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Insights into the unique roles of extracellular vesicles for gut health modulation: Mechanisms, challenges, and perspectives

Qiming Wu [a,1], Juntao Kan [a,1], Caili Fu [b,1], Xin Liu [b], Zhengying Cui [b], Sixu Wang [b], Yi Le [b], Zhanming Li [c], Qin Liu [d,*], Yuyu Zhang [e,*], Jun Du [a,*]

[a] Nutrilite Health Institute, Shanghai 200031, China
[b] Department of Food Science and Technology, National University of Singapore Suzhou Research Institute, Suzhou 215123, China
[c] Department of Food Quality and Safety, Jiangsu University of Science and Technology, Zhenjiang 212100, China
[d] Centre for Chinese Medicine Drug Development Limited, Hong Kong Baptist University, 999077, Hong Kong Special Administrative Region of China
[e] Key Laboratory of Geriatric Nutrition and Health, Beijing Technology and Business University, Beijing 100048, China

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ABSTRACT

Extracellular vesicles (EVs), which play significant regulatory roles in maintaining homeostasis and influencing immune responses, significantly impact gut microbiota composition and function, affecting overall gut health. Despite considerable progress, there are still knowledge gaps regarding the mechanisms by which EVs, including plant-derived EVs (PDEVs), animal-derived EVs (ADEVs), and microbiota-derived EVs (MDEVs), modulate gut health. This review delves into the roles and mechanisms of EVs from diverse sources in regulating gut health, focusing on their contributions to maintaining epithelial barrier integrity, facilitating tissue healing, eliciting immune responses, controlling pathogens, and shaping microbiota. We emphasize open challenges and future perspectives for harnessing EVs in the modulation of gut health to gain a deeper understanding of their roles and impact. Importantly, a comprehensive research framework is presented to steer future investigations into the roles and implications of EVs on gut health, facilitating a more profound comprehension of this emerging field.

1. Introduction

The gut, also known as the gastrointestinal (GI) tract, is a complex system with multiple functions. It is responsible for sensing, digesting, and absorbing nutrients (Jackson and McLaughlin, 2009; Rasoamanana et al., 2012), initiating immune response (Pabst, 1987), regulating metabolism (Tremaroli and Bäckhed, 2012), defending against infections (Iacob et al., 2019), controlling the gut microbiota (Lozupone et al., 2012), producing hormones (Martin et al., 2020), and establishing bidirectional interaction with the brain along the gut-brain axis (Mayer et al., 2022). The gut barrier and the gut microbiota are crucial components within this complex system (Bischoff, 2011). The gut barrier consists of microbial, chemical, mechanical, and immune barriers. It prevents harmful substances from entering the body while allowing essential nutrients to pass through (Chen et al., 2021a; Vancamelbeke and Vermeire, 2017). The gut microbiota is a diverse ecosystem comprising numerous microorganisms, including bacteria, archaea, fungi, viruses, and protozoa. It holds a bidirectional relationship with the gut barrier and influences its function (Natividad and Verdu, 2013). The communication between the gut microbiota and the gut barrier is dynamic and complex, and the specific molecular and cellular mechanisms still need to be determined. Disruption of this interaction can lead to increased intestinal permeability, systemic inflammation, autoimmune disorders, and higher susceptibility to infections, further causing various health problems beyond the GI tract like metabolic syndrome (Festi et al., 2014), mental health disorders (Malan Muller et al., 2018), and some chronic inflammatory conditions. Therefore, maintaining a healthy gut is essential for overall health and disease prevention.

Extracellular vesicles (EVs) contain a lipid bilayer membrane and are crucial carriers for bioactive substances like proteins, nucleic acids, lipids, and metabolites (Van Niel et al., 2018). They possess the unique ability to selectively fuse with target cell membranes or be internalized via endocytic mechanisms, facilitating the delivery of surface proteins and encapsulated components (Gandek et al., 2023). EVs have a wide variety of sources since nearly all cells can produce them. Plant-derived EVs (PDEVs) are derived from fruits, vegetables, and grains.

* Corresponding authors.
E-mail addresses: qinliu@hkbu.edu.hk (Q. Liu), zhangyuyu@btbu.edu.cn (Y. Zhang), Eric.Du@amway.com (J. Du).
[1] Contributed equally.

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Animal-derived EVs (ADEVs) primarily originate from stem cells, intestinal epithelial cells (IECs), as well as from breast milk, cow's milk, and blood cells. Microbial-derived EVs (MDEVs) are mostly released by microorganisms including bacteria, archaea, and fungi (Brown et al., 2015). These EVs differ in size, composition, and cargo (Table 1), enabling them to play unique roles in various biological processes.

