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Review Paper

Nanovesicles: Diagnostic and Therapeutic Tools in Nanoscale Medicine

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Abstract The use of nanovesicles (NVs) has contributed to nanotechnology in the development of new concept medicine to compete with diseases of deleterious and infectious to human health. Due to their properties of size, morphology, and biocompatibility NVs have great impact on public health especially in the development of new therapeutic and prophylaxis approaches in addition to the device for biosensors to diagnose human diseases. Recent data also strongly suggest that NVs are regarded as innovative materials in developing for vaccines and diagnostic tools. In this review, we focus on the basic concepts and recent applications of NVs to utilize or engineer them as therapeutic materials.

Keywords: Nanovesicles, Diagnosis, Therapy, Vaccine, Cancer, Drug delivery

I. Introduction

Nanovesicles (NVs) are one of the biomaterials used in nanotechnology field, in which the design and production of structures, devices, and systems in terms of atoms and molecules at the nanoscale level. These nanoparticles are produced from most of living cells including bacteria, archaea, and eukaryotes by secreting out nanoscale objects through their outmost envelope [1,2]. These nanoparticles have been developed for diverse applications in medicine, including deleterious and infectious diseases with the action against specific targets. In addition, these materials are currently used for biosensors in both accurate and cost-effective manner. The biogenesis of such vesicles have been well studied although it still needs to be fully investigated. The cell-derived particles are morphologically spherical and bilayered structure, and ranged from 20 to 500 nm in size [2,3]. The size variation might be due to the various growth conditions [4]. The components of NVs are almost all of cellular materials including lipids, proteins, carbohydrates, nucleic acids, metabolites, virulence factors, or compounds involved in the cell-cell communications [1-3,5-8]. Recently, the development of artificial NVs with adjusted biocompatibility and carrier property are used in modulating the specific biological activity such as horizontal gene transfer, pathogenesis, resistance to antibiotics, immune response, and intra- and interspecies communications [3,9]. More interestingly, the use of NVs as vaccines and drug delivery systems with artificial ways also have been increased and therefore, factors treating hard-to-treat diseases could be intentionally loaded onto NVs as designed fashion [4]. The aim of this review is to discuss the basics and advances made on the studies of NVs for the potential use of them in nanoscale medicine.

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II. Classification, composition, and functions of NVs

NVs are generated through plasma membrane of the host. Therefore, their membrane definitely consists of the same elements with mother cell. The composition of membrane and species are described in Table 1.

NVs are grouped into several types based on the nature of their biogenesis. Generally, there are three types based on their intracellular origins: apoptotic bodies, microvesicles and exosomes (Table 2). Apoptotic bodies (1~5,000 nm) are released by cells undergoing programmed cell death and enclose DNA, RNA, especially rRNA[10], oncogenes [11], T-cell epitopes [12], B-cell autoantigens [13], and histone proteins [14,15]. During apoptosis, apoptotic bodies transfer their contents to macrophages, which leads to phagocytosis. Microvesicles (100~1,000 nm), referred also to ectosomes, plasma membrane-derived vesicles, micro-particles or shedding vesicles, are generated by budding and fission of the plasma membrane near the outmost cell envelope. For that reason, they have similar composition to the plasma membrane of the mother cell. In contrast to apoptotic bodies, microvesicles have found not to contain considerable amount of any RNA [10]. One fascinating feature of microvesicles is the easily detectable size as a cancer biomarker [16]. Additionally, microvesicles have been suggested as disease-modulating molecules in

Table 1. Composition of the membrane of host cells.

