We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Extracellular Vesicles and Their Interplay with Biological Membranes

Jacob Yeo Xian Ping, Yub Raj Neupane and Giorgia Pastorin

Abstract

Most cells secrete vesicles into the extracellular environment to interact with other cells. These extracellular vesicles (EVs), have undergone a paradigm shift upon the discovery that they also transport important material including proteins, lipids and nucleic acids. As natural cargo carriers, EVs are not recognised by the immune system as foreign substances, and consequently evade removal by immune cells. These intrinsic biological properties of EVs have led to further research on utilising EVs as potential diagnostic biomarkers and drug delivery systems (DDSs). However, the internalisation of EVs by target cells is still not fully understood. Moreover, it is unclear whether EVs can cross certain biological membranes like the blood-brain barrier (BBB) naturally, or require genetic modifications to do so. Hence, this review aims to evaluate the relationship between the composition of EVs and their association with different biological membranes they encounter before successfully releasing their cargo into target cells. This review identifies specific biomarkers detected in various EVs and important biological barriers present in the gastrointestinal, placental, immunological, neurological, lymphatic, pulmonary, renal and intracellular environments, and provides a recommendation on how to engineer EVs as potential drug carriers based on key proteins and lipids involved in crossing these barriers.

Keywords: biological barriers, diagnostic biomarkers, drug delivery, engineering, extracellular vesicles, bioengineering

1. Introduction

Extracellular vesicles (EVs), phospholipid bilayer-enclosed vesicles consisting of proteins, lipids and nucleic acids, were once thought of as merely how cells may discard their waste materials and debris. However, recent discoveries have proven them to be indispensable to cells even in normal physiological functions and as diagnostic biomarkers for various diseases [1]. EVs are secreted by various cells and can be isolated from diverse biological sources like saliva, breast milk and blood serum [2].

Over the years, EVs have been researched as promising diagnostic biomarkers for pathological conditions. This is because their concentration and composition correlate with disease progression, a unique characteristic that sets them apart from other types of paracrine secretions [3, 4]. EVs have also been explored as possible carriers for

drug delivery. Recent studies have shown promising results regarding the utilisation of EVs as drug delivery systems (DDSs) to treat various conditions, such as cardiovascular diseases [2, 5], osteoporosis [2, 6] and brain tumours [2, 7]. In light of this, EVs are seen as a more desirable strategy for drug delivery compared to other conventional nanoparticles like liposomes, micelles and polymeric nanoparticles [8, 9]. Conventional DDSs have been extensively used for their ability to protect drugs from inactivation in the external environment. However, plasma proteins risk adsorbing onto the surfaces of these non-EV nanoparticles upon injection into the body, making them an easy target of immune cells and decreasing their uptake by their target cells [10]. Although these nanoparticles may undergo modification to avoid immune cell removal, they still lack biocompatibility due to their non-biological origins. EVs, on the other hand, can evade phagocytosis by immune cells naturally, in addition to being highly selective for designated target sites, due to their biological origins and cell-specific surface properties inherited from the parent cells that secrete them.

Although EVs are promising in their diagnostic and therapeutic applications, it is still unclear whether they can cross membranes like the blood-brain barrier (BBB) naturally or when genetically modified, or only when the membranes become more permeable in certain conditions like injury [11, 12]. Furthermore, the uptake of EVs by target cells is still not fully understood at a microscopic level, be it *via* endocytosis, membrane fusion or other mechanisms [3]. The ability to pass through biological membranes is an important factor to consider when engineering EVs to deliver drugs to specific cells. As there remains a lack of understanding on how EVs can cross significant biological membranes before reaching their target sites, this review aims to identify potential key proteins and lipids that play a dominant role in the functions of EVs, and evaluate the relationship of these key components on EVs with different biological membranes, so that a recommendation can be given on how to best engineer EVs as potential drug carriers.

2. EVs—classification and key components

Classified by their biogenesis, size, morphology and function, there are three main EV categories—exosomes, microvesicles and apoptotic bodies (**Figure 1**) [16–18].

Although exosomes, microvesicles and apoptotic bodies are distinct from one another, there is a partial overlap among their respective size range and composition. Although many different methods have been previously deployed to isolate EVs from their sample sources (a notable example being ultracentrifugation in isolating and purifying exosomes and microvesicles [19–22]), these methods are unable to provide an accurate attribution of unique characteristics to each EV category. This is due to the complex nature of EVs, such that different size ranges can be derived from the same EV source depending on the isolation technique used [23]. As such, this review will mainly elaborate on EVs in general, unless otherwise stated.

Apart from biogenesis, size and morphology, each EV category possesses its own unique set of key proteins, lipids and nucleic acids (**Table 1**). Being able to differentiate EV categories based on their key components is vital in understanding their specific roles in both normal and pathological conditions. In general, all EVs possess cell adhesion proteins [13, 14, 17, 18, 24–28], heat-shock proteins [13, 14, 18, 25, 28–30], biogenesis-associated proteins [13, 14, 17, 18, 24, 25, 28], fusion proteins [13, 14, 18, 25], cell-type specific proteins [13, 14, 18, 27, 28], cytoskeletal proteins [13, 18], signalling molecules [13, 14, 28, 31], enzymes [13, 25, 28], messenger ribonucleic acid (mRNA), micro ribonucleic acid (miRNA), non-coding ribonucleic

Figure 1.

*Biogenesis, size, morphology and function of exosomes, microvesicles and apoptotic bodies. (A) **Exosomes** (spheroid shape, 30–200 nm) are mainly involved in regulating intercellular communication. Their formation begins when the plasma membrane undergoes endocytosis to generate an early endosome. Intraluminal vesicles (ILVs) within the endosome are then formed from the inward budding of the endosomal membrane, resulting in a multivesicular body (MVB). This process is facilitated by either endosomal sorting complex required for transport (ESCRT)-dependent or -independent mechanisms [1, 13]. The MVB finally fuses with the plasma membrane to release the ILVs as exosomes. (B) **Microvesicles** (irregular shape, 100–1000 nm), like exosomes, also regulate intercellular communication. They are formed via budding from the plasma membrane directly without going through endocytic processes. (C) **Apoptotic bodies** (variable shape, usually >1000 nm but can be as small as 50 nm [14, 15]) are formed only during cell apoptosis, during which the post-apoptotic cell bulges outwards to form vesicles for easier removal by macrophages (created with BioRender.com).*

acid (RNA), phosphatidylethanolamine, sphingolipids and higher levels of phosphatidylserine (PS) than the cell plasma membrane [24, 25, 28, 35].

The distinct protein, lipid and nucleic acid profiles of each category might be correlated with its formation processes and functions. Both exosomes and microvesicles consist of key protein components which are responsible for cell-to-cell communication [18], such as glycoproteins [18, 28], membrane signalling receptors, growth factors and cytokines [18, 25], while apoptotic bodies do not. This is most likely because exosomes and microvesicles are meant to reach target cells, while apoptotic bodies are merely the means for discarding dead cells. Microvesicles and apoptotic bodies consist of other cytoplasmic proteins which seem to be less prominent in exosomes [13]. This might be due to the similar “budding/bulging” nature of the biogenesis of microvesicles and apoptotic bodies from the cytoplasmic membrane directly, a characteristic that differs from the endocytic-driven biogenesis of exosomes. Unlike exosomes and microvesicles, apoptotic bodies are composed of chromosomal deoxyribonucleic acid (DNA) fragments, chromatin remnants, cytosol portions, degraded proteins and cell organelles from dead cells [25, 35], indicative of their role in removing dead cells.

EVs also possess additional key features according to the specific cell line they originate from (**Table 2**). In general, cancer cells consist of higher levels of sphingolipids, glycerophospholipids, sterol lipids, ceramide, phosphatidic acid and matrix metalloproteinases like a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), while non-cancer cells consist of higher levels of prenol lipids, glycerolipids and fatty acids [24, 83].

Components	Exosomes	Microvesicles	Apoptotic bodies	Reference(s)
Proteins				
Tetraspanins	CD9, CD63, CD81, CD37, CD82, CD53, TSPAN 6, TSPAN 8, TSPAN 29, TSPAN 30	CD40 ligands, CD82	CD40 ligands, CD82	[13, 14, 24, 25]
Cell adhesion proteins	Integrins (integrin-alpha, integrin-beta), selectins (P-selectin), lactadherin, ICAM	integrins, selectins (P-selectin), fibronectin, PECAM-1	integrins, fibronectin, PECAM-1	[13, 14, 17, 18, 24–28]
Heat shock proteins	Hsc70, Hsp20, Hsp27, Hsp60, Hsp70, Hsp90	Hsp70, Hsp90	Hsp70, Hsp90	[13, 14, 18, 25, 28–30]
Biogenesis-associated proteins	ESCRT proteins (Alix, Tsg101), VPS4, clathrin, ubiquitin, syntenin, VPS32, PLD	ESCRT proteins (Alix, Tsg101), VPS4, ERK, PLD	VPS4, ERK, PLD	[13, 14, 17, 18, 24, 25, 28]
Fusion proteins	Flotillin 1 and 2, annexins, GTPases, Rab GTPases, dynamin, syntaxin	Flotilin-2, Rab GTPases, annexins	Rab GTPases, annexins (Annexin V)	[13, 14, 18, 25]
Cell-type specific proteins	MHC class I, MHC class II, APP, PMEL, TCR, CXCR4, HSPG, CD86, PrP, WNT	MHC class I, MHC class II, LFA1, CD14	MHC class I, LFA1, CD14	[13, 14, 17, 18, 27, 28]
	Actin, tubulin, cofilin	Actin, tubulin	Actin, tubulin	[13, 18]
Cytoskeletal proteins	Protein kinases, beta-catenin, 14-3-3, G proteins	ARF6, Rab11, ROCK	ARF6, Rab11, ROCK	[13, 14, 28, 31]
Signalling molecules	PLA2, peroxidases, pyruvate kinase, enolase, GADPH, ATPases	GADPH	GADPH	[13, 25, 28]
Other enzymes	Glycoproteins	Glycoproteins		[18, 28]
	• e.g. beta-galactosidase, O-linked glycans, N-linked glycans	• e.g. Glycoprotein Ib		
Additional proteins	Growth-factors and cytokines	Growth factors and cytokines		[18, 25]
	• e.g. TNF- α , TGF- β , TNF-related apoptosis-inducing ligand			
	Membrane signalling receptors	Membrane signalling receptors		
	• e.g. FasL, TNF receptor, Tfr			
	Phosphoproteins	High phosphoproteins		[28, 32]
	Ribosomal proteins	GTP-binding protein ARF6		[28, 33]
	Lysosomal proteins	Chemokines		[25]
	• e.g. Lamp2b			

Components	Exosomes	Microvesicles	Apoptotic bodies	Reference(s)
Lipids	High phosphatidylserine	High phosphatidylserine		[14, 25]
	Phosphatidylethanolamine	Phosphatidylethanolamine		[24, 25, 28, 32]
	Sphingolipids	Sphingolipids		[18]
	• e.g. sphingomyelin, gangliosides			
	High cholesterol			[14, 28, 34]
	High diacylglycerol			[14]
	Ceramides			[13, 24, 28]
	Phosphatidylcholine			[28]
	Phosphatidylinositol			[18]
Nucleic acids	LBPA			[13]
	mRNA		mRNA	[25, 35]
	miRNA		miRNA	
	Non-coding RNA		Non-coding RNA	• e.g. small nucleolar RNAs, microRNAs, piwi-interacting RNAs, other long non-coding RNAs
	• e.g. small nucleolar RNAs, microRNAs, piwi-interacting RNAs, other long non-coding RNAs			
	DNA with histones		Chromosomal DNA fragments with histones, chromatin remnants, cytosol portions, degraded proteins, cell organelles	

Abbreviations: ADP: adenosine diphosphate, APP: amyloid-beta precursor protein, ARF: ADP ribosylation factor, CXCR: CXC chemokine receptor, DNA: deoxyribonucleic acid, ERK: extracellular signal-regulated kinase, ESCRT: endosomal sorting complex required for transport, FasL: Fas ligand, GTP: guanosine triphosphate, HSPG: heparan sulphate proteoglycan, ICAM: intercellular adhesion molecule, Lamp: lysosome-associated membrane protein, LFA: lymphocyte function-associated antigen, MHC: major histocompatibility complex, PECAM: platelet endothelial cell adhesion molecule, piwi: P-element induced wimpy testis, PLA2: phospholipase A2, PLD: phospholipase D, PMEL: premelanosome protein, PrP: prion protein, Rab: Ras-associated binding, TCR: T-cell receptor, RNA: ribonucleic acid, ROCK: Rho-associated protein kinase, TSPAN: tetraspanin, Tsg: tumour suppressor gene, VPS: vacuolar protein sorting-associated protein, WNT: wingless/integrated, GADPH: glyceraldehyde 3-phosphate dehydrogenase, TDP: transactive response DNA-binding protein, TfR: transferrin receptor, TGF: transforming growth factor, TNF: tumour necrosis factor.