Research suggests that EVs from different sources have the potential to be crucial regulators of overall well-being and might provide protection against gut-related diseases (Zhang et al., 2024). These EVs can engage with intestinal and immune cells to modulate immune responses and inflammatory reactions (Ocansey et al., 2020). Moreover, they can shape the composition and metabolic functions of the gut microbiota through different mechanisms, thereby affecting the gut immune system and the integrity of the gut barrier (Díaz-Garrido et al., 2021). Furthermore, studies have emphasized the crucial role of EVs in intercellular communication and signaling within the gut microenvironment (Kim et al., 2021). By enabling the cellular exchange of information, EVs are vital for regulating a variety of physiological and pathological processes within the gut (Pitt et al., 2016). This complex communication network mediated by EVs shows their potential importance in maintaining gut health and provides a promising direction for further exploration in intestinal research.

Although EVs hold great promise for promoting gut health, developing EV-based interventions for gut health applications still faces several significant challenges that require in-depth investigation and further exploration. The exact impact of EVs on gut modulation remains an unanswered question, and filling the knowledge gaps is crucial for understanding the underlying mechanisms of EVs and their effects on gut health, as well as the interaction among EVs, gut microbiota, and host immunity.

In this review, we explored the functions and mechanisms by which EVs modulate gut health. Moreover, we focused on and discussed in detail the unsolved challenges and perspectives regarding the exploration of EVs' roles in gut health. Furthermore, this review has also proposed a research framework to address future inquiries, with the main goal of deepening our comprehension of the roles and implications of EVs on gut health.

2. Multifaceted roles on gut health of EVs from various sources

EVs, which are derived from diverse sources such as animals, plants, and microbiota, play a crucial role in gut health. Animal-derived EVs (ADEVs), for example, those found in milk, fish, breast milk, honey, and the human body, can generate exosomes, apoptotic bodies, and microvesicles. Plants-derived EVs (PDEVs), including those from apple, coconut, broccoli, ginger, garlic, and lemon, produce exosomes and microvesicles. The microbiota-derived EVs (MDEVs) themselves release exosomes and microvesicles. Collectively, these EVs are of great importance for regulating the balance of the intestinal microbiota, restoring the integrity of the gut barrier, and modulating immune responses, thus contributing to overall gut health (Fig. 1).

2.1. Roles of plant-derived EVs

EVs from plant sources are of importance in modulating gut microbiota, enhancing intestinal barrier integrity, modulating immune responses, and facilitating tissue repair. *In vitro* and *in vivo* investigations have both shown their potential for anti-cancer effects (Rome, 2019) (Table 2). These PDEVs generally have a diameter in the range of 30 to 500 nm and encapsulate proteins, miRNAs, and metabolites such as flavonoids and vitamins (Shao et al., 2023). Moreover, PDEVs are attracting attention because of their biocompatibility, low immunogenicity, and the potential to pass through biological barriers, for example, the blood-brain barrier, thus providing promising opportunities for the delivery of bioactive materials (Alzahrani et al., 2023).

Numerous investigations have highlighted the potential of PDEVs, for example, those derived from tea leaves, in enhancing gut microbiota diversity. These tea leaves EVs have been determined to enhance the *Bacteroidetes/Firmicutes* ratio and enhance the overall abundance of fecal bacteria, such as *Alloprevotella, Rikenellaceae*_RC9_gut_group, and Norank_f_*Bacteroidales*_S24–7_group (Chen et al., 2023). Besides, studies indicate that PDEVs can mitigate acute colitis, promote intestinal repair, and prevent chronic colitis. In a study involving mice with induced colitis, treatment with ginger-derived EVs resulted in a decrease in pro-inflammatory cytokines, among which are tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β and a rise in anti-inflammatory cytokines like IL-10 and IL-22. This suggests that ginger-derived EVs

Table 1
Comparison of EVs from plant, animal, and microbiota sources (Cuesta et al., 2021; Shao et al., 2023; Wang et al., 2022b).

