentrifugation contain impurities like cell debris and protein aggregates.

FujiFilm has introduced a companion product, the PS Capture Exosome Flow Cytometry Kit, which uses Tim4-immobilized magnetic beads binding to PS. It also employs an antibody against an exosome surface marker protein, plus a fluorescence-labeled secondary antibody. The advantage to this approach lies in its ability to characterize exosomes without the purification step, directly from samples of interest.

The performance of PS-affinity on the recovery and purity of isolated exosomes from a K562 culture has also been studied.[4] Investigators collected exosomes from serum-free cell culture supernatant MagCapture, ultracentrifugation, and polymer-based precipitation. Recovered materials were analyzed by silver staining and western blots using anti-CD63, anti-Flotillin-2, and anti-Lamp-1 antibodies.

Products from each trial were further analyzed by mass spectrometry and compared with the percentage of human-derived peptides from K562 cells.

Under these conditions, MagCapture detected three times as many human-derived peptides as ultracentrifugation and seven times as many as polymer-based precipitation. Furthermore, the percentage of proteins originating from the

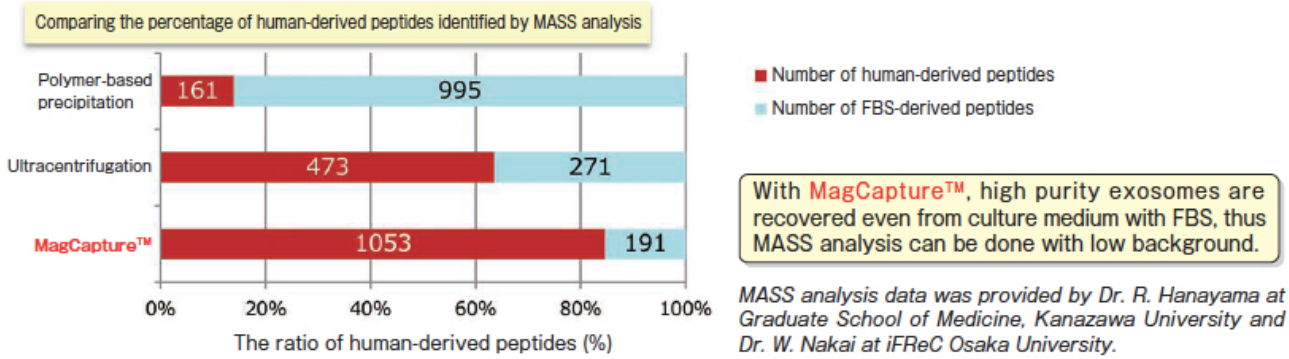

Figure 2. Comparing the percentage of human-derived peptides identified by MASS analysis

medium or additives was reduced from 86% for the preciptation method and 34% for centrifugation to 15% for MagCapture. Figure 2 shows these results.

Conclusion

Given the potential for exosomes, both as high-value potential diagnostics and as therapy-delivery systems, researchers will require ever more sensitive, discriminating, and flexible analytical tools. Among existing methods, the PS-Affinity method provides the most attractive combination of sensitivity, discrimination, quantitation/characterization, and user-friendliness. We look forward to seeing this technique expanded and broadened, to incorporate the analysis of very low-concentration species and non-traditional samples.

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Studying Exosomes in Practice

Due to their role in gene expression, neural regulation, immune control, and more, exosomes have become a major research focus.

Exosomes are a form of extracellular vesicle (EV) that originate from a specific endosome inside the cell called a "multi-vesicular body" (MVB). Given that recent research has shown that these vesicles are involved in processes such as intercellular gene expression, viral transmission, neural regulation, and immune system control, to name a few, they've become a hot area of study. Despite the increased interest, there is still a lot to be learned. In order to expand research possibilities, many companies are developing new products and techniques to isolate and identify exosomes.

One example of this is a kit from FujiFilm called the MagCapture™ Exosome Isolation Kit PS. Since the presence of phosphatidylserine (PS) is a marker of EVs, this kit uses beads with Tim4—the receptor for PS—to isolate EVs. To release the EVs from the beads, all that's needed is to add EDTA for removing Ca^{2+}. This allows for isolation of EVs without damaging the vesicular membrane. This article will discuss two examples of studies being done on exosomes using this new technology.