Host	Size (nm in diameter)	Composition of the membrane	Examples on study	Reference
Gram-negative bacteria	10-300	Outer membrane(OM), lipopolysaccharide, porin, peptidoglycan(thin), lipoprotein, inner membrane(IM)	*E.coli*, *A. baumannii*, *M. xanthus*, *H. pylori*, *F. philomiragis*, *F. tularensis* supsp., *P. aeruginosa*, *K. pneumonia*, *N. meningitidis*, *C. jejuni*, *P. syringae*, *S. enterica* serovar *Typhimurium*	[1,2,9]
Gram-positive bacteria	20–250	Peptidoglycan(thick), lipoteichoic acid, teichoic acid, lipoprotein, cell membrane	*S. aureus*, *Bacillus spp*., *S. coelicolor*,. *perfringens*, *L. monocytogenes*, *T. thermosulfurgenes*	[2,21,22]
Archaea	90–230	A single cytoplasmic membrane, which is usually enclosed by a protein crystal structure known as S-layer	Sulfolobus spp.	[2,23,24]
Mycobacteria	50-300	Glycolipids, porin, lipoarabinomannan, mycolic acids, arabinogalacan, peptidoglycan, cell membrane, lipoprotein	M. tuberculosis, M. bovis	[2]
Fungi	Not determined	Mannoproteins, β-glucan, chitin, cell membrane, lipoprotein	C. neoformans, S. cerevisiae	[2]

Table 2. Classification of NVs by the biogenesis.

Nomenclature	Size (nm in diameter)	Biogenesis	Properties	Functions	Reference
Apoptotic bodies	1,000-5,000	Consequence of the apoptosis	- Releasing from dying cells - Containing rRNA	- Horizontal transfer of genes and proteins - Phosphatidylserine externalization	[10,14,15, 25,26]
Microvesicles	100-1,000	Outward budding/ blebbing/fission from the plasma membrane	- Similar composition to the plasma membrane of the mother cell - Products of platelets, red blood cells and endothelial cells - Readily detectable size to be a biomarker of cancers - Do not contain considerable amounts of RNA	- Procoagulant activity - Contribution to the pathogenesis of rheumatoid arthritis and pro-invasive character of tumors - Fetomaternal communication	[10,14-16, 25,26]
Exosomes	50 -100	Releasing of the late multi-vesicular-endosomes as a result of the inward budding of the endosomal vesicle though the plasma membrane	- Containing various kinds of proteins, lipids, and nucleic acids	- Modulation of immune responses - Horizontal transfer of nucleic acids, such as mRNA and miRNA, receptors, virulence factors - Usage as drug carriers	[10,14,15, 19,25,26]

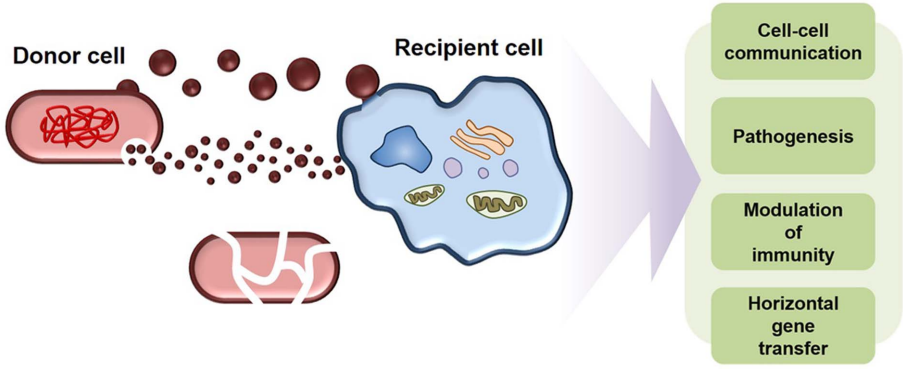

Figure 1. Schematic representation of known roles of NVs. NVs (1-5,000 nm) (circles not in scale) are released from donor cells and they are affecting to func-tions of recipient cells in four modes of action: 1) cell-cell communication, 2) patho-genesis, 3) modulation of immunity, and 4) horizontal gene transfer.

that they show a pro-coagulant activity, contribute to the pathogenesis of rheumatoid arthritis, and are associated in the pro-invasive character of tumors and fetomaternal communication.