Table 1.
Classification of key components of EVs by their main categories.

EV source	EV source subtype	Component(s)	Reference(s)
Bacteria	Gram-positive	ABC transporters, mobility-related proteins (FliC, PilQ), multidrug efflux pumps, porins (Omps, OprF, PorA, PorB)	[24, 36]
	Gram-negative	Beta-lactamase, coagulation factor, penicillin-binding protein	[24, 37–39]
	Myxobacteria	Chaperonin GroEL1, GroEL2, hydrolase, peptidase	[24, 40, 41]
Blood cells	Platelets	CD31, CD41, CD42a, CD62, C-type lectin, CXCR4, GPIIb/IIIa, PF4, SDF-1 α	[24, 42–44]
	Erythrocytes	Glycophorin A, stomatin	[24, 34]
	Reticulocytes	Galectin-5	[42, 45]
Bone cells	Osteoblasts	Cadherin-11	[42, 46]
Cancer cell lines	Breast cancer cells (MM231, MM231LN)	Rab-5b, actin, integrin beta 1, cavolin-1	[47]
	Breast cancer cells (MCF7)	Actin, Rab-5b	[47]
	Breast cancer cells (MCF10A)	Integrin beta 1	[47]
	Cervical cancer cells (HeLa)	EGF	[42, 48, 49]
	Colon cancer cells (LIM1863—EpCAM apical exosomes)	CD44, CD46, CD59, CLDN7, EpCAM, HMGB2, HMGB3, Muc-13, sucrase isomaltase	[50]
	Colon cancer cells (LIM1863—A33 basolateral exosomes)	ADP-ribosylation factor, AP1G1, AP1M1, AP1M2, AP3B1, CLSTN1, CLTA, CLTB, COPB2, EEA1, GPA33, HLA-A, HLA-B, HLA-C, HLA-E, HLA-A29.1, Rab-13, REEP6	[50]
	Colorectal cancer cells (CRC line SW403, CRC28462)	Carcinoembryonic antigen, class I HLA	[51]
	Hepatoblastoma cancer cells (HepG2, K562)	TfR1, TfR2	[42, 52]
	Hepatocellular cancer cells (HKCI-C3, HKCI-8, MHCC97L, MIHA)	ADAM10, ARHGEF18, BROX, CAV1, CAV2, CD44, CDC42, CLDN3, EDIL3, EIF4A3, GNA11, GNA13, GNAQ, GNAS, GRB2, MET, RHOG, RRAS, SNTA1, TNFRSF21, TNFAIP2	[53]
	Myeloma cancer cells (RPMI-8226, CAG)	Fibronectin	[54]
	Nasopharyngeal cancer cells (C15)	Galectin-9, LMP1	[55]
	Nasopharyngeal cancer cells (C17)	Galectin-9	[55]
	Ovarian cancer cells (IGROV1, OVCAR-3)	Beta-actin, EpCAM, hnRNPA1, hnRNPK	[56]
	Prostate cancer cells (PC3)	Rab-5b, integrin beta 1, cavolin-1	[47]
	Prostate cancer cells (PC-3 M-luc)	Rab-5b, actin, Integrin beta 1	[47]
	Prostate cancer cells (22Rv1)	Rab-5b, actin	[47]
	Prostate cancer cells (PNT2)	Actin, integrin beta 1	[47]

EV source	EV source subtype	Component(s)	Reference(s)
Endothelial and epithelial cells		C-type lectin, galectin-3, Muc-1	[42]
Immune cells	B-cells	A2,3-linked sialic acid, CD169	[42, 57]
	T-cells	CXCR4, SDF-1 α	[42, 48, 58, 59]
	Dendritic cells	FLOT1, galectins, Lamp-1, MFG-E8, MHC class I and II, TNFR1, TNFR2	[24, 42, 60–62]
	Macrophages	C-type lectin, LFA-1	[42, 63, 64]
	Natural killer cells	Granzyme B, perforin	[65]
Mesenchymal stem cells		Alternative splicing and Golgi apparatus component mRNA encoding transcription factors, CD54, CD73, CD86, CD90, CD105, CD166, MHC class I and II, sialic acids	[24, 28, 42, 66–75]
Milk cells	Bovine milk cells	β -casein, β -lactoglobulin mRNA, CD59, MFG-E8, miR-30a, miR-92a, miR-223, Rab-1b, Rab-11a	[24, 76–80]
	Human breast milk cells	miR-17, miR-181a	[81]
Nervous cells	Astrocytes	MCP-1, MMP3, MMP9, TIMP-1	[65]
	Microglia	CD13, CD107a, CD107b	[65]
Placental cells		MHC class I chain-related proteins A and B, placental alkaline phosphatase, placental leucine aminopeptidase, pregnancy specific glycoprotein 3, RAET1 proteins/ULBP1–5, TGF β 1, TRAIL, trophoblast glycoprotein 5 T4	[82]

Abbreviations: ABC: adenosine triphosphate-binding cassette, ADAM: A disintegrin and metalloproteinase domain-containing protein, ADP: adenosine diphosphate, AP: adaptor related protein complex, ARFGEF: Rho/Rac guanine nucleotide exchange factor, BROX: BRO1 domain and CAAX motif containing, CA: carbohydrate antigen, CAV: caveolin, CLDN: claudin, CLSTN: calsyntenin, CLT: clathrin light chain, COP: coatomer protein complex, CXCR: CXC chemokine receptor, EDIL: EGF like repeats and discoidin domains, EEA: early endosome antigen, EGFR: epidermal growth factor receptor, EGF: Epidermal growth factor, EpCAM: epithelial cell adhesion molecule, FLOT: flotillin, GN: guanine nucleotide-binding protein, GP: glycoprotein, GRB: growth factor receptor-bound protein, HLA: human leukocyte antigen, HMG: high-mobility group, HNRNP: heterogeneous nuclear ribonucleoprotein, Lamp: lysosome-associated membrane protein, LDLR: low-density lipoprotein receptor, LDL: low-density lipoprotein, LFA: lymphocyte function-associated antigen, LMP1: Epstein-Barr virus latent membrane protein 1, MAPK: mitogen-activated protein kinase, MCP: membrane cofactor protein, MET: mesenchymal-epithelial transition factor, MFG-E: milk fat globule-EGF factor, MHC: major histocompatibility complex, miRNA: microRNA, MMP: matrix metalloproteinase, Muc: mucin, PF: platelet factor, RAET: retinoic acid early transcript, Rab: Ras-associated binding, REEP: receptor expression-enhancing protein, RHOG: Ras homolog family member G, RRAS: RAS-related protein R-Ras, SDF: stromal cell-derived factor, SNT: syntrophin, TfR: transferrin receptor, TNF: tumour necrosis factor, TNFR: tumour necrosis factor receptor, TNFRSF: TNF receptor superfamily, TNFAIP: TNF alpha-induced protein, TRAIL: tumour necrosis factor-related apoptosis-inducing ligand, TYRP: tyrosinase-related protein, ULBP: UL16 binding protein.

Table 2.
Classification of additional key components of EVs by their specific cell lines.

3. The EV journey—overcoming biological barriers

To reach their target sites, EVs need to overcome various biological barriers (**Figure 2**). Complementing these barriers are blood vessels (capillaries in particular). EVs can enter and extravasate from these vessels *via* diffusion, due to their lipidic nature which enables them to pass through the highly-lipidic capillary endothelium and their small size that enables them to pass or squeeze through fenestrations in the capillary wall [84].

Figure 2. Biological barriers encountered by extracellular vesicles (EVs). (A) **Neurological barriers** include the blood-brain barrier (BBB), blood-labyrinth barrier (BLaB) and blood-retinal barrier (BRB). (B) **The pulmonary barrier**, or blood-air barrier (BAB), guards against the invasion of pathogens in the lungs via its immune cell-rich lung mucosa, lung epithelial cells and ciliary action. (C) **Immunological barriers** eliminate pathogens and perceived foreign substances from the body via the mononuclear phagocyte system and the adaptive immune system. (D) **The placental barrier** consists of an inner blood-vessel-rich layer with the syncytiotrophoblast facing the bloodstream and an outer layer of trophoblasts. (E) **The lymphatic barrier**, or blood-lymph barrier (BLyB), is regulated by various mechanisms including extravasation, overcoming the interstitium, diffusion, and passage through the mucosal barrier. The collagen reticular network (RN) also hinders soluble substances from passing through. (F) **The renal barrier**, or glomerular filtration barrier, composes of the fenestrated endothelium, glomerular basement membrane and glomerular epithelium, and this hinders the passage of large molecules across the barrier. (G) **Gastrointestinal barriers** are associated with digestive enzyme degradation, harsh stomach acidic conditions and the small intestinal barrier. (H) **Cellular barriers** of the target cell include the plasma membrane, endosomal membrane and lysosomal membrane. EVs internalised by cells via endocytosis are packaged into endosomes which may risk fusing with lysosomes to undergo degradation (created with BioRender.com).

3.1 Gastrointestinal barriers

EVs administered orally need to overcome digestive enzymatic degradation, harsh stomach acidic conditions and the small intestinal barrier before entering the bloodstream for systemic absorption. As milk and plant-derived EVs are delivered into the body naturally *via* oral consumption, they might provide key insights into how EVs can be used and/or engineered for oral administration. *In vivo* evidence in rodents showed that unmodified bovine milk-derived EVs naturally containing immune-active proteins were able to cross the intestinal barrier *via* endocytosis to treat inflammatory bowel disease (IBD) [85], and were distributed significantly in the bloodstream 24 h post-oral consumption [86]. EVs can pass through the intestinal barrier *via* intestinal epithelial cell (IEC) mediated transendocytosis, a process that requires surface glycoproteins on both EVs and target cells, based on *in vitro* findings of skimmed bovine milk-derived EVs being internalised by human colon carcinoma Caco-2 cells and rodent small intestinal IEC-6 cells [87, 88]. Paracellular translocation is another possible mechanism by which EVs may cross the intestinal epithelium,

through tight junctions between adjacent epithelial cells [76, 87, 89]. Although *in vivo* evidence is lacking, it is possible that EVs might cross the intestinal epithelium paracellularly to a greater extent in pathological conditions like IBD as the tight junctions would be disrupted [90], making the intestinal epithelium more penetrable.