Glycome analysis of exosomes

The first study, published in *Scientific Reports* on March 2018 by Saito et al., focused on the exosomal glycome—the array of sugars on the plasma membrane. Previously, the team had shown that the glycome of human induced pluripotent stem cells (hiPSCs) is distinct from the glycome of non-hiPSCs. In the present study, the researchers were interested in determining whether there is a difference between the glycome of EVs originating from hiPSCs and the glycome of EVs originating from non-hiPSCs.

Using the MagCapture kit, the scientists were able to specifically isolate EVs from the cell culture media of different cell lines. To characterize the glycome, they used both flow cytometry and a technique called "lectin microarray," in which the cell media is poured over an array of lectins, or proteins that bind specifically to certain sugars. According to their analyses, the glycomes of the EVs looked like the glycome of the cell they originated from, whether it was a hiPSC or a non-hiPSC.

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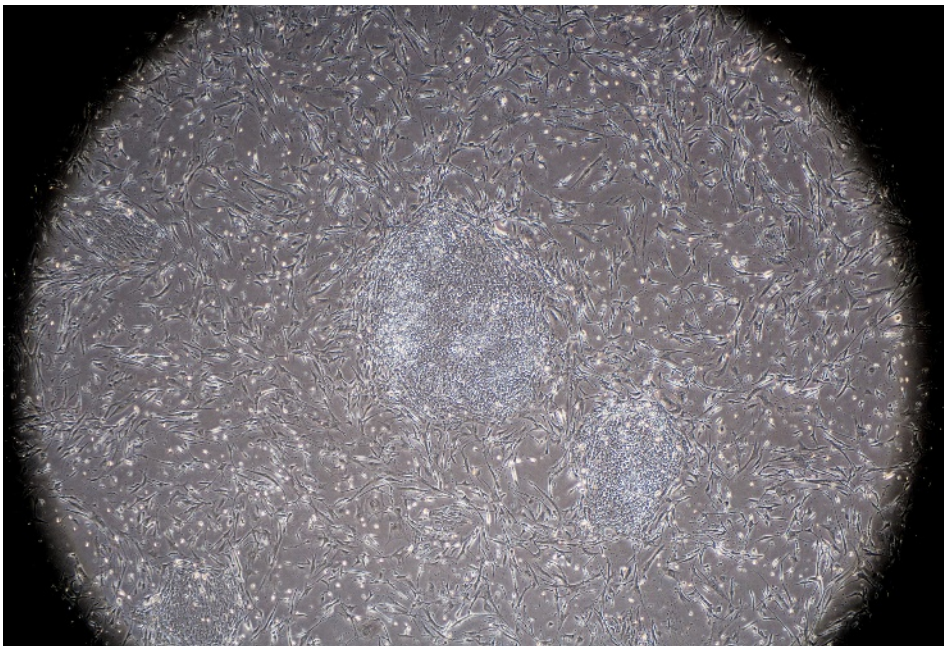
*1 Thickness of layers excluding the base. 20 microns thick means nearly 20 layers included.
*2 YFujifilm's iPS cells are being utilized in the world's first clinical trial using iPS cells conducted in the UK by the Australian company Cynata.



One small and one large colony of human induced pluripotent stem cells (iPSCs) in culture on top of mouse feeder cells. This view is as it appears through an inverted biological phase contrast microscope with 200x magnification. © Jan Bruder, Dreamstime.com