Features		PDEVs	ADEVs	MDEVs
Sources		Fruits, vegetables, and grains	Various animal cells and secretions	Bacteria, fungi, and other microorganisms
Size (nm)		30-500	30-1500	20-200
Compositions	Proteins	TMPs, cell wall modifying proteins, and CPs	TMPs, lipid-anchored proteins, and intracavity soluble proteins	Periplasmic proteins, lipoproteins, CPs, enzymes, toxins, and outer membrane proteins
	Nucleic acids	Mainly RNAs and a small number of DNAs	miRNAs, mRNAs, lncRNAs, and a small number of DNAs	DNAs and RNAs, including some small RNAs
	Lipids	Glycerin, phospholipids, sphingolipids, and plant sterols	Glycerin, phospholipids, sphingolipids, and cholesterols	Phospholipids, sphingolipids, and lipopolysaccharides
	Carbohydrates	Polysaccharides and oligosaccharides	Glycoproteins and glycosaminoglycans	Polysaccharides
	Metabolites	Anthocyanins, gingerols, folic acid, limonins, carotenoids, vitamins, and sulforaphane	Carboxylic acids, amino acids, sugars, carnitine, biogenic amines, vitamins, and cyclic alcohols	SCFAs
Cargos	Nucleic Acids	miRNAs	mRNAs, miRNAs, and lncRNAs	Small RNAs and DNAs
	Proteins	Actin, proteolytic enzymes, aquaporins, reticulin heavy chains, and HSPs	Targeted fusion proteins, HSPs, membrane transporters apoptosis-linked gene 2-interacting protein X, a cluster of differentiation 9, a cluster of differentiation 63, and tumor susceptibility gene 101	Enzymes, toxins, and signaling molecules
	Lipids	Digalactosyldiacylglycerol, phosphatidylglycolamine, and phosphatidic acid	Cholesterols, sphingomyelins, glycosphingolipids, and ceramides	Phospholipids and cholesterols
	Metabolites	Flavonoids, chlorophylls, and curcuminoids	Hormones and metabolic intermediates	Carbohydrates and SCFAs

PDEVs: plant-derived EVs. ADEVs: animal-derived EVs. MDEVs: microbiota-derived EVs. HSPs: heat shock proteins. SCFA: short-chain fatty acid. TMPs: transmembrane proteins. CPs: cytoplasmic proteins.

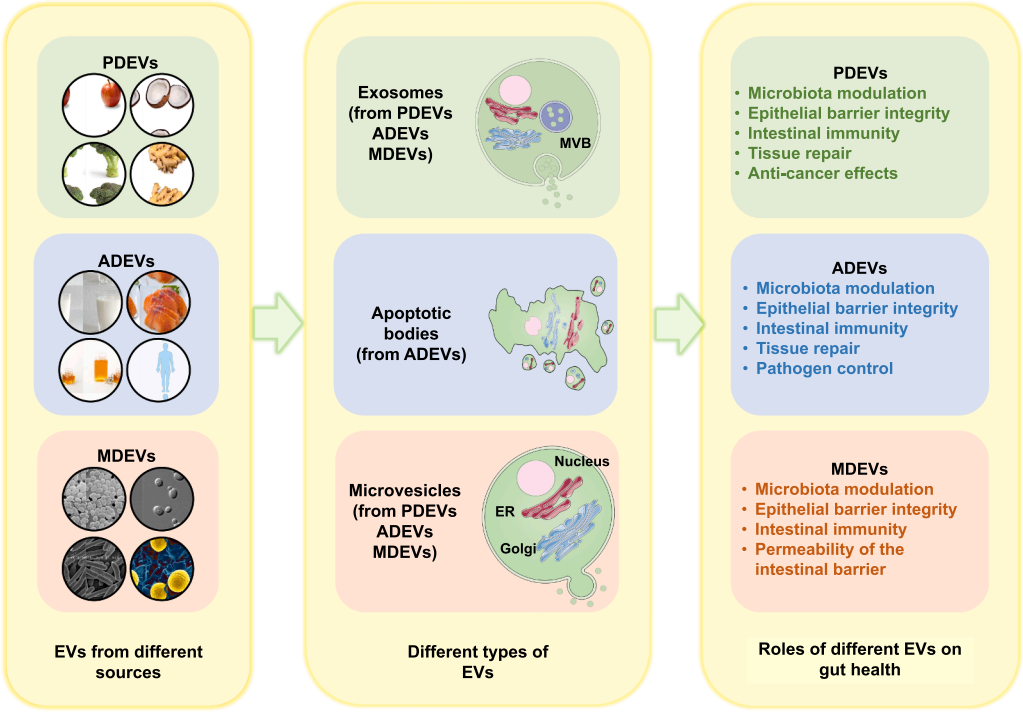

Fig. 1. Three different types of EVs from plants, animals, and microbiota have diverse roles in maintaining gut health.
PDEVs: plant-derived EVs. ADEVs: animal-derived EVs. MDEVs: microbial-derived EVs. MVB: multivesicular bodies. ER: endoplasmic reticulum. EVs: extracellular vesicles.

not only mitigate inflammatory damage while promoting healing but also maintain the intestinal barrier (Zhang et al., 2016a). Additionally, a study has identified for the first time EVs derived from lemon juice (*Citrus limon* L.). Researchers found that lemon EVs can suppress the growth of chronic myeloid leukemia tumors *in vivo*, which is achieved by targeting tumor sites and triggering apoptotic cell processes mediated by TNF-related apoptosis-inducing ligand, suggesting anti-cancer effects (Raimondo et al., 2015). Furthermore, the potential of EVs from various fruits as delivery treatment vehicles to improve the bioavailability of oral curcumin is currently being evaluated in human colon cancer patients, further emphasizing their promise in cancer therapy (Wu et al., 2017).