Milk-derived EVs have been shown to withstand acidic and enzymatic conditions [87, 91]. However, their ability to do so might be dependent on the milk source, as EVs from processed milk would have undergone more damage than those from unprocessed milk and hence possess less integrity [87, 92–97]. Although bovine milk-derived EV surface proteins CD9 and CD81 were found to be partially degraded by acidification at pH 4.6 in one study [98], these findings did not demonstrate whether these EVs can survive stomach acidic conditions, which are usually characterised by a much lower pH. Moreover, the study was focused on evaluating the effectiveness of acidification in ultracentrifugation to isolate EVs. Thus, these conditions would have differed vastly from true gastrointestinal conditions. Although the underlying mechanism is unclear, the ability of both processed and unprocessed milk-derived EVs to withstand harsh conditions might be correlated with milk calcium content [87]. This could be due to the adhering of milk calcium to the surface of EVs, which might strengthen their membrane integrity against acidic and enzymatic degradation. Another hypothesis is that calcium might influence milk-derived EV biogenesis pathways in alveoli cells to increase the expression of certain proteins or transporters in secreted EVs that enable them to withstand gastrointestinal conditions.

Fruit and vegetable-derived EVs have been shown to withstand gastrointestinal conditions and eventually be internalised by rodent intestinal tissue *in vivo*, though their passage across the intestinal barrier into the bloodstream cannot be concluded in some studies [77, 99–102]. Grape EVs derived *via* cold-pressing have been discovered to enter rodent IECs *via* macropinocytosis [100], while a previous analysis of grapefruit EVs derived *via* homogenization revealed their internalisation by intestinal macrophages *via* macropinocytosis and clathrin-mediated endocytosis [101]. Watermelon EVs were also observed to be taken up by human IECs in an *in vitro* experiment *via* clathrin-mediated endocytosis, causing the cells to multiply rapidly and their basal secretome to change [103]. Ginger EVs were found to accumulate in rodent liver tissue 12 h post-oral consumption, implying that the EVs were able to withstand gastrointestinal conditions and cross the intestinal barrier into the bloodstream while remaining intact [104]. Though unconfirmed, the uptake of plant-derived EVs *via* clathrin-mediated endocytosis and macropinocytosis probably indicates that these EVs possess receptor tyrosine kinases, G protein-coupled receptors (GPCRs) and transferrin receptors [105], while passage across the intestinal barriers into the bloodstream might imply that these plant-derived EVs undergo transendocytosis like milk-derived EVs, a mechanism which requires EVs to possess surface glycoproteins [87, 88]. The ability of milk and plant-derived EVs to withstand and overcome gastrointestinal conditions and barriers makes them highly suitable as DDSs *via* the oral route as a non-invasive alternative to intravenous DDSs.

3.2 Placental barrier

The placenta supports foetal growth and development while secreting female hormones [106–111]. The placental barrier (PB) is suggested to be selectively penetrable, given that drugs administered to pregnant women can either cause adverse side effects in both the mother and the fetus or not penetrate the PB at all. It consists of an inner blood-vessel-rich layer with the syncytiotrophoblast facing the bloodstream

and an outer layer of trophoblasts [106, 112–114]. Occurring in large amounts during pregnancy [115, 116], placental exosomes exert their functions during foetal growth and development, being involved in processes like angiogenesis regulation and cell migration [106, 116–126]. This implies that they can overcome the PB, though the underlying mechanism is unclear. Placental exosomes have also been tested as diagnostic biomarkers for foetal development [106, 115] and gestational diabetes [106, 127].

Although placental EVs may be used to pass through the PB, the use of non-placental EVs to deliver drugs across the PB is a potential area for exploration. The placenta can respond to signals from immune cells and exert an inflammatory response during infection. An *in vitro* study revealed that THP-1 monocyte-derived exosomes were internalised by human placental trophoblast cells *via* clathrin-mediated endocytosis, exerting a pro-inflammatory effect that caused the cells to release cytokines [128]. Provided that this mechanism can be proven *in vivo*, packaging drugs in EVs derived from immune cells might be one way to deliver drugs across the PB. Another possible method to deliver drug-containing EVs across the PB might be *via* administering EVs that target IECs instead of placental cells, as IECs can communicate with the placenta [103]. IECs that internalise watermelon EVs can secrete watermelon EV contents *via* the formation of intestinal exosomes, which are shown to be taken up by placental cells *via* clathrin-mediated endocytosis [103]. This concept, however, is deduced from a few *in vitro* studies and has yet to be proven in a single *in vivo* experiment. Nevertheless, being able to deploy non-placenta-derived EVs to treat placental pathological conditions like chorioamnionitis may offer some flexibility in EV engineering, as researchers would not need to adhere strictly to using placental EVs.

3.3 Immunological barriers

The body is heavily guarded by immune cells responsible for eliminating pathogens and perceived foreign substances. As such, nanoparticles injected into the bloodstream risk being removed by phagocytes of the mononuclear phagocyte system (including those in the liver and spleen), or the adaptive immune system *via* antibody production [8]. Thus, in conventional non-EV drug therapy, immune cells can potentially hinder the therapeutic effects of nanoparticles by decreasing their systemic circulation half-life [10]. EVs, on the other hand, can evade removal by immune cells naturally. CD47 is a prominent component found on EVs that binds to signal regulatory protein alpha (SIRP α) on dendritic cells and macrophages, which inhibits phagocytosis *via* a “don’t eat me” signal [129–131]. Other EV components found on both cancer and non-cancer cell-derived EVs like CD24, CD31 and PD-L1 have been associated with exerting a similar “don’t eat me” signal, with PD-L1 also inhibiting T-cell activation [130, 132–134].

A recent study on an *in vivo* rodent tumour model seems to suggest that it may be possible for EVs to be phagocytosed by Kupffer cells in the liver and eliminated *via* biliary excretion, given that the fluorescent markers tagged to the U937 human myeloid leukaemia EVs used in the study were found to accumulate in the liver and eventually detected in the faeces [10]. However, these fluorescent markers were also predominantly detected in CT26 mouse colon adenocarcinoma cells targeted by the EVs, probably because the EVs might have already undergone disintegration in the cells, and the fluorescent marker component might have been excreted *via* exocytosis before being transported to the liver *via* systemic circulation. In other words, the accumulation of dyes associated with EVs in the liver is not synonymous with a

definite uptake of EVs by Kupffer cells. Nevertheless, EVs may still be removed by immune cells, as shown in another study where melanoma, myoblast, fibroblast, aortic endothelial and macrophage-like cell exosomes from rodents were eliminated by rodent liver macrophages *in vivo*, most likely due to the presence of PS on EVs which is recognised by macrophages [135]. As to whether these EVs possess high amounts of CD47, CD24, CD31 or PD-L1, the study did not include such findings.

3.4 Neurological barriers

The blood-brain barrier (BBB) is characterized by an innermost layer of endothelial cells (which prevents blood and extracellular fluid from mixing), pericytes surrounding the endothelial cells and astrocyte end-feet acting as a sheath in the outermost layer. Though the movement of substances across the BBB is tightly regulated [136], different EVs have been observed to cross the BBB. One study demonstrated the ability of exosomes to carry miR-193b-3p across the BBB to exert an anti-inflammatory effect on rodent brain cells with subarachnoid haemorrhage [137], although the mechanism of crossing was unclear. Other studies involving the BBB in zebrafish showcased the ability of various human breast cancer cell EVs and brain endothelial cell EVs to cross the BBB *via* clathrin-mediated endocytosis [7, 138] and macropinocytosis [138], a notable surface protein that enabled clathrin-mediated endocytosis being CD63 [7]. Another study conducted on rodent BBB showed that human and rodent EVs derived from both cancer and non-cancer cells were able to cross the BBB *via* adsorptive-mediated transcytosis, which correlated with the presence of CD46 on the surface of EVs [11].

Modifications have also been made to EVs to enhance their ability to cross the BBB. In one experiment, after overexpressing the rabies virus glycoprotein (RVG) peptide on their surface, dendritic exosomes became significantly localized in rodents' brain cells [139]. Mouse L929 fibroblastic cell exosomes loaded with methotrexate and functionalized with LDL peptide in another experiment showed enhanced BBB exosome extravasation in rodents [140]. When miR-210-loaded mesenchymal stromal cell-derived exosomes were coupled with c(RGDyK) peptide in another experiment, they displayed enhanced targeting of rodent ischaemic brain cells, indicating greater angiogenesis and improving animal survival significantly [141]. Another experiment showed that RGE-Exo EVs demonstrated greater accumulation and duration of accumulation in murine glioma tumour cells than free exosomes [142].

Apart from surface components, the size of EVs might also be a crucial factor in determining whether EVs can cross the BBB, as deduced from another study where intranasal administration of exosomes to rodent microglial cells *via* the extra-neuronal pathway showed rapid translocation of exosomes to target cells, in contrast to larger microparticles of at least 500 nm in diameter which did not reach these target cells [143]. However, surface components of EVs might be a more vital factor than the size of EVs in enabling passage across the BBB, as proven by how larger brain endothelial EVs can penetrate the BBB better than smaller EVs of the same cell source, due to the higher levels of CD63 in the larger EVs [7, 106].

The blood-labyrinth barrier (BLaB) and blood-retinal barrier (BRB) are two other neurological barriers pertaining to the ear and eye, respectively. The BLaB consists of five layers, namely, the blood-endolymph barrier, blood-perilymph barrier, cerebrospinal-fluid-perilymph barrier, middle-ear-labyrinth barrier and endolymph-perilymph barrier [106, 144]. The BRB consists of the retinal vascular endothelium and the retinal pigment epithelium (RPE) [106]. These two barriers share similarities

with each other and the BBB, though the number of EV studies on these two barriers is smaller than that involving passage across the BBB [106]. Nevertheless, the utilization of EVs as potential drug carriers targeting the ear and eye with negligible side effects is worth further research, especially when current drug treatments have resulted in adverse side effects [106]. EVs from RPE cells are involved in the progression of age-related macular degeneration *via* regulating the production of pigment granule and lipid balance in RPE cells [106, 145]. They also promote vascular leakage *via* miR-105 which interferes with the tight cellular junctions of barriers [106, 146]. It is hoped that these seemingly destructive EV mechanisms can be manipulated to enable drug delivery across the BLaB and BRB, by modifying these EVs in a way that does not harm the barriers yet still permits their passage across them.

3.5 Lymphatic barrier

The process of crossing the blood-lymph barrier (BLyB) is regulated by various mechanisms including extravasation, overcoming of the interstitium, diffusion and passage through the mucosal barrier [106, 147]. In addition, the collagen reticular network (RN) hinders soluble substances from passing through [106, 148–153]. However, EVs possess certain characteristics that enable them to cross the BLyB. For instance, human ovarian cancer cell exosomes were found to be able to travel from the periphery to the lymph node in just a matter of minutes in rodents due to their small size [106, 154], and their lipidic rather than soluble nature seemed to enable them to cross the RN [106, 155].

Although EVs already possess intrinsic advantages that enable them to cross the BLyB, methods like microfluidic surface engineering have been conducted on EVs to modify them further as potential drug carriers for lymphoma treatment or other diseases related to the lymphatic system [106, 156, 157]. A recent study explored the modification of exosomes derived from bovine serum. α -D-mannose was added to the exosomes containing immune stimulators to enable them to interact with the mannose receptors on dendritic cells for uptake, and the exosomes were PEGylated. This has been found to enhance the internalisation of the exosomes by murine dendritic cells and to increase their localisation in the lymph nodes, paving the way for efficient delivery of immune stimulators *via* EVs *in vivo* as a potential form of drug therapy [158].