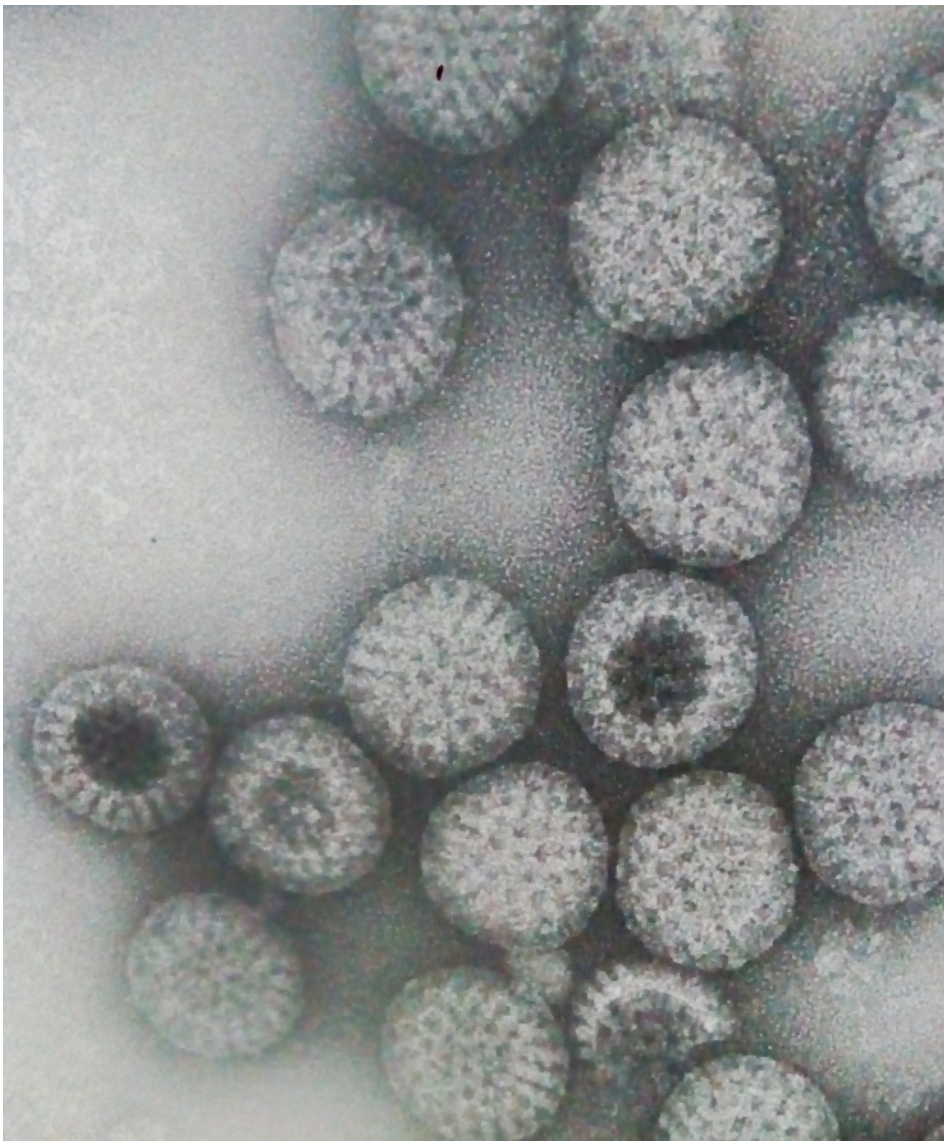
The group also created an innovative sandwich assay to isolate a subgroup of EVs—hiPSC-derived EVs. Previously, the team had shown that a particular lectin called rBC2LCN binds specifically to carbohydrates that are on hiPSC membranes and not on non-hiPSC membranes. As the glycome of the EV membranes reflects the glycome of the cells they originated from, the scientists decided to use rBC2LCN in combination with Tim4 to specifically isolate EVs that originated from hiPSCs. They applied their assay to detect hiPSC-derived EVs during endodermal differentiation.

Being able to specifically target and isolate EVs and subgroups of EVs is important for future clinical developments. According to the authors, since rBC2LCN is a lectin specific to sugars on the hiPSC membrane, it has already been applied to detect and eliminate unwanted stem cells residing in cell therapy products that come from hiPSCs. Such an application is important because stem cells residing in regenerative therapies can increase the probability of tumor development. Using rBC2LCN (or other lectins specific to other subgroups of EVs) in combination with Tim4 to isolate or target certain EVs could be useful for diagnostic and therapeutic purposes.

The role of exosomes in viral transmission

Exosomes also contain proteins, mRNAs, and miRNAs that could all affect gene expression in faraway cells, meaning that they are potentially a therapeutic target for genetic and gene expression disorders. But it's not just endogenous genetic material that can get transferred through exosomes—a study published in August 2018 in *Cell Host & Microbe* by Santiana et al. discusses how EVs are also involved in shuttling viruses between cells and even between organisms.

The accepted view of viral transmission has historically been that standalone viral particles are the optimal unit of infection. Therefore, as the authors of this second study set out to research how rotaviruses exit cells, they assumed that they did so as single units. However, after finding that rotavirus could exit the cell without membrane lysis, they investigated whether it could be released within EVs. Using MagCapture and similar methods, they isolated EVs and found rotavirus particles within them, which they verified using electron microscopy.