Exosomes (50-100 nm) are one of the extensively studied NVs after being introduced in 1970 by Rose Johnstone and her colleagues [17]. They are originated from endosome and released by exocytosis of the late multi-vesicular-endosome (MVEs) as a consequences of the fusion with the plasma membrane. These smallest particles among NVs are composed of proteins, such as heat shock proteins (Hsp), major histocompatibility complex (MHC)-II, membrane transport and fusion proteins (GTPases. Annexins and flotillin), and tetraspanins. They also contain lipids, such as sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and GM3, and nucleic acids, such as mRNA, miRNA, non-coding RNA [15,18]. For the function of exosomes, they are involved in modulation of immune responses and horizontal transfer of nucleic acids, such as mRNA and miRNA, receptors, virulence factors [14,15,19] (Figure 1). Interestingly, they are secreted by all types of cells and could be found in most body fluids, including blood, saliva, and urine, widely used in diagnosis of diseases [15]. Now, exosomes are regarded as therapeutic drug carriers and delivery vehicles across biological membranes.

NVs adhere to, directly fuse with the plasma membrane or be endocytosed into the recipient cells [20]. The understanding of how NVs are generated and work will have a great impact in the field of the development of biotechnological applications.

III. Applications of NVs

Many applications of NVs have been suggested and studied. Among them vaccine development, drug delivery, biomarker and diagnostic applications (Figure 2) which we think that widely used for human healthcare in the future will be discussed in detail.

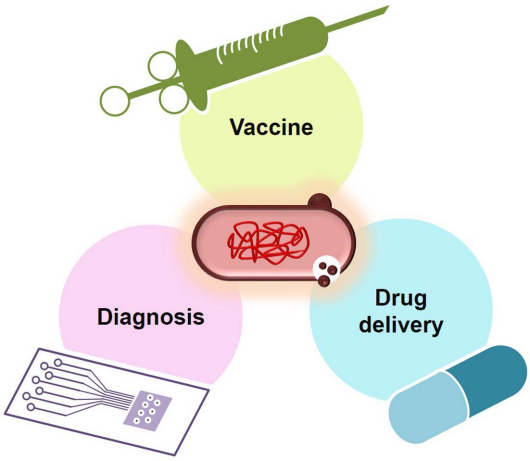

Figure 2. Three major applications of NVs. Naturally prepared or artificially made NVs could be utilized in the development of vaccine, drug delivery, and diagnosis. Details are described in the main text.

1. Vaccine development

Immunotherapy is to utilize immune responses to defeat harmful cells or to protect healthy tissues from immune destruction. Traditionally, live-attenuated vaccines were widely used due to its similarity to a natural infection and elicit strong cellular and antibody responses and confer lifelong immunity with only single or double doses. Due to such vaccines use diseases-causing microbes, scientists produce inactivated vaccines by killing such microbes with chemicals, heat, or radiation, resulting the more stable and safer than live vaccines. Moreover, acellular vaccines such as inactivated toxins or proteins have been developed. However, the weaker immune response of such vaccines strongly requires the use of adjuvants in vaccine preparation. Although many innovative platforms of vaccines have been widely developed still researchers look for new effective vaccine platforms. For instance, combinatorial vaccines with known adjuvants to be safer and more effective treatments for specific cancers, viruses, infectious diseases are significant challenges for protecting public health from potential pandemics or ongoing ones [27].

Especially, the establishment of immunity requires interaction between T-cells and adenomatous polyposis coils (APCs), antigen-specific lymphocytes. Therefore, antigen presentation is a key step in therapeutic use of vaccines. As an artificial antigen-presenting system, acellular technology has been recently well developed such as magnetic beads, artificial liposomes, and NVs.

NVs have been potentially used as vaccines in cancers and infections of pre-clinical studies [28]. In cancer treatment, APC- and tumor-derived NVs play a key role in anti-tumor immune response. In case of bacterial infections, it has been shown that mice injected with NVs of *M. tuberculosis* and *M. bovis* BCG in which many immunogenic proteins are enriched, manifest an immune response to vesicle components [29,30]. It has also found that vesicles from *Klebsiella pneumoniae* induce humoral, or antibody-generating, and cellular T-cell-engaging immune systems in a recent study. For the current practice, a vesicle-based vaccine to *Neisseria meningitides* has been already approved for its efficacy in Europe [31].