3.6 Pulmonary barrier

Located in the lungs, the blood-air barrier (BAB) possesses characteristics that enable it to guard against pathogenic invasion. For instance, the lung mucosa is a rich source of immune cells [106, 159], and lung epithelial cells can sense a wide range of bacteria and viruses *via* a broad array of membrane-bound, endosomal and cytosolic pattern-recognition receptors (PRR) [106, 160]. In response to the presence of pathogens, the BAB regulates paracellular flow, cell-to-cell communication, synthesis of mucus and the composition of periciliary fluid [160], which complements the removal of foreign substances *via* ciliary action [106, 161]. While the passage of EVs across the BAB is still largely unexplored [106], exosomes derived from bronchoalveolar lavage fluid (BALF) have been discovered to possess a similar protein profile to mesenchymal derived dendritic cells, given that they carry CD54, CD63, CD86 and in particular, MHC classes I and II [162, 163], implying their involvement in triggering

an immune response against pathogenic invasion in the BAB. In light of this, BALF exosomes might potentially be used as diagnostic biomarkers for pathogenic detection, in addition to engineering them as personalised medicine for effective drug delivery across the BAB.

3.7 Renal barrier

The nephron's ability to efficiently filter out waste materials from the blood into the urine is attributed to the high pressure in the glomerulus due to high blood flow, as well as the presence of the glomerular filtration barrier consisting of three layers—fenestrated endothelium, glomerular basement membrane and glomerular epithelium [66, 164–166]. Despite the tiny pores (2.5–2.8 nm) of the glomerular basement membrane [166, 167] which are smaller than the smallest EVs (30 nm [16–18]), and the presence of filter proteins lining the slits in the glomerular epithelium [165, 166, 168], the urine is surprisingly a rich source of EVs from both renal and non-renal sources. While the majority of EVs found in urine originate from the kidney, urinary bladder, testis, prostate, epididymis and seminal vesicle [169–172], studies have also identified EVs from outside the urinary tract, such as those carrying biomarkers of acute myocardial infarction [173]. EVs injected into the bloodstream of rodents in one study were found to accumulate in the kidneys, and eventually the urine, without undergoing degradation, as indicated by their ability to be internalized by HEK293 cells after being retrieved from the urine and introduced to the cells [166]. The presence of EVs in urine might imply that EVs can squeeze through the tiny pores of the glomerular filtration barrier due to their fluid membranes, or undergo mechanisms like transcytosis to reach the glomerular filtrate. It is also logical to deduce that EVs can cross the glomerular filtration barrier better in pathological conditions like diabetic nephropathy when the glomerular filtration barrier becomes more porous due to injury [166].

3.8 Cellular barriers

EVs that eventually reach the target cell has to overcome the plasma membrane, escape the endosome and evade lysosomal degradation to release their cargo into the cytosol (**Figure 3**).

The plasma membrane is an intricate structure consisting of various domains formed *via* different mechanisms. Some of these mechanisms include the formation of plasma membrane protein fences to reduce lateral diffusion in the plasma membrane, the arrangement of plasma membrane proteins into a scaffold that interacts with certain plasma membrane lipids, and protein-lipid interaction to form lipid rafts [174]. These domains represent the first barrier that a freshly-secreted EV needs to cross and determine the way the target cell internalizes EVs. Environmental factors also influence the interaction of EVs with the plasma membrane. For instance, the uptake of EVs *via* fusion with the cell membrane is observed to occur at a higher rate under acidic conditions [175], while endocytosis is shown to be hindered by neutral pH or high cholesterol levels [14, 176].

EVs can be internalised by target cells *via* endocytosis, be it caveolae-dependent endocytosis, flotillin-dependent endocytosis, ARF6-dependent endocytosis or other forms of endocytosis [105]. Clathrin-mediated endocytosis (CME), or “receptor-mediated endocytosis”, plays an especially prominent role in the uptake of small EVs [105],

Figure 3.

Internalisation, lysosomal degradation and lysosomal escape mechanisms of extracellular vesicles (EVs) in target cells. Upon reaching the target cell, EVs may be internalised by the cell via endocytosis or direct fusion with the plasma membrane. EVs that are internalised via endocytosis are packaged into endosomes, which may fuse with lysosomes to degrade the EVs. To escape lysosomal degradation, endocytosed EVs may undergo retrograde trafficking to the trans-Golgi network, endocytic recycling to be secreted out of the cell, or another mechanism altogether. Endocytosed EVs that do not undergo lysosomal degradation fuse with the endosomal membrane via the mediation of soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins to release their cargo into the cytosol. EVs that fuse directly with the plasma membrane to release their cargo into the cytosol evade endosomal and lysosomal activity completely (created with BioRender.com).

as supported by recent studies on the uptake of human epidermoid carcinoma EVs [177] and rat pheochromocytoma EVs [178] by human cervical carcinoma (HeLa) cells and rat bone marrow-derived mesenchymal stromal cells respectively. During CME, a temporary membrane scaffold forms as a result of membrane binding of Bin/amphiphysin/Rvs (BAR) domain-containing proteins which recruit clathrin. Clathrin then binds to the cytoplasmic tails of membrane proteins with the help of adaptor proteins, resulting in a clathrin-coated pit that internalises the EV [105]. EVs internalised *via* endocytosis are packaged into endosomes. These EVs then proceed to release their cargo into the cytoplasm by fusion of their membranes with the endosomal membrane, a process mediated by soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins which join the cytosolic sides of the EV and endosomal membranes together [3].

In general, the CME of EVs is initiated through the mediation of lectins, tetraspanins, cell adhesion proteins and other receptor-ligand interactions [18, 179]. For instance, exosomes from macrophages possess C-type lectin, which interacts with the C-type lectin receptor found on dendritic and brain endothelial cells [179, 180]. Galectin-5 on red blood cell (RBC) EVs enables them to be internalised by macrophages [45, 179]. Integrins on tumour EVs have been associated with the uptake of these EVs by lung fibroblasts and liver macrophages [42, 179]. Exosomes and target cells can exploit the interaction between intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function-associated antigen 1 (LFA-1) in exosomal uptake [179–181]. Heparan sulphate proteoglycans on target cells bind to EV fibronectin to facilitate uptake of EVs [54, 179, 182]. The high levels of outward-facing PS on the surface of exosomes also enable the recognition and

uptake of these exosomes by antigen-presenting cells *via* T-cell immunoglobulin and mucin domain (TIM) receptors located on the antigen-presenting cells' surface [63, 179, 183].

EVs internalised *via* endocytosis might risk being degraded by lysosomes in the cytosol [3]. The fusion of the membrane of endosomes containing the endocytosed EV with the lysosomal membrane is mediated by SNARE proteins and involves the active transport of vesicles along the cytoskeleton [18]. EV size may play a role in determining the fate of EVs upon uptake *via* endocytosis, as EVs larger than 100 nm may require macropinocytosis for their uptake, which tends towards lysosomal degradation more than other internalisation mechanisms accessible to smaller EVs [177, 184, 185]. Endocytosed EVs might escape lysosomes *via* pathways similar to those of viruses, like the CD81 positive lysosome-associated membrane protein 1 (Lamp-1) negative route in dendritic cells which resembles that of HIV-1 uptake [186]. A study showed that HEK293 exosomes internalised by human fibroblastic, hepatic and renal cells were transported to the endoplasmic reticulum where they released their cargo, a pathway that might be a potential escape route from lysosomal degradation [187]. EVs may also evade lysosomal degradation *via* endocytic recycling out of the cell [188] or retrograde trafficking from the endosomal pathway to the trans-Golgi network [189].

EVs have also been reported to deliver their cargo into target cells *via* direct membrane fusion with the cell membrane, with EV surface proteins syncytin-1 and syncytin-2 seemingly playing a significant role in this [190–192]. Originally found on the plasma membranes of placental trophoblast cells [190, 191], gamete cells [190, 193] and various cancerous and non-cancerous cells known to fuse directly with other cells [190, 194–197], these proteins have also been detected on EVs secreted from these cells [190, 192]. In light of this, incorporating these surface proteins into EVs to increase their uptake *via* direct membrane fusion might be a possible way to evade endocytosis and lysosomal degradation completely.

4. Recommendation—EVs as drug carriers

In providing a recommendation to engineer EVs as DDSs, EV engineering methods to overcome specific barriers can be deployed. Natural evasion of immune cells is a highly favourable quality and should mark all engineered EVs regardless of the barrier(s) they target. CD47, CD24, CD31 and PD-L1 are prominent surface proteins that achieve this quality and should be incorporated into engineered EVs in high amounts if not originally present [129–134]. Fusing EVs with liposomes to create hybrid DDSs can also increase their drug loading capacity without risking cargo aggregation [198, 199].

The choice of the source of EVs depends on its availability and the ability of its EVs to overcome respective biological barriers associated with the disease. Milk and plant-derived EVs, which are highly available in nature and able to overcome gastrointestinal barriers [76, 77, 85–105], can be engineered for drug delivery *via* the oral route to treat IBD. The patient's own EVs might also be used as a form of personalised medicine. EVs from the patient may be chosen based on whether their cell of origin matches the target cell for better selectivity, but there are exceptions. For instance, immune and intestinal cell-derived EVs can be internalized by placental cells [103, 128]. Breast milk might also be a possible EV drug carrier source to treat

both gastrointestinal and neurological conditions, as milk-derived EVs can cross gastrointestinal barriers [76, 85–90] and the BBB [87, 96] respectively. Human Type O RBC EVs loaded with antisense oligonucleotides were also found to target human leukaemia and breast cancer cells *in vitro* and *in vivo*. This is advantageous as RBCs are widely accessible from blood banks and lack DNA, which ensures that no oncogenic material is transferred from EVs to target cells [200]. This offers diverse compatible EV sources to choose from for a single ailment, enhancing the flexibility of the engineering process.

A summary of the recommendation is shown in **Figure 4**.

Figure 4.

Recommendation on how to engineer extracellular vesicles (EVs) to overcome various biological barriers for drug delivery applications. (1) **Gastrointestinal barriers** can be overcome by deploying milk and plant-derived EVs to withstand the harsh acidic and enzymatic conditions, while also ensuring that EVs possess surface glycoproteins to enable trans-endocytosis across the small intestinal barrier. (2) **The placental barrier** can be overcome directly via the use of placental and THP-1 monocyte EVs, or indirectly via engineering watermelon EVs to target intestinal epithelial cells (IECs) which can communicate with the placenta. (3) **Immunological barriers** can be overcome by having a high proportion of CD47, CD24, CD31 and PD-L1 to produce the “don’t eat me” signal, and probably a low level of phosphatidylserine (PS) on the surface of EVs to minimize the chances of being engulfed by macrophages. (4) **Neurological barriers** can be overcome minimally by incorporating high amounts of CD46 and CD63 into EVs as the quantity of these tetraspanins are positively correlated with the ability of EVs to cross the blood-brain barrier (BBB). (5) **The lymphatic barrier** can be overcome by EVs naturally as their lipidic nature enables them to cross the reticular network of the blood-lymph barrier (BLyB). Adding α -D-mannose and PEGylating EVs may also enhance their passage across the barrier. (6) **The pulmonary barrier** may be targeted by EVs derived from bronchoalveolar lavage fluid (BALF), which possess MHC classes I and II, CD54, CD63 and CD86. (7) **The renal barrier** can be overcome by EVs naturally, probably due to their small size and fluid lipid membranes which might allow them to squeeze through the tiny pores of the glomerular filtration barrier. (8) **Cellular barriers** can be overcome by EVs naturally via retrograde trafficking, endocytic recycling, direct fusion with the plasma membrane or other mechanisms. Engineering EVs with a size of <100 nm might help to reduce the chances of EVs being internalised via macropinocytosis which tends to lead to lysosomal degradation more than other mechanisms accessible to smaller EVs. Incorporating syncytin-1 and -2 into EVs might also enable them to fuse with the plasma membrane directly, allowing them to evade the endosomal and lysosomal membranes completely (created with BioRender.com).

