European Journal of Pharmaceutics and Biopharmaceutics 118 (2017) 56–61

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Review article

Extracellular vesicles – A promising avenue for the detection and treatment of infectious diseases?

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ARTICLE INFO

Article history:
Received 14 July 2016
Revised 6 March 2017
Accepted in revised form 6 April 2017
Available online 7 April 2017

Keywords:
Antibiotic resistance
Exosomes
Biosensing
Drug therapy
Immune response

ABSTRACT

Extracellular vesicles (EVs) have gained increasing attention as novel disease biomarkers and as promising therapeutic agents. These cell-derived, phospholipid-based particles are present in many – if not all – physiological fluids. They have been shown to govern several physiological processes, such as cell-cell communication, but also to be involved in pathological conditions, for example tumour progression. In infectious diseases, EVs have been shown to induce host immune responses and to mediate transfer of virulence or resistance factors. Here, we discuss recent developments in using EVs as diagnostic tools for infectious diseases, the development of EV-based vaccines and the use of EVs as potential anti-infective entity. We illustrate how EV-based strategies could open a viable new avenue to tackle current challenges in the field of infections, including barrier penetration and growing resistance to antimicrobials.

Contents

1. Introduction

In the past decade, extracellular vesicles (EVs) have attracted significant attention in the fields of molecular biochemistry, biosensing and drug delivery due to their involvement in many physiological and pathological processes [1,2]. EVs are cell-derived particles which are naturally produced by most eukaryotic and prokaryotic cells [3]. Based on their intracellular origin and biochemical composition the most common populations of EVs are classified exosomes (size 50–200 nm) or shedding microvesicles (100 nm – up to several μm) [4]. In this perspective, these will be collectively termed EVs as suggested by the International Society for Extracellular Vesicles (www.isev.org) and in recent reviews from the field [1,4,5]. EVs consist of a lipid bilayer membrane decorated with various surface and membrane proteins and are often referred to as natural liposomes. In recent years, EVs have attracted enormous scientific attention as novel therapeutic agents and biomarkers with first clinical applications already in the pipeline [6]. In nature, EVs are very efficient mediators of intercellular communication [3] and transfer protein and nucleic acid based cargoes (*e.g.*, micro RNA) highly selective from one cell to another [7], even over long distances [8]. For example, EVs from T cells are targeted unidirectional to antigen-presenting cells (APC) [9]. EVs may not only transport biomolecules to distant tissue, they are also postulated to bypass immune activation and to exhibit improved

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http://dx.doi.org/10.1016/j.ejpb.2017.04.005
0939-6411/

stability under physiological conditions [3]. The potential of utilising EVs for medical applications has been exploited, for example by developing EV-based selective anti-inflammatory drug vehicles in different mouse models of inflammation [10]. Furthermore, EVs from immune and non-immune cells are involved in immune regulation processes [11] and they have been employed as immunemodulators to treat severe forms of graft-*versus*-host disease in humans [12]. Although EV applications have resulted in first (pre)-clinical trials, significant drawbacks such as their reproducible large-scale production under good manufacturing practice (GMP) standards, industrially applicable robust analytics and safety validation need to be overcome [5,13]. Here, basic and industrial investigation is needed as discussed in this perspective paper and outlined in recent extensive reviews [1,3,4]

Interestingly, not only eukaryotic cells use EVs as messenger tools. Bacterial EVs (so-called outer membrane vesicles, OMVs) have been identified as key mediators in the communication of bacteria and within biofilms [14]. Indeed, *Pseudomonas aeruginosa* release signalling molecules into EVs to enable specific interbacterial communication by transfer of both soluble and insoluble factors, such as quorum sensing molecules [15]. OMVs are strongly involved in pathogen-mediated infections, such as bacterial, viral, fungal and parasite infections [16]. They can mediate host immune activation and have been shown to transfer resistance genes or virulence factors [15]. For example, OMVs from known pathogenic bacteria, such as *Staphylococcus aureus*, *Mycobacteria tuberculosis* and fungi, such as *Cryptococcus neoformans* contain hydrolytic factors, toxins, proteins, DNA or cell wall components [14]. The OMV release mechanism is currently discussed in three diverging hypotheses including pressure release from the cell wall, cell-wall modifying enzymes and EV-specific release channels [17]. Once liberated, OMVs can contribute to the formation of biofilms [18,19] and transfer virulence factors [20] or even DNA [21] to mammalian cells. When taken up into mammalian cells, OMVs may also transfer their toxic and pro-inflammatory cargoes and manipulate the host immune response, induce proliferation, DNA damage or other deteriorating effects. For a detailed discussion on these mechanisms and effects the reader is referred to a recent review [22]. In this perspective paper, we focus on recent efforts in developing novel EV-based detection and therapy avenues. These approaches may pose potent future strategies to counteract decreasing options for treating and diagnosing severe infections, especially when facing growing antimicrobial resistance.