2.2. Roles of animal-derived EVs

Research on animal-derived EVs (ADEVs) has shown their significant effects (Table 3) on gut tissue repair, regulation of the balance of gut microbiota, restoration of intestinal barrier integrity, modulation of immune responses, and control of pathogens (Williams et al., 2023; Zeng et al., 2021). ADEVs usually have a range from 30 to 1500 nm and encapsulate various RNA species, including miRNAs, mRNAs, and lncRNAs that are essential in cell-to-cell communication.

Research investigating the impacts of breast milk-derived EVs (mEVs) on intestinal mucosal injury and inflammation in a murine model of necrotizing enterocolitis (NEC) has yielded notable findings. Western blot analysis showed that the quantities of tight junction proteins (TJPs) such as Zonula Occludens-1 (ZO-1), Claudin-1, and Occludin in the NEC-induced groups were less than those of the control group. ZO-1 is a major component of tight junctions that helps maintain the stability and integrity of cell-to-cell connections. Immunofluorescence revealed increased Claudin-1 expression in EVs-treated intestinal tissue. ELISA assays detected higher quantities of pro-inflammatory cytokines, for example, IL-6 and TNF-α in NEC-induced groups lacking EVs treatment. Histological examinations indicated significant mucosal injury in NEC-induced groups, which was alleviated by EV pretreatment. These results suggest that breast mEVs have a protective role against NEC-induced intestinal damage and inflammation (He et al., 2021). Besides, human breast milk-derived EVs treatment significantly alleviates NEC by targeting the miR-148a-3p/p53/SIRT1 axis, reducing inflammation, enhancing tight junctions, and inhibiting NF-κB-mediated apoptosis (Guo et al., 2022a). Additionally, studies have shown that breast mEVs contain miRNAs, which modulate gene expression governing growth, inflammation, and the initiation of Tregs, which can consequently confer protection against autoimmunity and NEC (Pérez-Escamilla et al., 2023).

The experiment demonstrates that EVs from bovine milk influence the gut microbiome in mice by changing the composition of microbial communities in the cecum. Specifically, C57BL/6 mice fed with different diets, namely EVs/RNA-sufficient (ERS) and EVs/RNA-depleted (ERD), demonstrated that the abundance of the *Tenericutes* in the ERS group was more than three times higher than in the ERD group at 15 and 47 weeks ($P < 0.05$). Additionally, at 47 weeks, the abundance of the *Verrucomicrobiaceae* was markedly decreased in the ERS group than in the ERD group ($P < 0.05$). Furthermore, four operational taxonomic units (OTUs) from the *Lachnospiraceae* were more than twice as abundant in the ERS group at both 7 and 47 weeks ($P < 0.05$). These findings suggest that bovine mEVs significantly impact the gut microbiome in mice by regulating the abundance of various bacterial phyla, families, and OTUs (Zhou et al., 2019). Oral administration of these mEVs also appears to modulate the gut microbiota composition and regulate the generation of short-chain fatty acids (SCFAs) (Du et al., 2021). Additionally, oral administration of mammalian EVs supports the restoration of intestinal barrier integrity across various layers, including the mucosal, epithelial, and immune barriers. The mechanisms by which EVs protect this barrier integrity contribute to mitigating intestinal inflammation and disorders related to the gut-liver axis (Tong et al., 2023).

EVs can also be obtained from various animal cell types, like IECs and stem cells, and their functions closely resemble those of the cells that secrete them (Ocansey et al., 2022). For example, IEC-derived EVs can participate in antigen presentation and induce tolerogenic immune

Table 2
A summary of the impact of PDEVs on gut health.