5. Conclusion—the future of EVs

Through critically analysing the relationship between the key components of EVs and the biological barriers EVs overcome, this review is the first to put together a recommendation in such a manner (**Figure 4**) to engineer EVs as suitable DDSs based on various studies. The implementation of EV drug carriers would revolutionise the global worldview of therapeutic treatments, as EVs unlock a whole new realm of endless possibilities in achieving the ideal therapeutic for patients, one of maximum efficacy and biocompatibility with negligible side effects. Even now, efforts have been made to transform the notion of personalised medicine into a reality, and having EVs as fully-approved personalised DDSs is worth pursuing. As past findings are limited due to the complex nature of EVs and various biological membranes, it is hoped that the mechanisms of EVs and their interactions with various biological membranes can continue to be more fully delved into, and that EV engineering can be carried out *via* efficacious yet sustainable methods, bearing in mind the availability and accessibility of natural EV sources, and hence the cost-effectiveness of the engineering processes.

Author details

Jacob Yeo Xian Ping, Yub Raj Neupane and Giorgia Pastorin^{*†}
Department of Pharmacy, National University of Singapore, Singapore

*Address all correspondence to: phapg@nus.edu.sg

† Equal contribution.

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Abels ER, Breakefield XO. Introduction to extracellular vesicles: Biogenesis, RNA cargo selection, content, release, and uptake. *Cellular and Molecular Neurobiology*. 2016;**36**(3): 301-312
- [2] Meng W, He C, Hao Y, Wang L, Li L, Zhu G. Prospects and challenges of extracellular vesicle-based drug delivery system: Considering cell source. *Drug Delivery*. 2020;**27**(1):585-598
- [3] Mathieu M, Martin-Jaular L, Lavieu G, Théry C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nature Cell Biology*. 2019;**21**(1):9-17
- [4] Yáñez-Mó M, Siljander PR-M, Andreu Z, Zavec AB, Borràs FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. *Journal of Extracellular Vesicles*. 2015;**4**(1):27066
- [5] Beltrami C, Besnier M, Shantikumar S, Shearn AIU, Rajakaruna C, Laftah A, et al. Human pericardial fluid contains exosomes enriched with cardiovascular-expressed MicroRNAs and promotes therapeutic angiogenesis. *Molecular Therapy*. 2017;**25**(3):679-693
- [6] Lu J, Yang J, Zheng Y, Chen X, Fang S. Extracellular vesicles from endothelial progenitor cells prevent steroid-induced osteoporosis by suppressing the ferroptotic pathway in mouse osteoblasts based on bioinformatics evidence. *Scientific Reports*. 2019;**9**(1):16130
- [7] Yang T, Martin P, Fogarty B, Brown A, Schurman K, Phipps R, et al. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in *Danio rerio*. *Pharmaceutical Research*. 2015;**32**(6):2003-2014
- [8] Busatto S, Pham A, Suh A, Shapiro S, Wolfram J. Organotropic drug delivery: Synthetic nanoparticles and extracellular vesicles. *Biomedical Microdevices*. 2019;**21**(2):46
- [9] Elsharkasy OM, Nordin JZ, Hagey DW, de Jong OG, Schiffelers RM, Andaloussi SE, et al. Extracellular vesicles as drug delivery systems: Why and how? *Advanced Drug Delivery Reviews*. 2020;**159**:332-343
- [10] Goh WJ, Zou S, Ong WY, Torta F, Alexandra AF, Schiffelers RM, et al. Bioinspired cell-derived nanovesicles versus exosomes as drug delivery systems: A cost-effective alternative. *Scientific Reports*. 2017;**7**(1):14322
- [11] Banks WA, Sharma P, Bullock KM, Hansen KM, Ludwig N, Whiteside TL. Transport of extracellular vesicles across the blood-brain barrier: Brain pharmacokinetics and effects of inflammation. *International Journal of Molecular Sciences*. 2020;**21**(12):4407
- [12] Saint-Pol J, Gosselet F, Duban-Deweer S, Pottiez G, Karamanos Y. Targeting and crossing the blood-brain barrier with extracellular vesicles. *Cell*. 2020;**9**(4):851
- [13] van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nature Reviews. Molecular Cell Biology*. 2018;**19**(4): 213-228
- [14] Antimisiaris SG, Mourtas S, Marazioti A. Exosomes and exosome-inspired vesicles for targeted drug delivery. *Pharmaceutics*. 2018;**10**(4):218

- [15] Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: Current perspectives and future challenges. *Acta Pharmaceutica Sinica B*. 2016;**6**(4):287-296
- [16] Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annual Review of Cell and Developmental Biology*. 2014;**30**(1):255-289
- [17] El Andaloussi S, Mäger I, Breakefield XO, MJA W. Extracellular vesicles: Biology and emerging therapeutic opportunities. *Nature Reviews. Drug Discovery*. 2013;**12**(5): 347-357
- [18] Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: From biogenesis to uptake and intracellular signalling. *Cell Communication and Signaling: CCS*. 2021;**19**(1):47
- [19] Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cell*. 2019;**8**(7):727
- [20] Cvjetkovic A, Lötval J, Lässer C. The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. *Journal of Extracellular Vesicles*. 2014;**3**(1): 23111
- [21] Duong P, Chung A, Bouchareychas L, Raffai RL. Cushioned-density gradient ultracentrifugation (C-DGUC) improves the isolation efficiency of extracellular vesicles. *PLoS One*. 2019;**14**(4):e0215324
- [22] Takov K, Yellon DM, Davidson SM. Comparison of small extracellular vesicles isolated from plasma by ultracentrifugation or size-exclusion chromatography: Yield, purity and functional potential. *Journal of Extracellular Vesicles*. 2019;**8**(1):1560809
- [23] Kanada M, Bachmann MH, Hardy JW, Frimannson DO, Bronsart L, Wang A, et al. Differential fates of biomolecules delivered to target cells via extracellular vesicles. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;**112**(12): E1433-E1442
- [24] Dang XTT, Kavishka JM, Zhang DX, Pirisinu M, Le MTN. Extracellular vesicles as an efficient and versatile system for drug delivery. *Cell*. 2020;**9**(10):2191
- [25] Sluijter JPG, Verhage V, Deddens JC, van den Akker F, Doevendans PA. Microvesicles and exosomes for intracardiac communication. *Cardiovascular Research*. 2014;**102**(2): 302-311
- [26] Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: Microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood*. 1999;**94**(11):3791-3799
- [27] van Dommelen SM, Vader P, Lakhal S, Kooijmans SAA, van Solinge WW, Wood MJA, et al. Microvesicles and exosomes: Opportunities for cell-derived membrane vesicles in drug delivery. *Journal of Controlled Release*. 2012;**161**(2):635-644
- [28] Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular vesicles: Composition, biological relevance, and methods of study. *Bioscience*. 2015;**65**(8):783-797
- [29] Géminard C, De Gassart A, Blanc L, Vidal M. Degradation of AP2 during

reticulocyte maturation enhances binding of hsc70 and Alix to a common site on TFR for sorting into exosomes: AP2 degradation induces TfR sorting in reticulocyte exosomes. *Traffic*. 2004;**5**(3):181-193

[30] Théry C, Boussac M, Véron P, Ricciardi-Castagnoli P, Raposo G, Garin J, et al. Proteomic analysis of dendritic cell-derived exosomes: A secreted subcellular compartment distinct from apoptotic vesicles. *Journal of Immunology*. 2001;**166**(12):7309-7318

[31] van der Pol E, Böing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacological Reviews*. 2012;**64**(3):676-705

[32] Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nature Cell Biology*. 2011;**13**(4):423-433

[33] Di Vizio D, Morello M, Dudley AC, Schow PW, Adam RM, Morley S, et al. Large oncosomes in human prostate cancer tissues and in the circulation of mice with metastatic disease. *The American Journal of Pathology*. 2012;**181**(5):1573-1584

[34] Llorente A, Skotland T, Sylvänne T, Kauhanen D, Róg T, Orłowski A, et al. Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. *Biochimica et Biophysica Acta*. 2013;**1831**(7):1302-1309

[35] György B, Szabó TG, Pásztói M, Pál Z, Misják P, Aradi B, et al. Membrane vesicles, current state-of-the-art: Emerging role of extracellular vesicles. *Cellular and Molecular Life Sciences*. 2011;**68**(16):2667-2688

[36] Lee E-Y, Choi D-Y, Kim D-K, Kim J-W, Park JO, Kim S, et al. Gram-positive bacteria produce membrane vesicles: Proteomics-based characterization of *Staphylococcus aureus*-derived membrane vesicles. *Proteomics*. 2009;**9**(24):5425-5436

[37] Bottero D, Gaillard ME, Errea A, Moreno G, Zurita E, Pianciola L, et al. Outer membrane vesicles derived from *Bordetella parapertussis* as an acellular vaccine against *Bordetella parapertussis* and *Bordetella pertussis* infection. *Vaccine*. 2013;**31**(45):5262-5268

[38] Jang SC, Kim SR, Yoon YJ, Park K-S, Kim JH, Lee J, et al. In vivo kinetic biodistribution of nano-sized outer membrane vesicles derived from bacteria. *Small*. 2015;**11**(4):456-461

[39] Lee E-Y, Choi D-S, Kim K-P, Ghoo YS. Proteomics in gram-negative bacterial outer membrane vesicles. *Mass Spectrometry Reviews*. 2008;**27**(6):535-555

[40] Berleman JE, Allen S, Danielewicz MA, Remis JP, Gorur A, Cunha J, et al. The lethal cargo of *Myxococcus xanthus* outer membrane vesicles. *Frontiers in Microbiology*. 2014;**5**:474

[41] Goes A, Lapuhs P, Kuhn T, Schulz E, Richter R, Panter F, et al. Myxobacteria-derived outer membrane vesicles: Potential applicability against intracellular infections. *Cell*. 2020;**9**(1):194

[42] Hoshino A, Costa-Silva B, Shen T-L, Rodrigues G, Hashimoto A, Tesic Mark M, et al. Tumour exosome integrins determine organotropic metastasis. *Nature*. 2015;**527**(7578):329-335

[43] Tao S-C, Guo S-C, Zhang C-Q. Platelet-derived extracellular vesicles: An emerging therapeutic approach.