Rotavirus particles photographed under transmission electron microscope. © Edgloris Marys, Dreamtime.com.

To determine whether these EVs were exosomes originating from MVBs or microvesicles originating from the plasma membrane, the team used antibodies against CD63—a biomarker of exosomes—and found that it was not present, indicating that the rotavirus EVs were unlikely to be exosomes. However, vesicles containing a second virus that was tested in this study, the norovirus, could be isolated using antibodies against CD63 and two other exosomal markers—CD9 and CD81—indicating that they likely are exosomes. But no matter the origin, all the viral-carrying EVs were found to remain intact within the intestines of hosts and to be passed on to new organisms via fecal–oral transmission.

Overall, the study found that in the case of rotavirus and norovirus, free viral particles are not the optimum infectious agent. In fact, these viruses hijack natural cellular processes to escape in clusters within EVs. Cloaked by vesicles, these viruses are protected from breakdown and arrive at a new host cell in large enough numbers to initiate expression of the viral genome.

Whether it be to analyze the glycome or describe viral transmission or something else entirely, scientists all over the world are delving into the realm of exosomes. And techniques like MagCapture and antibody detection of biomarkers continue to be developed to make deeper research possible. As the knowledge gap in this area shrinks, the potential for new innovation in the treatment of many diseases continues to grow.

Solutions for Isolation, Detection, and Analysis of Extracellular Vesicles

FujiFilm Wako has created a number of tools for extracellular vesicle research.

FUJIFILM Wako Solutions for Isolation, Detection, and Analysis of Extracellular Vesicles

On the basis of PS Affinity, PhosphatidylSerine (PS) affinity-based method, FUJIFILM Wako has created effective tools for extracellular vesicles research and it delivers High-Quality Purification, High-Sensitive Detection, and High Reproducibility.

- **MagCapture™ Exosome Isolation Kit PS** can easily isolate intact and high purity exosomes and other extracellular vesicles from cell culture medium and body fluids at high yield without ultracentrifugation
- **PS Capture™ Exosome ELISA Kit (Streptavidin HRP)** can easily detect surface marker proteins of extracellular vesicles with 50 to 1,000 times higher sensitivity than Western blot
- **PS Capture™ Exosome Flow Cytometry Kit** can detect surface marker proteins at a high sensitivity by flow cytometry and realize direct qualitative analysis of surface marker proteins without purification of extracellular vesicles
- **EV-Save™ Extracellular Vesicle Blocking Reagent** can strongly suppresses adsorption of extracellular vesicles to laboratory tools and save valuable extracellular vesicles from loss
- **Exosomal Antibodies**: High quality antibodies are developed by DNA immunization. These antibodies introduce high sensitivity and high specificity.

Resources

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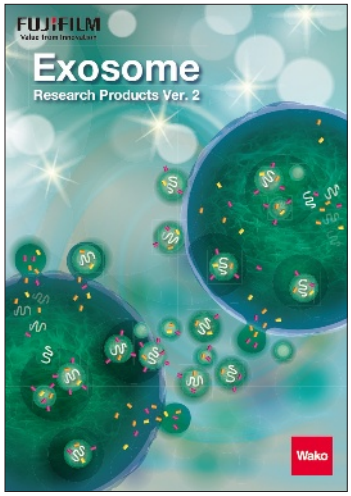
Exosome Research

Research on extracellular vesicles (EVs) has been advancing at an accelerating pace. The number of scientific articles on EVs published in 2011 was about 200, the number increased to more than 1,000 in 2016. Although exosomes had long been considered to be involved in the release of unnecessary cell contents, exosomes are increasingly being studied as mediators of cell-cell communication involved in transporting biomolecules such as lipids, proteins, and RNAs in vivo.

→United States

→Europe

→Asia



Exosome Research Products

Conventional methods for exosome purification involve ultracentrifugation and various commercial purification kits that use polyethylene glycol precipitation. Unfortunately these methods produce large amounts of contaminants and careful analysis is required to determine whether experimental results obtained are actually due to the actions of exosome constituents. Clearly, a technology that facilitates easy purification of exosomes at a high purity is needed. This resource reviews one such option: Tim4-immobilized magnetic beads, which were developed in collaboration with Professor Rikinari Hanayama of Kanazawa University Graduate School of Medical Sciences.

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