Sources of PDEVs	Cargo/Components	Performances / Mechanisms	Refs.
Ginger	mdo-miR7267–3p	Increase I3C and IL-22 production. Improve barrier function and ameliorate colitis. Enhance organoid formation and protect against DSS-induced colitis.	Teng et al. (2018)
	N/A	Significantly enhance the expression of HO-1, IL-6, and IL-10. Maintain intestinal homeostasis.	Mu et al. (2014)
	mRNAs	Increase Foxa2 expression in gut epithelial. Restore intestinal epithelial cell function and prevent EV-mediated insulin resistance.	Kumar et al. (2022)
Mulberry bark	N/A	Prevent DSS-induced colitis. Facilitate HSP family A (Hsp70) member 8 -mediated activation of the AhR signaling pathway.	Sriwastva et al. (2022)
Lemon	IL-22 messenger RNA	Improve the stress survival of gut bacteria through RNase P-mediated specific tRNA decay.	Lei et al. (2021)
	mRNAs and tRNAs	Increase bile resistance and gut survivability of *Lactobacillus rhamnosus* GG and *Streptococcus thermophilus* ST-21. Induce IL-22 through increased AhR ligands.	Lei et al. (2020)
Tea leaf	miR-4057	Enhance the community abundance and diversity of gut microbiota. Increase the abundance of the total fecal bacteria. Lead to higher *Bacteroidetes/Firmicutes* ratios.	Chen et al. (2023)
	Lipids and proteins	Prevent or alleviate IBD and colitis-associated colon cancer. Suppress the expression of pro-inflammatory cytokines, and boost the secretion of anti-inflammatory IL-10 from macrophages.	Zu et al. (2021)
Blueberry	Proteins, lipids, mRNAs, and/or miRNAs	Protect against TNF-α induced oxidative stress, which is involved in the inflammatory response, and reverse the impact of TNF-α-induced mRNA expression of IL-6, IL1RL1, MAPK1, ICAM1, TRL8, and TNF.	De Robertis et al. (2020)
Apple	miRNAs	Modulate the mRNA expression of intestinal transporters. Decrease OATP2B1 expression in Caco-2 cells at the levels of mRNA, protein content, and transport activity.	Fujita et al. (2018)
Tartary buckwheat	miRNAs	Increase the diversity of fecal microorganisms and the SCFA levels. Significantly promote the growth of *Escherichia coli* and *Lactobacillus rhamnosus* in the gut.	Liu et al. (2022)
Hemp (*Cannabis sativa* L.)	N/A	Protect against DSS-induced gut leak. Improve intestinal barrier proteins, and reduce NF-κB activation and oxidative stress markers in DSS-induced colitis.	Eom et al. (2022)
Coconut	miRNAs	Ensure the survival and metabolic activity of the probiotic bacterium WCFS1. Repress rpoC and yegH expression levels in MG1655 while elevating those of CcpA.	Yu et al. (2019)
Grapefruit	Chemotherapeutic agents, miRNAs, DNA vectors and proteins	Enhance the levels of IL-10 and IL-22.	Zhang et al. (2016b)
	N/A	Inhibit IL-1β and TNF-α production in intestinal macrophages. Ameliorate DSS-induced mouse colitis. Maintain intestinal macrophage homeostasis and attenuate inflammatory responses.	Wang et al. (2014)
Grape	Proteins, lipids, and miRNAs	Taken up by intestinal stem cells and stimulate the proliferation of these cells.	Mu et al. (2014)
	Lipids	Target intestinal stem cells, improve organoid formation, and induce Lgr5+ stem cells. Protect against DSS-induced colitis, modulate intestinal tissue renewal and remodeling, and involve β-catenin signaling pathways.	Ju et al. (2013)

N/A: not available in the references.
I3C: indole-3-carboxaldehyde. SCFAs: short-chain fatty acids. IL: interleukin. DSS: dextran sulfate sodium. AhR: aryl hydrocarbon receptor. HSPs: heat shock proteins. HO-1: heme oxygenase 1. EV: extracellular vesicle. IBD: inflammatory bowel disease. CcpA: catabolite control protein A. NF-κB: the nuclear factor-kB. TNF: tumor necrosis factor.

responses (Van Niel et al., 2003; Van Niel et al., 2001). Mesenchymal stem cell (MSC)-derived colonic EVs have shown effectiveness in alleviating colitis by reducing inflammation. They have exhibited macrogenomic and metabolomic characteristics and have upregulated the expression of the colonic farnesoid X receptor (FXR) (Wu et al., 2022). In addition, MSC-derived EVs positively impact gut microbial composition, enhance cellular functions, and reduce disease-associated bacteria, thus offering potential therapeutic targets (Ocansey et al., 2022).

2.3. Roles of microbial-derived EVs

EVs from microbiota (MDEVs) are crucial in the survival and interactions of gut microorganisms and have multiple functions, such as cell signaling, gene transfer, biofilm assembly, antimicrobial activity, and modulation of host immune responses (Huang et al., 2023; Zhang et al., 2022). MDEVs usually range in size from 20 to 200 nm and encapsulate nucleic acids (including RNAs and DNAs) and various SCFAs. Through the delivery of encapsulated bioactive molecules, EVs interact with other microorganisms, host cells, and the host immune system, influencing microbial ecosystems and taking part in the preservation of the intestinal barrier (Table 4).