International Journal of Biological Sciences. 2017;**13**(7):828-834

[44] Zaldivia MTK, McFadyen JD, Lim B, Wang X, Peter K. Platelet-derived microvesicles in cardiovascular diseases. *Frontiers in Cardiovascular Medicine*. 2017;**4**:74

[45] Barrès C, Blanc L, Bette-Bobillo P, André S, Mamoun R, Gabius H-J, et al. Galectin-5 is bound onto the surface of rat reticulocyte exosomes and modulates vesicle uptake by macrophages. *Blood*. 2010;**115**(3):696-705

[46] Bilen MA, Pan T, Lee Y-C, Lin S-C, Yu G, Pan J, et al. Proteomics profiling of exosomes from primary mouse osteoblasts under proliferation versus mineralization conditions and characterization of their uptake into prostate cancer cells. *Journal of Proteome Research*. 2017;**16**(8):2709-2728

[47] Yoshioka Y, Konishi Y, Kosaka N, Katsuda T, Kato T, Ochiya T. Comparative marker analysis of extracellular vesicles in different human cancer types. *Journal of Extracellular Vesicles*. 2013;**2**(1):20424

[48] Nakase I, Kobayashi NB, Takatani-Nakase T, Yoshida T. Active macro-pinocytosis induction by stimulation of epidermal growth factor receptor and oncogenic Ras expression potentiates cellular uptake efficacy of exosomes. *Scientific Reports*. 2015; **5**(10300). <https://www.nature.com/articles/srep10300>

[49] Kooijmans SAA, Aleza CG, Roffler SR, van Solinge WW, Vader P, Schiffelers RM. Display of GPI-anchored anti-EGFR nanobodies on extracellular vesicles promotes tumour cell targeting. *Journal of Extracellular Vesicles*. 2016;**5**(1):31053

[50] Tauro BJ, Greening DW, Mathias RA, Mathivanan S, Ji H, Simpson RJ. Two

distinct populations of exosomes are released from LIM1863 colon carcinoma cell-derived organoids. *Molecular & Cellular Proteomics*. 2013;**12**(3):587-598

[51] Huber V, Fais S, Iero M, Lugini L, Canese P, Squarcina P, et al. Human colorectal cancer cells induce T-cell death through release of proapoptotic microvesicles: Role in immune escape. *Gastroenterology*. 2005;**128**(7):1796-1804

[52] Calzolari A, Raggi C, Deaglio S, Spasi NM, Stafsnes M, Fecchi K, et al. TfR2 localizes in lipid raft domains and is released in exosomes to activate signal transduction along the MAPK pathway. *Journal of Cell Science*. 2006;**119**(Pt 21): 4486-4498

[53] He M, Qin H, Poon TC, Sze SC, Ding X, Co NN. Hepatocellular carcinoma-derived exosomes promote motility of immortalized hepatocyte through transfer of oncogenic proteins and RNAs. *Carcinogenesis*. 2015;**36**: 1008-1018

[54] Purushothaman A, Bandari SK, Liu J, Mobley JA, Brown EE, Sanderson RD. Fibronectin on the surface of myeloma cell-derived exosomes mediates exosome-cell interactions. *The Journal of Biological Chemistry*. 2016;**291**(4):1652-1663

[55] Keryer-Bibens C, Pioche-Durieu C, Villemant C, Souquère S, Nishi N, Hirashima M, et al. Exosomes released by EBV-infected nasopharyngeal carcinoma cells convey the viral latent membrane protein 1 and the immunomodulatory protein galectin 9. *BMC Cancer*. 2006;**6**(1):283

[56] Liang B, Peng P, Chen S, Li L, Zhang M, Cao D, et al. Characterization and proteomic analysis of ovarian cancer-derived exosomes. *Journal of Proteomics*. 2013;**80**:171-182

- [57] Saunderson SC, Dunn AC, Crocker PR, McLellan AD. CD169 mediates the capture of exosomes in spleen and lymph node. *Blood*. 2014;**123**(2):208-216
- [58] Blanchard N, Lankar D, Faure F, Regnault A, Dumont C, Raposo G, et al. TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *Journal of Immunology*. 2002;**168**(7):3235-3241
- [59] Pienimaeki-Roemer A, Kuhlmann K, Böttcher A, Konovalova T, Black A, Orsó E, et al. Lipidomic and proteomic characterization of platelet extracellular vesicle subfractions from senescent platelets: PLT extracellular vesicle fractions. *Transfusion*. 2015;**55**(3):507-521
- [60] Miksa M, Wu R, Dong W, Das P, Yang D, Wang P. Dendritic cell-derived exosomes containing milk fat globule epidermal growth factor-factor VIII attenuate proinflammatory responses in sepsis. *Shock*. 2006;**25**(6):586-593
- [61] Morelli AE, Larregina AT, Shufesky WJ, Sullivan MLG, Stolz DB, Papworth GD, et al. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood*. 2004;**104**(10):3257-3266
- [62] Obregon C, Rothen-Rutishauser B, Gerber P, Gehr P, Nicod LP. Active uptake of dendritic cell-derived exovesicles by epithelial cells induces the release of inflammatory mediators through a TNF-alpha-mediated pathway. *The American Journal of Pathology*. 2009;**175**(2):696-705
- [63] Baglio SR, van Eijndhoven MAJ, Koppers-Lalic D, Berenguer J, Loughheed SM, Gibbs S, et al. Sensing of latent EBV infection through exosomal transfer of 5'pppRNA. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;**113**(5):E587-E596
- [64] Chakraborty S, Núñez D, Hu S-Y, Domingo MP, Pardo J, Karmenyan A, et al. FRET based quantification and screening technology platform for the interactions of leukocyte function-associated antigen-1 (LFA-1) with intercellular adhesion molecule-1 (ICAM-1). *PLoS One*. 2014;**9**(7):e102572
- [65] Mueller SK, Nocera AL, Bleier BS. Exosome function in aerodigestive mucosa. *Nanomedicine*. 2018;**14**(2):269-277
- [66] Kwon SH. Extracellular vesicles in renal physiology and clinical applications for renal disease. *The Korean Journal of Internal Medicine*. 2019;**34**(3):470-479
- [67] Eirin A, Riester SM, Zhu X-Y, Tang H, Evans JM, O'Brien D, et al. MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells. *Gene*. 2014;**551**(1):55-64
- [68] Haga H, Yan IK, Borrelli DA, Matsuda A, Parasramka M, Shukla N, et al. Extracellular vesicles from bone marrow-derived mesenchymal stem cells protect against murine hepatic ischemia/reperfusion injury. *Liver Transplantation*. 2017;**23**(6):791-803
- [69] Katakowski M, Buller B, Zheng X, Lu Y, Rogers T, Osobamiro O, et al. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. *Cancer Letters*. 2013;**335**(1):201-204
- [70] Lou G, Song X, Yang F, Wu S, Wang J, Chen Z, et al. Exosomes derived from miR-122-modified adipose tissue-derived MSCs increase chemosensitivity of hepatocellular carcinoma. *Journal of Hematology & Oncology*. 2015;**8**(1):122

- [71] Ramos TL, Sánchez-Abarca LI, Muntión S, Preciado S, Puig N, López-Ruano G, et al. MSC surface markers (CD44, CD73, and CD90) can identify human MSC-derived extracellular vesicles by conventional flow cytometry. *Cell Communication and Signaling: CCS*. 2016;**14**(1):2
- [72] Munoz JL, Bliss SA, Greco SJ, Ramkissoon SH, Ligon KL, Rameshwar P. Delivery of functional anti-miR-9 by mesenchymal stem cell-derived exosomes to glioblastoma multiforme cells conferred chemosensitivity. *Molecular Therapy: Nucleic Acids*. 2013;**2**(e126):e126
- [73] Pascucci L, Coccè V, Bonomi A, Ami D, Ceccarelli P, Ciusani E, et al. Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: A new approach for drug delivery. *Journal of Controlled Release*. 2014;**192**:262-270
- [74] Shimbo K, Miyaki S, Ishitobi H, Kato Y, Kubo T, Shimose S, et al. Exosome-formed synthetic microRNA-143 is transferred to osteosarcoma cells and inhibits their migration. *Biochemical and Biophysical Research Communications*. 2014;**445**(2):381-387
- [75] Shimoda A, Tahara Y, Sawada S-I, Sasaki Y, Akiyoshi K. Glycan profiling analysis using evanescent-field fluorescence-assisted lectin array: Importance of sugar recognition for cellular uptake of exosomes from mesenchymal stem cells. *Biochemical and Biophysical Research Communications*. 2017;**491**(3):701-707
- [76] Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Medicine*. 2013;**11**(1):200
- [77] Mu J, Zhuang X, Wang Q, Jiang H, Deng Z-B, Wang B, et al. Interspecies communication between plant and mouse gut host cells through edible plant derived exosome-like nanoparticles. *Molecular Nutrition & Food Research*. 2014;**58**(7):1561-1573
- [78] Arntz OJ, Pieters BCH, Oliveira MC, Broeren MGA, Bennink MB, de Vries M, et al. Oral administration of bovine milk derived extracellular vesicles attenuates arthritis in two mouse models. *Molecular Nutrition & Food Research*. 2015;**59**(9):1701-1712
- [79] Manca S, Giraud D, Zemleni J. The bioavailability and distribution of bovine milk exosomes is distinct from that of their cargos in mice. *The FASEB Journal*. 2017;**31**(S1). DOI: 10.1096/fasebj.31.1_supplement.148.2. https://www.researchgate.net/publication/352244149_The_Bioavailability_and_Distribution_of_Bovine_Milk_Exosomes_is_Distinct_from_that_of_their_Cargos_in_Mice
- [80] Reinhardt TA, Lippolis JD, Nonnecke BJ, Sacco RE. Bovine milk exosome proteome. *Journal of Proteomics*. 2012;**75**(5):1486-1492
- [81] Kosaka N, Izumi H, Sekine K, Ochiya T. microRNA as a new immune-regulatory agent in breast milk. *Silence*. 2010;**1**(1):7
- [82] Mincheva-Nilsson L, Baranov V. The role of placental exosomes in reproduction: Placental exosomes in reproduction. *American Journal of Reproductive Immunology*. 2010;**63**(6):520-533
- [83] Brzozowski JS, Jankowski H, Bond DR, McCague SB, Munro BR, Predebon MJ, et al. Lipidomic profiling of extracellular vesicles derived from

- p prostate and prostate cancer cell lines.
- Lipids in Health and Disease*
- . 2018;
- 17**
- (1):211
- [84] Barral J-P, Croibier A. Circulatory physiology. In: *Visceral Vascular Manipulations*. Elsevier; 2011. pp. 27-45
- [85] Tong L, Hao H, Zhang Z, Lv Y, Liang X, Liu Q, et al. Milk-derived extracellular vesicles alleviate ulcerative colitis by regulating the gut immunity and reshaping the gut microbiota. *Theranostics*. 2021;**11**(17):8570-8586
- [86] Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer Letters*. 2016;**371**(1):48-61
- [87] Sanwlani R, Fonseka P, Chitti SV, Mathivanan S. Milk-derived extracellular vesicles in inter-organism, cross-species communication and drug delivery. *Proteomes*. 2020;**8**(2):11
- [88] Wolf T, Baier SR, Zempleni J. The intestinal transport of bovine milk exosomes is mediated by endocytosis in human colon carcinoma Caco-2 cells and rat small intestinal IEC-6 cells. *The Journal of Nutrition*. 2015;**145**(10):2201-2206
- [89] Tulkens J, Vergauwen G, Van Deun J, Geurickx E, Dhondt B, Lippens L, et al. Increased levels of systemic LPS-positive bacterial extracellular vesicles in patients with intestinal barrier dysfunction. *Gut*. 2020;**69**(1):191-193
- [90] Lee B, Moon KM, Kim CY. Tight junction in the intestinal epithelium: Its association with diseases and regulation by phytochemicals. *Journal of Immunology Research*. 2018;**2018**:1-11
- [91] Izumi H, Kosaka N, Shimizu T, Sekine K, Ochiya T, Takase M. Bovine milk contains microRNA and messenger RNA that are stable under degradative conditions. *Journal of Dairy Science*. 2012;**95**(9):4831-4841
- [92] Baddela VS, Nayan V, Rani P, Onteru SK, Singh D. Physicochemical biomolecular insights into buffalo milk-derived nanovesicles. *Applied Biochemistry and Biotechnology*. 2016;**178**(3):544-557
- [93] Baier SR, Nguyen C, Xie F, Wood JR, Zempleni J. MicroRNAs are absorbed in biologically meaningful amounts from nutritionally relevant doses of cow milk and affect gene expression in peripheral blood mononuclear cells, HEK-293 kidney cell cultures, and mouse livers. *The Journal of Nutrition*. 2014;**144**(10):1495-1500
- [94] Howard KM, Jati Kusuma R, Baier SR, Friemel T, Markham L, Vanamala J, et al. Loss of miRNAs during processing and storage of cow's (*Bos taurus*) milk. *Journal of Agricultural and Food Chemistry*. 2015;**63**(2):588-592
- [95] Kirchner B, Pfaffl MW, Dimpler J, von Mutius E, Ege MJ. microRNA in native and processed cow's milk and its implication for the farm milk effect on asthma. *The Journal of Allergy and Clinical Immunology*. 2016;**137**(6):1893-1895, e13
- [96] Manca S, Upadhyaya B, Mutai E, Desaulniers AT, Cederberg RA, White BR, et al. Milk exosomes are bioavailable and distinct microRNA cargos have unique tissue distribution patterns. *Scientific Reports*. 2018;**8**(1). DOI: 10.1038/s41598-018-29780-1. <https://www.nature.com/articles/s41598-018-29780-1>
- [97] Zhao Z, Yu S, Xu M, Li P. Effects of microwave on extracellular vesicles and microRNA in milk. *Journal of Dairy Science*. 2018;**101**(4):2932-2940

