Journal of Controlled Release 289 (2018) 56–69

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Review article

Extracellular vesicles: intelligent delivery strategies for therapeutic applications

Alice Pinheiro[a,b,c], Andreia M. Silva[a,b,d], José H. Teixeira[a,b,d], Raquel M. Gonçalves[a,b,d], Maria I. Almeida[a,b], Mário A. Barbosa[a,b,d], Susana G. Santos[a,b,*]

[a] i3S – Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal
[b] INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal
[c] FEUP – Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal
[d] ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal

ARTICLE INFO

Keywords:
Exosomes
Biodistribution
Delivery system
Nanoparticles

ABSTRACT

Extracellular vesicles (EV), in particular exosomes, have been the object of intense research, due to their potential to mediate intercellular communication, modulating the phenotype of target cells. The natural properties and functions of EV are being exploited as biomarkers for disease diagnosis and prognosis, and as nano-biocarriers for the development of new therapeutic strategies. EV have been particularly examined in the field of cancer, but are also increasingly investigated in other areas, like immune-related diseases and regenerative medicine.

In this review, the therapeutic use of EV as drug delivery systems is described, balancing the advantages and drawbacks of different routes for their *in vivo* administration. Systemic and local delivery of EV are discussed, tackling the persisting difficulties in the assessment of their pharmacokinetics, pharmacodynamics and biodistribution *in vivo*. Finally, we discuss the future perspectives for incorporating EV into delivery systems and their use for an improved and controlled release of EV *in vivo*.

1. Introduction

In the last decades, research on cell secreted Extracellular Vesicles (EV) has expanded exponentially. Their role in cancer has been by far the most described and explored, but their contribution to the processes of inflammation and tissue repair has also been under intense scrutiny. These have been recently reviewed by us [1].

Three main classes of EV secreted by cells have been reported and characterized, based on their origin, release mechanism and properties: apoptotic bodies, microvesicles and exosomes. The last two types of vesicles have deserved most attention from the scientific community due to their biological properties [2]. Microvesicles originate from the plasma membrane and their size can range from 50 nm to 1 μm (Fig. 1). Exosomes are smaller, with sizes in the range of 40–100 nm, are derived from the intraluminal bud of multivesicular endosomes (MVE), and are released after fusion of these MVE with the plasma membrane (Fig. 1) [3]. These nanovesicles are secreted by most types of cells, including tumor cells [4,5], playing a key role in intercellular communication, without cell-cell contact. In this review, exosomes will be particularly emphasized. However, the exact distinction of these different vesicle populations is still controversial, and partly hindered by methodological limitations, and thus wherever appropriate the designation EV will be preferred.

Upon secretion, the different types of EV are present in body fluids or supernatants of *in vitro* cell cultures, and thus to study each population, it is necessary to isolate them separately. Different methods have been developed and optimized, mainly intended to isolate exosomes, achieving different degrees of purity and yield. These methods include differential centrifugation with a final step of ultracentrifugation [6], ultracentrifugation in density gradients [6,7], phase separation [8], size-exclusion chromatography [9] and immunoprecipitation, particularly based on the commonly used exosomal marker CD63 [6]. However, difficulties in isolating pure EV populations still persist, with most methods leading to the co-precipitation of non-EV contaminants. Consequently, it is of utmost importance that the vesicles isolated are extensively characterized, using standardized methods [7,10–12]. So far, the main techniques used for EV characterization are transmission electron microscopy [6,13], atomic force microscopy [14], nanoparticle tracking analysis [13,15,16], dynamic light scattering [17], flow cytometry (directly on EV or upon their capture on beads) and western

* Corresponding author at: INEB – Instituto de Engenharia Biomédica, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal.
E-mail address: susana.santos@ineb.up.pt (S.G. Santos).

https://doi.org/10.1016/j.jconrel.2018.09.019
Received 29 June 2018; Received in revised form 20 September 2018; Accepted 21 September 2018
Available online 24 September 2018