In the intestinal environment, EVs produced by *Escherichia coli* Nissle 1917 attach to receptors on the surface of IECs and trigger nucleotide oligomerization domain 1 (NOD1) signaling pathways (Olivo Martínez et al., 2024). Epithelial cells are induced to increase the synthesis and redistribution of TJPs, such as ZO-1 and Claudin-14. The upregulation of Claudin-14 is associated with decreased intestinal permeability, preventing harmful substances from crossing the intestinal barrier (Kozieł et al., 2021). This mechanism shows that the EVs critically influence the integrity of the gut barrier by modulating the expression of TJPs, thus influencing intestinal health.

Besides, EVs are involved in cytotoxin-associated gene A (CagA)-positive *Helicobacter pylori* infection. These small EVs, released by cells and containing functional molecules, serve as mediators of intercellular communication. Studies indicated that CagA-positive *Helicobacter pylori* infection triggers the release of EVs containing CagA, which disrupts the intestinal mucosal barrier integrity through a CagA-mediated mechanism. Specifically, CagA-positive EVs promote the expression of Claudin-2, a TJP associated with impaired mucosal barrier function. By targeting IECs, CagA-positive EVs induce upregulation of Claudin-2 and hinder the repair process of damaged intestinal mucosa by affecting Claudin-2 transcription levels (Guo et al., 2022b).

EVs derived from *Lactobacillus rhamnosus* GG have a protective effect on alcohol-related liver disease (ALD) by regulating the intestinal miR-194-FXR signaling pathway. These nanoparticles interact with the gut microbiota, enhancing the population of beneficial bacteria carrying

Table 3
A summary of the impact of ADEVs on gut health.

Sources of ADEVs	Cargo/ Components	Performances / Mechanisms	Refs.
Milk	N/A	Elicit changes in bacterial communities.	Zhou et al. (2019)
	N/A	Alter gut microbiota composition. Regulate local intestinal immunity. Increase the expression level of IgA and sIgA in the GI tract.	Tong et al. (2020)
	N/A	Improve gut barrier integrity in chemical-induced acute and chronic intestinal inflammation. Modulate lamina propria immune cells, raising the proportions of intestinal resident macrophages and Treg cells, and the generation of IL-10.	Tong et al. (2023)
Porcine milk	miR-4334, miR-219, miR-338	Protect the IECs from LPS-induced injury. Decrease cell inflammation and apoptosis.	Xie et al. (2019)
IECs	miR-23a-3p	Regulate gut injury after intestinal ischemia/reperfusion.	Yang et al., (2022a)
	N/A	Induce M1 polarization and IL-1β secretion in mesenteric lymph nodes.	Xi et al. (2022)
Human placental MSCs	N/A	Modulate gut microbiota and suppress inflammation.	Yang et al. (2022b)
CD11c myeloid cells	miR-146a	Reduce intestinal inflammation during colitis. Transfer from gut immune cells to myeloid and T-cells via a Rab27-dependent mechanism, and target Traf6, IRAK-1, and NLRP3 in macrophages.	Bauer et al. (2022)
Human breast milk	N/A	Protect intestinal tissue against necrotizing enterocolitis. Maintain the intestinal epithelial TJPs ZO-1, Claudin-1, and Occludin.	He et al. (2021)
	N/A	Protect the intestine against damage by IR injury. Counteract the increased expression of inflammatory cytokine TNF-α and decreased intestinal proliferation by IR.	Wang et al., (2022a)
Honey	Lipids, proteins, and small RNAs	Alleviate inflammation and liver damage. Inhibit NLRP3 inflammasome activation.	Chen et al. (2021b)
MSCs	N/A	Mitigate colitis in mice. Modulate the gut metabolomics–FXR axis.	Ocansey et al. (2022)

N/A: not available in the references. IL: interleukin. IgA: immunoglobulin A. sIgA: secretory immunoglobulin A. ZO: Zonula Occludens. TNF: tumor necrosis factor. IR: ischemia-reperfusion. ADEVs: animal-derived EVs. MSCs: Mesenchymal stem cells. LPS: lipopolysaccharide. FXR: farnesoid X receptor. IECs: intestinal epithelial cells. TJPs: tight junction proteins.

bile salt hydrolase, which lowers bile acid levels and increases intestinal taurine concentration. They also upregulate the expression of the Tug1 gene which inhibits miR-194 expression, thus restoring FXR activity in the intestine. Activated FXR suppresses intrahepatic bile acid synthesis and lipid production through upregulation of the fibroblast growth factor 15 signaling pathway, thereby alleviating ALD damage. This study revealed that EVs, through modulation of the miR-194-FXR signaling pathway, restore intestinal barrier function and have a protective effect, providing a novel therapeutic strategy for ALD treatment (Jiang et al., 2023). Besides, studies have suggested the anti-tumor potential of *Lactobacillus rhamnosus* GG EVs *in vitro* and *in vivo*, mainly by promoting apoptosis of cancer cells (Lu et al., 2023), there is also

Table 4
A summary of the impact of MDEVs on gut health.