- [98] Rahman MM, Shimizu K, Yamauchi M, Takase H, Ugawa S, Okada A, et al. Acidification effects on isolation of extracellular vesicles from bovine milk. *PLoS One*. 2019;**14**(9):e0222613
- [99] Deng Z, Rong Y, Teng Y, Mu J, Zhuang X, Tseng M, et al. Broccoli-derived nanoparticle inhibits mouse colitis by activating dendritic cell AMP-activated protein kinase. *Molecular Therapy*. 2017;**25**(7):1641-1654
- [100] Ju S, Mu J, Dokland T, Zhuang X, Wang Q, Jiang H, et al. Grape exosome-like nanoparticles induce intestinal stem cells and protect mice from DSS-induced colitis. *Molecular Therapy*. 2013;**21**(7):1345-1357
- [101] Wang B, Zhuang X, Deng Z-B, Jiang H, Mu J, Wang Q, et al. Targeted drug delivery to intestinal macrophages by bioactive nanovesicles released from grapefruit. *Molecular Therapy*. 2014;**22**(3):522-534
- [102] Zhang M, Viennois E, Prasad M, Zhang Y, Wang L, Zhang Z, et al. Edible ginger-derived nanoparticles: A novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. *Biomaterials*. 2016;**101**:321-340
- [103] Timms KM. Investigating the Effect of Plant-Derived Extracellular Vesicles on Human Placental Function. New York: The University of Manchester; 2018
- [104] Zhuang X, Deng Z-B, Mu J, Zhang L, Yan J, Miller D, et al. Ginger-derived nanoparticles protect against alcohol-induced liver damage. *Journal of Extracellular Vesicles*. 2015;**4**(1): 28713
- [105] Doherty GJ, McMahon HT. Mechanisms of endocytosis. *Annual Review of Biochemistry*. 2009;**78**(1): 857-902
- [106] Elliott RO, He M. Unlocking the power of exosomes for crossing biological barriers in drug delivery. *Pharmaceutics*. 2021;**13**(1):122
- [107] Wood CE, Keller-Wood M. The critical importance of the fetal hypothalamus-pituitary-adrenal axis. *F1000Research*. 2016;**5**:115
- [108] Condon JC, Jeyasuria P, Faust JM, Mendelson CR. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;**101**(14): 4978-4983
- [109] Gao L, Rabbitt EH, Condon JC, Renthal NE, Johnston JM, Mitsche MA, et al. Steroid receptor coactivators 1 and 2 mediate fetal-to-maternal signaling that initiates parturition. *The Journal of Clinical Investigation*. 2015;**125**(7): 2808-2824
- [110] Genbacev O, Zhou Y, Ludlow JW, Fisher SJ. Regulation of human placental development by oxygen tension. *Science*. 1997;**277**(5332):1669-1672
- [111] Mikaelsson MA, Constância M, Dent CL, Wilkinson LS, Humby T. Placental programming of anxiety in adulthood revealed by Igf2-null models. *Nature Communications*. 2013;**4**:2311
- [112] Houser BL. Decidual macrophages and their roles at the maternal-fetal interface. *The Yale Journal of Biology and Medicine*. 2012;**85**(1):105-118
- [113] Pemathilaka RL, Reynolds DE, Hashemi NN. Drug transport across the human placenta: Review of placenta-on-a-chip and previous approaches. *Interface Focus*. 2019;**9**(5):20190031

- [114] Robbins JR, Zeldovich VB, Poukchanski A, Boothroyd JC, Bakardjiev AI. Tissue barriers of the human placenta to infection with *Toxoplasma gondii*. *Infection and Immunity*. 2012;**80**(1):418-428
- [115] Miranda J, Paules C, Nair S, Lai A, Palma C, Scholz-Romero K, et al. Placental exosomes profile in maternal and fetal circulation in intrauterine growth restriction—Liquid biopsies to monitoring fetal growth. *Placenta*. 2018;**64**:34-43
- [116] Sarker S, Scholz-Romero K, Perez A, Illanes SE, Mitchell MD, Rice GE, et al. Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. *Journal of Translational Medicine*. 2014;**12**(1):204
- [117] Burnett LA, Nowak RA. Exosomes mediate embryo and maternal interactions at implantation and during pregnancy. *Frontiers in Bioscience (Scholar Edition)*. 2016;**8**(1):79-96
- [118] Chang G, Mouillet J-F, Mishima T, Chu T, Sadovsky E, Coyne CB, et al. Expression and trafficking of placental microRNAs at the feto-maternal interface. *The FASEB Journal*. 2017;**31**(7):2760-2770
- [119] Makiya R, Stigbrand T. Placental alkaline phosphatase has a binding site for the human immunoglobulin-G Fc portion. *European Journal of Biochemistry*. 1992;**205**(1):341-345
- [120] Makiya R, Stigbrand T. Placental alkaline phosphatase is related to human IgG internalization in HEp2 cells. *Biochemical and Biophysical Research Communications*. 1992;**182**(2):624-630
- [121] Mitchell MD, Peiris HN, Kobayashi M, Koh YQ, Duncombe G, Illanes SE, et al. Placental exosomes in normal and complicated pregnancy. *American Journal of Obstetrics and Gynecology*. 2015;**213**(4):S173-S181
- [122] Salomon C, Ryan J, Sobrevia L, Kobayashi M, Ashman K, Mitchell M, et al. Exosomal signaling during hypoxia mediates microvascular endothelial cell migration and vasculogenesis. *PLoS One*. 2013;**8**(7):e68451
- [123] Salomon C, Torres MJ, Kobayashi M, Scholz-Romero K, Sobrevia L, Dobierzewska A, et al. A gestational profile of placental exosomes in maternal plasma and their effects on endothelial cell migration. *PLoS One*. 2014;**9**(6):e98667
- [124] She QB, Mukherjee JJ, Chung T, Kiss Z. Placental alkaline phosphatase, insulin, and adenine nucleotides or adenosine synergistically promote long-term survival of serum-starved mouse embryo and human fetus fibroblasts. *Cellular Signalling*. 2000;**12**(9-10):659-665
- [125] She QB, Mukherjee JJ, Huang JS, Crilly KS, Kiss Z. Growth factor-like effects of placental alkaline phosphatase in human fetus and mouse embryo fibroblasts. *FEBS Letters*. 2000;**469**(2-3):163-167
- [126] Stefaner I, Stefanescu A, Hunziker W, Fuchs R. Expression of placental alkaline phosphatase does not correlate with IgG binding, internalization and transcytosis. *The Biochemical Journal*. 1997;**327**(2):585-592
- [127] Liu J, Wang S-Z, Wang Q-L, Du J-G, Wang B-B. Gestational diabetes mellitus is associated with changes in the concentration and bioactivity of placental exosomes in the maternal circulation across gestation. *European Review for Medical and Pharmacological Sciences*. 2018;**22**(7):2036-2043

- [128] Holder B, Jones T, Sancho Shimizu V, Rice TF, Donaldson B, Bouqueau M, et al. Macrophage exosomes induce placental inflammatory cytokines: A novel mode of maternal-placental messaging. *Traffic*. 2016;**17**(2):168-178
- [129] Matozaki T, Murata Y, Okazawa H, Ohnishi H. Functions and molecular mechanisms of the CD47-SIRP α signalling pathway. *Trends in Cell Biology*. 2009;**19**(2):72-80
- [130] Parada N, Romero-Trujillo A, Georges N, Alcayaga-Miranda F. Camouflage strategies for therapeutic exosomes evasion from phagocytosis. *Journal of Advanced Research*. 2021;**31**:61-74
- [131] Weiskopf K. Cancer immunotherapy targeting the CD47/SIRP α axis. *European Journal of Cancer*. 2017;**76**:100-109
- [132] Barkal AA, Brewer RE, Markovic M, Kowarsky M, Barkal SA, Zaro BW, et al. CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature*. 2019;**572**(7769):392-396
- [133] Brown S, Heinisch I, Ross E, Shaw K, Buckley CD, Savill J. Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. *Nature*. 2002;**418**(6894):200-203
- [134] Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature*. 2017;**545**(7655):495-499
- [135] Charoenviriyakul C, Takahashi Y, Morishita M, Matsumoto A, Nishikawa M, Takakura Y. Cell type-specific and common characteristics of exosomes derived from mouse cell lines: Yield, physicochemical properties, and pharmacokinetics. *European Journal of Pharmaceutical Sciences*. 2017;**96**:316-322
- [136] Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: An overview: Structure, regulation, and clinical implications. *Neurobiology of Disease*. 2004;**16**(1):1-13
- [137] Lai N, Wu D, Liang T, Pan P, Yuan G, Li X, et al. Systemic exosomal miR-193b-3p delivery attenuates neuroinflammation in early brain injury after subarachnoid hemorrhage in mice. *Journal of Neuroinflammation*. 2020;**17**(1):74
- [138] Morad G, Carman CV, Hagedorn EJ, Perlin JR, Zon LI, Mustafaoglu N, et al. Tumor-derived extracellular vesicles breach the intact blood-brain barrier via transcytosis. *ACS Nano*. 2019;**13**(12):13853-13865
- [139] Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJA. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotechnology*. 2011;**29**(4):341-345
- [140] Ye Z, Zhang T, He W, Jin H, Liu C, Yang Z, et al. Methotrexate-loaded extracellular vesicles functionalized with therapeutic and targeted peptides for the treatment of glioblastoma multiforme. *ACS Applied Materials & Interfaces*. 2018;**10**(15):12341-12350
- [141] Zhang H, Wu J, Wu J, Fan Q, Zhou J, Wu J, et al. Exosome-mediated targeted delivery of miR-210 for angiogenic therapy after cerebral ischemia in mice. *Journal of Nanobiotechnology*. 2019;**17**(1):29
- [142] Jia G, Han Y, An Y, Ding Y, He C, Wang X, et al. NRP-1 targeted and cargo-loaded exosomes facilitate

simultaneous imaging and therapy of glioma in vitro and in vivo. *Biomaterials*. 2018;**178**:302-316

[143] Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Molecular Therapy*. 2011;**19**(10): 1769-1779

[144] Sun W, Wang W. Advances in research on labyrinth membranous barriers. *Journal of Otology*. 2015;**10**(3):99-104

[145] Klingeborn M, Dismuke WM, Bowes Rickman C, Stamer WD. Roles of exosomes in the normal and diseased eye. *Progress in Retinal and Eye Research*. 2017;**59**:158-177

[146] Zhou W, Fong MY, Min Y, Somlo G, Liu L, Palomares MR, et al. Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. *Cancer Cell*. 2014;**25**(4): 501-515

[147] Thomas SN, Schudel A. Overcoming transport barriers for interstitial-, lymphatic-, and lymph node-targeted drug delivery. *Current Opinion in Chemical Engineering*. 2015;**7**:65-74

[148] Anderson AO, Anderson ND. Lymphocyte emigration from high endothelial venules in rat lymph nodes. *Immunology*. 1976;**31**(5):731-748

[149] Anderson AO, Anderson ND. Studies on the structure and permeability of the microvasculature in normal rat lymph nodes. *The American Journal of Pathology*. 1975;**80**(3):387-418