Above indicates that parts of NVs from tumor cells or pathogens are able to provide protective capability against immunity-requiring diseases [32]. Thus, vesicle-based vaccines are promising way to cope with diseases derived from vesicle-generating-cells.

2. Drug delivery

Because NVs are comprised of cellular membranes with multiple adhesive proteins on the surface they are potentially specialized in cell-cell communications and provide an exceptional ability for the delivery of various therapeutic agents to target cells. The surface of NVs is similar to that of their mother cells: negatively charged and have cations like

Mg^{2+} or Ca^{2+} which stabilize the membrane. For that reason, NVs readily attach and interact with the moiety of their surfaces on the membrane of target cells [3]. Moreover, NVs are less hazardous than antibodies or proteins and easier to produce in terms of therapeutic device [33]. NVs also can carry different types of molecules including proteins and nucleic acids in more stable way than they would be if released directly to body fluids. This feature that NVs can carry cargoes safely has attracted the attention of researchers who develop biomaterial-based drugs [34]. Several remarkable approaches have been developed to prepare NVs with therapeutic agents (reviewed in [35]). The pre-treatments before the isolation of exosomes include 1) the incorporation of purified drugs into NVs from parental cells by *ex vitro*, 2) the use of NVs released from parental cells with drugs, and 3) the transfection of NVs from donor cells with drug-encoding DNA or therapeutic compounds. Indeed, NVs have combined benefits of both synthesized nanomaterials and cell-mediated drug delivery systems even though limitations are not studied well so far.

3. Biomarkers and diagnosis

i. Biomarker for cancers

NVs, especially exosomes, have been considered as strong candidates of biomarkers, which means they deliver convincing molecules to detect diseases. Exosomal double-stranded DNA (exoDNA) and miRNA (ex-miRNA) identified in exosomes represent the whole genomic DNA and can be utilized to detect mutations of the mother tumor cells of melanoma, breast, lung, prostate, ovarian, and pancreatic cancers [36-39]. Proteomic analyses of body fluids such as urine, blood, and saliva have also shown that NVs contain protein biomarkers of acute kidney injury (AKI) and prostate and pancreatic cancers [40-42]. Although NVs are now highly promising non-penetrating biomarkers in cancer diagnosis, still further studies are needed on the development of new nanotechnologies to use NVs from any body fluids and solids from patients having cancers, as easier and more accurate biomarkers compared to other ones that are clinically used at present.

ii. Diagnostic device: NV-Based Bioelectronics

One group devised an artificial nose in the diagnosis of lung cancer by utilizing selectivity and sensitivity of human olfactory receptor system [43]. In their study, NVs generated from artificial olfactory cells were used as biomarkers. Olfactory receptor(OR), G protein, and Ca^{2+} ion channel subsequently operates upon stimulating factors like heptanal strike OR. After this chain of reaction, influx of Ca^{2+} ions are suddenly increase to unusually high level and ions are further bound to Fura-2, a ratiometric and sensitive indicator fluorescence dye for measuring intracellular calcium. These changes are monitored in response to excitation strength and represent the changes in olfactory memory network very rapidly and accurately. This system is one of the promising platforms that integrate both biotechnology and nanotechnology in detecting cellular sensing signals. Other systems with better imaging and lower detection limit of flow of cellular materials need to be developed in the future.

IV. Conclusions

NVs has attracted so much attention over the decades. Regardless of their potential in many applications, still there are many hurdles to overcome to use NVs in fancier therapeutic and diagnostic tools for diseases compared to present platforms. For instance, the composition of NVs is very different by the cell types and the biogenesis pathways. This inhibits the stable diagnosis of NVs as biomarkers for disease in one-shot try for any patients. Therefore, new platform to utilize NVs independent to cellular types and their synthetic pathways is needs to be developed. Second, considerable studies about the characteristics of NVs and its related functions are essential. At present, some interesting features are utilized in specific role of NVs in nowadays applications. Therefore, more comprehensive ways of utilizing all beneficial features of NVs in applications are limited. If we overcome above hurdles, NVs can be a superb therapeutic device for vaccine, drug delivery, biomarker, and be settled as a potential material for nanomedicine.

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