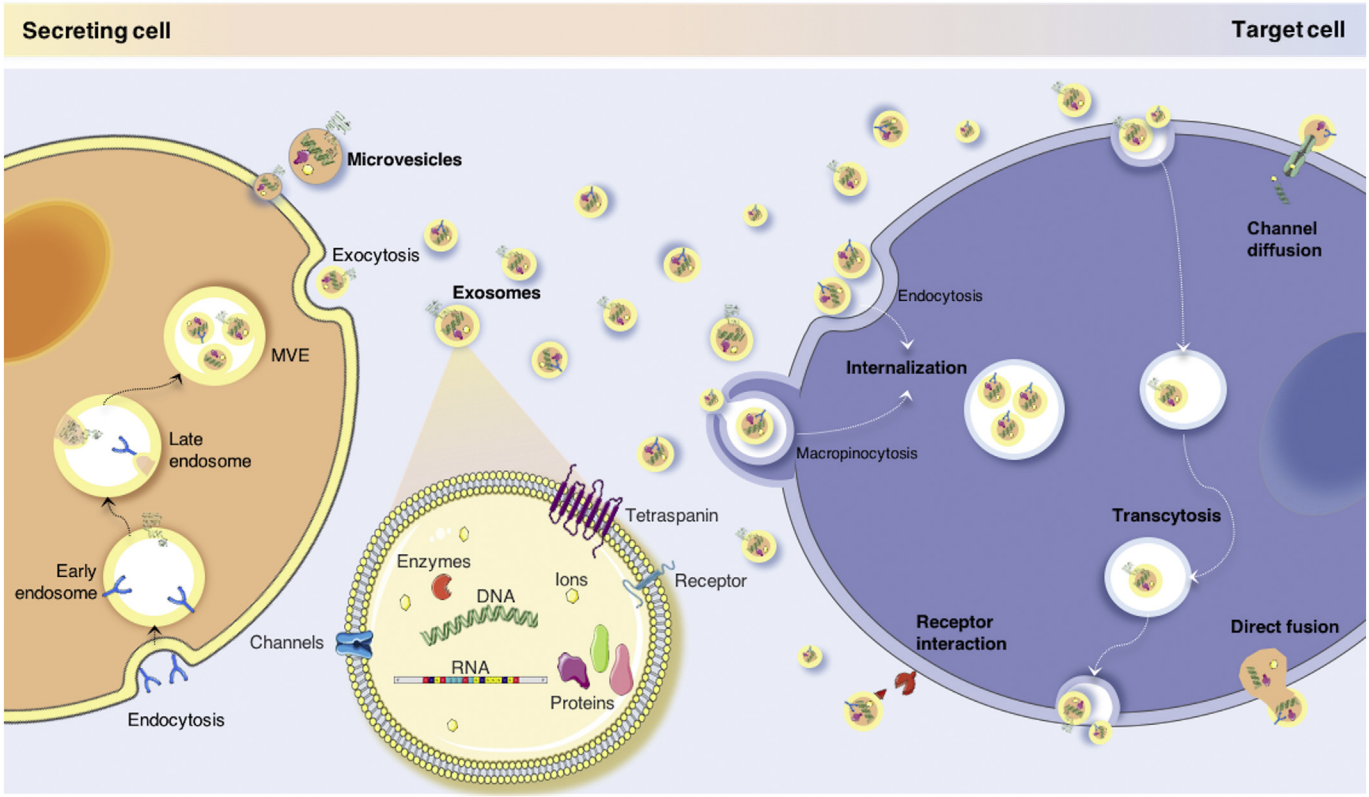

Fig. 1. EV biogenesis and interaction with target cells. Extracellular vesicles (EV) are a family of vesicular components with a lipid bilayer secreted by cells. The most representative vesicles of this family are exosomes, small vesicles (40–100 nm), originated within the multivesicular endosomes (MVE), and microvesicles, 50 nm – 1 μm vesicles originated by budding off of the plasma membrane, that upon secretion may be found in different biological fluids. They contain a biomolecular cargo that render them storage vessels of biomarkers of different diseases, but also natural vehicles of therapeutic molecules, reported to target different cells depending on their cell of origin. Upon interaction with a target cell, EV may directly release their content into cell's cytoplasm, or may be internalized by different mechanisms, being processed within the endocytic pathway for cargo delivery, or transported across a polarized cell by transcytosis.

blotting of common protein markers (*e.g.*, CD63, CD81, CD9) [18,19], and more recently, tunable resistive pulse sensing [13,15], surface plasmon resonance [20], and Raman spectroscopy [21].

Independently of their origin, EV are lipidic vesicles that carry a protein content in their core, as well as on their surface, and may also contain different types of RNAs, including messenger RNA and microRNAs (miRNAs), DNA, and even metabolites (Fig. 1) [22]. Nonetheless, each type of EV has a detailed biomolecule signature. Importantly, these biomolecules are reported to be functional, when delivered to target cells [23,24].

In this way, EV have been reported to participate in different homeostatic biological processes, including delivery of active biomolecules to target cells, antigen presentation to trigger an immune response, and cell waste management, initially thought to be their only function [25]. They may also contribute for the spreading of infecting pathogens and pathologies, namely cancer metastasis [26]. EV have also been greatly explored as biomarkers of disease, because their composition is related to the cell from which they derive. Characterization of EV levels and composition in biofluids, particularly in blood, urine and saliva, can contribute for the diagnosis, prognosis and monitoring of therapeutics in various diseases, cancer being the most explored [3,17,27,28].

EV have been increasingly investigated in the nanomedicine field, both as messengers in cell-cell communication and as intelligent systems for targeted delivery. Many studies have been reporting their use to deliver molecules such as miRNAs, siRNAs, proteins or drugs [2]. However, despite the increasing research in EV field, difficulties in their isolation, in terms of purity and yield, are obstacles to their successful translation into clinical applications. Furthermore, basic techniques for characterization of EV administered in *in vivo* models need substantial improvement, before going into clinical applications. The potential therapeutic role of EV makes it necessary to invest in quantification and characterization methodologies, before and after *in vivo* administration, in order to attain a fast and precise characterization of EV [15,29]. On the other hand, it should be taken into account that EV are not retained for a long time in circulation when administered *in vivo*, reportedly due to the action of macrophages [30]. Furthermore, the mechanisms controlling the specific delivery of EV to a specific target are largely unknown and need to be further studied. Different approaches are being developed to overcome these barriers that limit the therapeutic use of EV, from vesicle functionalization to their incorporation into local delivery systems.

In this review, we discuss the different reported approaches for *in vivo* delivery of EV, including their systemic and local delivery, as vesicles suspensions or incorporated in more complex delivery systems. The limitations of each approach in terms of vesicles biodistribution and biological effects achieved are also appreciated. In parallel, different approaches to follow EV after administration in *in vivo* models are detailed. Finally, we compare the systems used for EV delivery with the ones commonly used for nanoparticles incorporation, suggesting some adaptations of the latter for the improved delivery of EV *in vivo*.

2. Therapeutic potential of EV: The importance of delivery methods

EV nanosize and natural ability to transfer information between cells, renders them promising candidates to be explored as nanodevices for therapeutic applications [31].

Naturally, EV carry active biomolecules that are loaded into their core or incorporated in their membrane through endogenous cell-