[150] Anderson AO, Shaw S. T cell adhesion to endothelium: The FRC

conduit system and other anatomic and molecular features which facilitate the adhesion cascade in lymph node. *Seminars in Immunology*. 1993;**5**(4):271-282

[151] Gretz JE, Anderson AO, Shaw S. Cords, channels, corridors and conduits: Critical architectural elements facilitating cell interactions in the lymph node cortex. *Immunological Reviews*. 1997;**156**(1):11-24

[152] Gretz JE, Kaldjian EP, Anderson AO, Shaw S. Sophisticated strategies for information encounter in the lymph node: The reticular network as a conduit of soluble information and a highway for cell traffic. *Journal of Immunology*. 1996;**157**(2):495-499

[153] Sainte-Marie G, Peng FS. Diffusion of a lymph-carried antigen in the fiber network of the lymph node of the rat. *Cell and Tissue Research*. 1986;**245**(3): 481-486

[154] Srinivasan S, Vannberg FO, Dixon JB. Lymphatic transport of exosomes as a rapid route of information dissemination to the lymph node. *Scientific Reports*. 2016;**6**(1):24436

[155] Gretz JE, Norbury CC, Anderson AO, Proudfoot AEI, Shaw S. Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex. *The Journal of Experimental Medicine*. 2000;**192**(10):1425-1440

[156] Zhao Z, McGill J, Gamero-Kubota P, He M. Microfluidic on-demand engineering of exosomes towards cancer immunotherapy. *Lab on a Chip*. 2019;**19**(10):1877-1886

[157] Zhu Q, Heon M, Zhao Z, He M. Microfluidic engineering of exosomes:

Editing cellular messages for precision therapeutics. *Lab on a Chip*. 2018;**18**(12):1690-1703

[158] Choi ES, Song J, Kang YY, Mok H. Mannose-modified serum exosomes for the elevated uptake to Murine dendritic cells and lymphatic accumulation. *Macromolecular Bioscience*. 2019; **19**(7):e1900042

[159] Anderson MJ, Parks PJ, Peterson ML. A mucosal model to study microbial biofilm development and anti-biofilm therapeutics. *Journal of Microbiological Methods*. 2013;**92**(2): 201-208

[160] Leiva-Juárez MM, Kolls JK, Evans SE. Lung epithelial cells: Therapeutically inducible effectors of antimicrobial defense. *Mucosal Immunology*. 2018;**11**(1):21-34

[161] Ganesan S, Comstock AT, Sajjan US. Barrier function of airway tract epithelium. *Tissue Barriers*. 2013;**1**(4):e24997

[162] Admyre C, Grunewald J, Thyberg J, Gripenbäck S, Tornling G, Eklund A, et al. Exosomes with major histocompatibility complex class II and co-stimulatory molecules are present in human BAL fluid. *The European Respiratory Journal*. 2003;**22**(4): 578-583

[163] Admyre C, Telemo E, Almqvist N, Lötval J, Lahesmaa R, Scheynius A, et al. Exosomes—Nanovesicles with possible roles in allergic inflammation. *Allergy*. 2008;**63**(4):404-408

[164] Kang YS, Li Y, Dai C, Kiss LP, Wu C, Liu Y. Inhibition of integrin-linked kinase blocks podocyte epithelial-mesenchymal transition and ameliorates proteinuria. *Kidney International*. 2010;**78**(4):363-373

[165] Patrakka J, Tryggvason K. Molecular make-up of the glomerular filtration barrier. *Biochemical and Biophysical Research Communications*. 2010;**396**(1):164-169

[166] Simeone P, Bologna G, Lanuti P, Pierdomenico L, Guagnano MT, Pieragostino D, et al. Extracellular vesicles as signaling mediators and disease biomarkers across biological barriers. *International Journal of Molecular Sciences*. 2020;**21**(7):2514

[167] Ota K, Ota Z, Shikata K, Makino H. The ultrastructural disruption of the glomerular basement membrane in diabetic nephropathy revealed by “tissue negative staining method”. *Journal of Diabetes and its Complications*. 1995;**9**(4):285-287

[168] Wu X, Gao Y, Xu L, Dang W, Yan H, Zou D, et al. Exosomes from high glucose-treated glomerular endothelial cells trigger the epithelial-mesenchymal transition and dysfunction of podocytes. *Scientific Reports*. 2017;**7**(1):9371

[169] Kalra H, Simpson RJ, Ji H, Aikawa E, Altevogt P, Askenase P, et al. Vesiclepedia: A compendium for extracellular vesicles with continuous community annotation. *PLoS Biology*. 2012;**10**(12):e1001450

[170] Pathan M, Fonseka P, Chitti SV, Kang T, Sanwlani R, Van Deun J, et al. Vesiclepedia 2019: A compendium of RNA, proteins, lipids and metabolites in extracellular vesicles. *Nucleic Acids Research*. 2019;**47**(D1):D516-D519

[171] Svenningsen P, Sabaratnam R, Jensen BL. Urinary extracellular vesicles: Origin, role as intercellular messengers and biomarkers; efficient sorting and potential treatment options. *Acta Physiologica (Oxford, England)*. 2020;**228**(1):e13346

- [172] Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. *Science*. 2015;**347**(6220):1260-1267.
- [173] Cheng Y, Wang X, Yang J, Duan X, Yao Y, Shi X, et al. A translational study of urine miRNAs in acute myocardial infarction. *Journal of Molecular and Cellular Cardiology*. 2012;**53**(5):668-676.
- [174] Ziolkowska NE, Christiano R, Walther TC. Organized living: Formation mechanisms and functions of plasma membrane domains in yeast. *Trends in Cell Biology*. 2012;**22**(3):151-158.
- [175] Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, et al. Microenvironmental pH is a key factor for exosome traffic in tumor cells. *The Journal of Biological Chemistry*. 2009;**284**(49):34211-34222.
- [176] Joshi BS, de Beer MA, Giepmans BNG, Zuhorn IS. Endocytosis of extracellular vesicles and release of their cargo from endosomes. *ACS Nano*. 2020;**14**(4):4444-4455.
- [177] Costa Verdera H, Gitz-Francois JJ, Schiffelers RM, Vader P. Cellular uptake of extracellular vesicles is mediated by clathrin-independent endocytosis and macropinocytosis. *Journal of Controlled Release*. 2017;**266**:100-108.
- [178] Tian T, Zhu Y-L, Zhou Y-Y, Liang G-F, Wang Y-Y, Hu F-H, et al. Exosome uptake through clathrin-mediated endocytosis and macropinocytosis and mediating miR-21 delivery. *The Journal of Biological Chemistry*. 2014;**289**(32):22258-22267.
- [179] Gonda A, Kabagwira J, Senthil GN, Wall NR. Internalization of exosomes through receptor-mediated endocytosis. *Molecular Cancer Research*. 2019;**17**(2):337-347.
- [180] Hao S, Bai O, Li F, Yuan J, Laferte S, Xiang J. Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumour immunity. *Immunology*. 2007;**120**(1):90-102.
- [181] Yuan D, Zhao Y, Banks WA, Bullock KM, Haney M, Batrakova E, et al. Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. *Biomaterials*. 2017;**142**:1-12.
- [182] Christianson HC, Svensson KJ, van Kuppevelt TH, Li J-P, Belting M. Cancer cell exosomes depend on cell-surface heparan sulfate proteoglycans for their internalization and functional activity. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(43):17380-17385.
- [183] Sims B, Farrow A, Williams S, Bansal A, Krendelchtchikov A, Gu L, et al. Role of TIM-4 in exosome-dependent entry of HIV-1 into human immune cells. *International Journal of Nanomedicine*. 2017;**12**:4823-4833.
- [184] de Abreu RC, Fernandes H, da Costa Martins PA, Sahoo S, Emanuelli C, Ferreira L. Native and bioengineered extracellular vesicles for cardiovascular therapeutics. *Nature Reviews. Cardiology*. 2020;**17**(11):685-697.
- [185] Conner SD, Schmid SL. Regulated portals of entry into the cell. *Nature*. 2003;**422**(6927):37-44.
- [186] Izquierdo-Useros N, Naranjo-Gómez M, Archer J, Hatch SC, Erkizia I, Blanco J, et al. Capture and transfer of HIV-1 particles by mature dendritic cells converges with the exosome-dissemination pathway. *Blood*. 2009;**113**(12):2732-2741.
- [187] Heusermann W, Hean J, Trojer D, Steib E, von Bueren S, Graff-Meyer A,

- et al. Exosomes surf on filopodia to enter cells at endocytic hot spots, traffic within endosomes, and are targeted to the ER. *Journal of Cell Biology*. 2016;**213**(2): 173-184
- [188] Grant BD, Donaldson JG. Pathways and mechanisms of endocytic recycling. *Nature Reviews Molecular Cell Biology*. 2009;**10**(9):597-608
- [189] Spooner RA, Smith DC, Easton AJ, Roberts LM, Lord JM. Retrograde transport pathways utilised by viruses and protein toxins. *Virology Journal*. 2006;**3**:26
- [190] Prada I, Meldolesi J. Binding and fusion of extracellular vesicles to the plasma membrane of their cell targets. *International Journal of Molecular Sciences*. 2016;**17**(8):1296
- [191] Pötgens AJG, Drewlo S, Kokozidou M, Kaufmann P. Syncytin: The major regulator of trophoblast fusion? Recent developments and hypotheses on its action. *Human Reproduction Update*. 2004;**10**(6): 487-496
- [192] Tolosa JM, Schjenken JE, Clifton VL, Vargas A, Barbeau B, Lowry P, et al. The endogenous retroviral envelope protein syncytin-1 inhibits LPS/PHA-stimulated cytokine responses in human blood and is sorted into placental exosomes. *Placenta*. 2012;**33**(11):933-941
- [193] Bjerregaard B, Lemmen JG, Petersen MR, Østrup E, Iversen LH, Almstrup K, et al. Syncytin-1 and its receptor is present in human gametes. *Journal of Assisted Reproduction and Genetics*. 2014;**31**(5):533-539
- [194] Bjerregard B, Ziolkiewicz I, Schulz A, Larsson L-I. Syncytin-1 in differentiating human myoblasts: Relationship to caveolin-3 and myogenin. *Cell and Tissue Research*. 2014;**357**(1): 355-362
- [195] Buslei R, Strissel PL, Henke C, Schey R, Lang N, Ruebner M, et al. Activation and regulation of endogenous retroviral genes in the human pituitary gland and related endocrine tumours: ERV and pituitary adenomas. *Neuropathology and Applied Neurobiology*. 2015;**41**(2):180-200
- [196] Maliniemi P, Vincendeau M, Mayer J, Frank O, Hahtola S, Karenko L, et al. Expression of human endogenous retrovirus-w including syncytin-1 in cutaneous T-cell lymphoma. *PLoS One*. 2013;**8**(10):e76281
- [197] Søre K, Andersen TL, Hobolt-Pedersen A-S, Bjerregaard B, Larsson L-I, Delaissé J-M. Involvement of human endogenous retroviral syncytin-1 in human osteoclast fusion. *Bone*. 2011;**48**(4):837-846
- [198] Lin Y, Wu J, Gu W, Huang Y, Tong Z, Huang L, et al. Exosome-liposome hybrid nanoparticles deliver CRISPR/Cas9 system in MSCs. *Advances in Science*. 2018;**5**(4):1700611
- [199] Sato YT, Umezaki K, Sawada S, Mukai S-A, Sasaki Y, Harada N, et al. Engineering hybrid exosomes by membrane fusion with liposomes. *Scientific Reports*. 2016;**6**(1):21933
- [200] Usman WM, Pham TC, Kwok YY, Vu LT, Ma V, Peng B, et al. Efficient RNA drug delivery using red blood cell extracellular vesicles. *Nature Communications*. 2018;**9**(1). DOI: 10.1038/s41467-018-04791-8. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6004015/>