Sources of MDEVs	Cargo/ Components	Performances / Mechanisms	Refs.
Enterobacteria	N/A	Promote gut inflammation and colon cancer.	Deng et al. (2015)
Escherichia coli Nissle 1917 and ECOR63	N/A	Protect against intestinal epithelial barrier dysfunction. Counteract EPEC-altered mRNA levels of Occludin and Claudin-14. Maintain subcellular localization of ZO-1 and Occludin related to TJ structures at the cell boundaries, and preserve F-actin at the inter-cellular junctions.	Alvarez et al. (2019)
	N/A	Strengthen intact intestinal epithelial barrier. Downregulate the leaky protein Claudin-2.	Alvarez et al. (2016)
Lactobacillus plantarum Q7	N/A	Alleviate DSS-induced colitis symptoms.	Hao et al. (2021)
Helicobacter pylori	CagA	Damage to the mucosal barrier integrity in chronic colitis. Facilitate CDX2-dependent Claudin-2 maintenance.	Guo et al. (2022a)
Lactobacillus reuteri DSM-17,938	N/A	Modulate gut motility.	West et al. (2020)
Lactobacillus plantarum JCM8341	N-acylated peptides from lipoprotein 1918	Modulate intestinal immunity.	Kurata et al. (2022)
Clostridium butyricum	N/A	Defend against DSS-induced colitis by adjusting the repolarization of M2 macrophages and altering the composition of gut microbiota.	Liang et al. (2022)
	miR-199a-3p	Mitigate bacterial dysbiosis in colitis mice. Reduce the abundance of the bacterial pathogens *E. coli* and *Shigella flexneri*. Adjust the microbial tryptophan metabolites, boost intestinal barrier integrity, and suppress the inflammatory response in colitis mice.	Ma et al. (2023)
Akkermansia muciniphila BAA-835	N/A	Improve colon histopathological damage.	Keshavarz Azizi Raftar et al. (2021)
Akkermansia muciniphila DSM 22,959	N/A	Restore gut microbiota balance by selectively promoting beneficial bacteria. Elicit a mucosal immunoglobulin A response by activating B cells and DCs in Peyer's patches. Maintain intestinal barrier integrity by stimulating the expression of TJPs and mucus, and can alleviate colitis while enhancing anti-PD-1 therapy	Wang et al. (2023)

(*continued on next page*)

Table 4 (*continued*)

Sources of MDEVs	Cargo/ Components	Performances / Mechanisms	Refs.
		against colorectal cancer.	
Faecalibacterium prausnitzii A2–165	N/A	Increase the intestinal barrier permeability via TJPs (ZO-1 and Occludin) as well as PPAR-α, PPAR-γ, and PPAR β/δ genes and their targeted gene (ANGPTL4) in the Caco-2 cell line.	Moosavi et al. (2020)
Novel *Odoribacter splanchnic* Strain	N/A	Attenuate of *Escherichia coli* LPS-induced inflammation. Counteract of the pro-inflammatory cytokine IL-8 production.	Hiippala et al. (2020)

N/A: not available in the references. EPEC: Enteropathogenic Escherichia coli. ZO: Zonula Occludens. DSS: dextran sulfate sodium.CDX2: caudal type homeobox 2. TLR: toll-like receptor. DC: dendritic cell. LPS: lipopolysaccharide. IL: interleukin. TJPs: tight junction proteins. PD-1: programmed death-1.

evidence that EVs can promote tumor cell proliferation through the secretion of IL-6/8/10 and by suppressing nitric oxide release as well as the inducible nitric oxide synthase pathway (Jiang et al., 2022).

Nonetheless, research on MDEVs remains in its infancy, demanding further investigations to elucidate their detailed composition and mechanisms of action.

3. The impact of EVs in regulating gut health

Inflammatory bowel diseases (IBD), namely ulcerative colitis (UC) and Crohn's disease (CD), as well as colorectal cancer (CRC), are witnessing an increasing incidence worldwide, and pose a serious threat to human health. As EVs possess the ability to modulate immune responses, promote tissue repair, and deliver therapeutic agents, they have drawn significant attention in the field of gut-related diseases. The potential of EVs as both therapeutic agents and diagnostic biomarkers is being actively explored in preclinical and clinical settings.