2. EV-based diagnostic approaches

Recent discoveries lead to increasing evidence that EVs may serve as biomarkers for diverse pathological conditions, ranging from cancer to sepsis. EVs feature particularly interesting characteristics, as they contain selected information on the composition of the donor as well as the acceptor tissues. The fingerprint analysis of EVs may hence allow probing of a much wider context and EV-based systems may outperform classical biomarkers and lead to better diagnostic performance (higher specificity and sensitivity). Profiling of secreted vesicles in body fluids, such as blood and urine, may give access to spatiotemporally resolved information and disease progression. The prospect of using EVs as biomarkers for both bacterial and viral diseases has been exploited in a number of studies [23,24]. Recently, it has been shown that CD11β-positive EVs can potentially be used as sensitive and specific biomarkers to differentiate between infectious and non-infectious inflammatory conditions in intensive care unit patients [25]. Similarly, exosomal CD81 has been shown to be associated with inflammatory activity and severity of fibrosis in patients suffering from hepatitis C [26]. A study by Singh and colleagues pointed out that *Mycobacterium tuberculosis* induces secretion of exosomes containing a unique set of host miRNA and mRNA from macrophages that could be used to diagnose tuberculosis [27]. Several recent studies highlight the potential of exosomal microRNA profiles for use as diagnostic biomarkers of disease through non-invasive blood tests [24]. Not only exosome composition but also the concentration of exosomes in body fluids may be indicative for the disease state. Interestingly, patients suffering from sarcoidosis presented with significantly higher exosome concentrations in broncho-alveolar lavage fluid (BALF) compared to healthy individuals, suggesting that exosomes may play a key role in disease progression and exosome concentration in BALF may thus be indicative for disease severity.

In addition to the potential of EVs as biomarkers, EV-based carriers may in future be applied as diagnostic imaging agents, either experimentally to work out tissue homing and specificity [28–30], or clinically to better diagnose pathogenic processes in distant tissues that cannot normally be imaged by classical contrast agents [29,30]. Suitable labelling of exosomes without affecting their properties is pivotal. Recently, Hwang and colleagues have demonstrated rapid radiolabeling of macrophage-derived exosome-mimetic vesicles with ^{99m}Tc for *in vivo* radionuclide imaging via SPECT/CT in living animals [28]. Hu and colleagues have employed electroporation to load exosomes with superparamagnetic iron oxide nanoparticles for magnetic resonance tracking [30]. Busato et al. [29] have labelled cells with ultra-small superparamagnetic iron nanoparticles and then isolated exosomes from previously labelled cells. These exosomes retained the nanoparticles and demonstrated preserved morphology and physiological characteristics. Detection limits of 3 μg and 5 μg were demonstrated by magnetic resonance imaging *in vitro* and *in vivo*, respectively. Surprisingly, there are very few studies directly comparing EVs to their synthetic liposome counterparts. Using labelled exosomes as imaging probes promises advantages including the bypassing of many issues such as immune activation, off-target effect and rapid *in vivo* degradation. However, at the same time, these apparent advantages need to justify the risks of bringing biologically active exosomes into the body for diagnostic purposes. Off target effects of such naturally derived imaging probes need to be excluded and more investigations on risks and benefits of EVs are needed. Nonetheless, the above studies pave the way for exosome based diagnostic imaging with the prospect to enable new insights into disease aetiology, *e.g.*, in the context inflammatory responses or biofilm formation. Additionally, these concepts may be further extended to theranostic applications, *e.g.*, using iron oxide labelled exosomes for magnetic hyperthermia.

3. EVs as anti-infective therapeutic tools

3.1. EVs as versatile vaccines

There are two potential avenues of using EVs for treatment of pathogen-mediated infections: EV-based vaccines as discussed in this section and EVs as anti-infective entities themselves as outlined in the next paragraph. EVs may be ideal candidates for vaccination, as they naturally transfer information between cells, are stable under biological conditions and show specific binding to immune-competent cells [3,9]. EV-based vaccines may be derived from pathogen primed human cells or directly from pathogens. APC-derived EVs, in particular from dendritic cells (DC) that were pulsed with pathogens appear to be a promising source of EV-vaccines. *Leishmania* antigens transferred onto DC-derived exosomes conferred efficient protozoan infection protection by induction of T cell proliferation [31]. Furthermore, bone marrow DC-derived exosomes previously pulsed with pathogens elicited protective immunoglobulin production in mice which were