Following the previous discussion on the specific roles of EVs from various sources in gut health, EVs play different roles in Preserving epithelial barrier function, regulating immune responses, promoting tissue regeneration, controlling pathogen invasion, and shaping the gut microbiota by transporting bioactive substances, including miRNAs, proteins, and metabolites (Fig. 2). This section will further elaborate on the overall impact of EVs from various sources in regulating gut health. miRNAs carried in EVs can effectively avoid the degradation of RNases and the influence of environmental changes, to transmit genetic information between cells and regulate inter-cell communication. This transfer of genetic information can affect the gene expression of target cells and thus affect diverse physiological processes within the gut. Proteins carried by EVs contribute to maintaining the structural and functional integrity of the intestinal epithelium, supporting cell proliferation, differentiation, and adhesion. These proteins have a crucial function in regulating the immune response, promoting tissue repair, and enhancing barrier function. In addition, metabolites transported by EVs, such as SCFAs and amino acids, are also of great importance in regulating IECs and immune responses.

3.1. EVs in gut-related diseases

3.1.1. Ulcerative colitis

EVs have shown up as a hopeful therapy strategy for UC, a chronic IBD. Recent systematic reviews and meta-analyses have highlighted the positive consequences of mammalian EVs in animal models of UC. A comprehensive review analyzed 69 studies involving 1271 animals and found that EVs significantly reduced the disease activity index (DAI) in 98 % of the cases and the colonic mucosal damage index (CMDI) in several studies. The meta-analysis of 40 studies confirmed that naïve EVs markedly decreased both DAI (SMD=3.00; 95 % CI: 3.52 to 2.48) and CMDI (SMD=2.10; 95 % CI: 2.85 to 1.35), underscoring their therapeutic potential. Nevertheless, the safety status of EVs has not been adequately examined (Li et al., 2024b), with a few studies that have evaluated adverse effects. In the treatment of UC, EVs from sources like *Lactobacillus plantarum* Q7 can regulate the gut microbiota and improve UC symptoms in mice, including relieving colon shortening, bloody stools, and tissue damage, along with reducing the gene expression level of pro-inflammatory factors (Hao et al., 2021). Additionally, macrophage EV-modified nanomedicines exert a crucial influence on regulating intestinal immune homeostasis, addressing the issue of low drug targeting, and specifically improving UC damage in mice (Cheng et al., 2023).

Although the majority of these studies are still in the preclinical phase, they lay a solid foundation for future clinical applications. EVs offer novel therapeutic avenues by modulating gut microbiota, reducing pro-inflammatory cytokine expression, and improving drug targeting. As research progresses, EVs are expected to become a significant component of UC treatment strategies, providing new hope for patients with this debilitating condition.

3.1.2. Crohn's disease

EVs, which can be derived from mesenchymal stem cells (MSCs), are shown to influence immune regulation and intestinal barrier integrity with an anti-inflammatory effect on CD (Ocansey et al., 2020; Valter et al., 2021). The current status of clinical trials regarding EVs on CD shows promising developments. Over 300 patients have been included in phase I, II, and III clinical trials (Djouad et al., 2009; Ghannam et al., 2010). In these trials, EVs derived from MSCs are being investigated for their efficacy and safety in the treatment of CD, especially in the perianal fistulizing form which is extremely difficult to handle. Preliminary results suggest that EVs could serve as an alternative or supplementary option to conventional biologic and surgical treatments. These trials are investigating the anti-inflammatory effects of EVs on CD, which are probably associated with their influence on immune regulation and intestinal barrier integrity. Nevertheless, further research is still required to comprehensively understand the long-term impacts, optimal dosage, and patient selection criteria for EV-based therapies in CD (Dadgar et al., 2022).

3.1.3. Colorectal cancer

The current status of clinical trials regarding EVs in CRC is in the developing stage. Small EVs, which are crucial for intercellular communication, are being recognized as biomarkers for CRC diagnosis. Although nucleic acids and proteins in CRC-derived EVs from blood have the potential to be used as biomarkers, only a few clinical trials have evaluated EV molecules in this regard. Two clinical trials (NCT03874559 and NCT04227886) have indicated the changes in EV molecules before and after concurrent chemoradiation therapies for rectal cancer. Moreover, the results of two trials (NCT04394572 and NCT04523389) are anticipated to clarify the role of EV proteins and miRNAs in the early diagnosis and prediction of treatment response in CRC. However, till now, there has been no clinical trial to explore the effectiveness of blood tests using EV molecules for screening (Chang et al., 2022). Hence, additional research in this field is needed.

In EV clinical trials across different GI-related diseases, common challenges include the long-term impacts of EV-based therapies are unclear, making it hard to assess potential risks over time. The heterogeneity of EVs in GI disorders poses a significant challenge, as variations in size, composition, and biological activity lead to inconsistent results and hinder standardized treatment protocols. The lack of reliable large-scale production methods for GI-specific EVs is also a hurdle. Ensuring

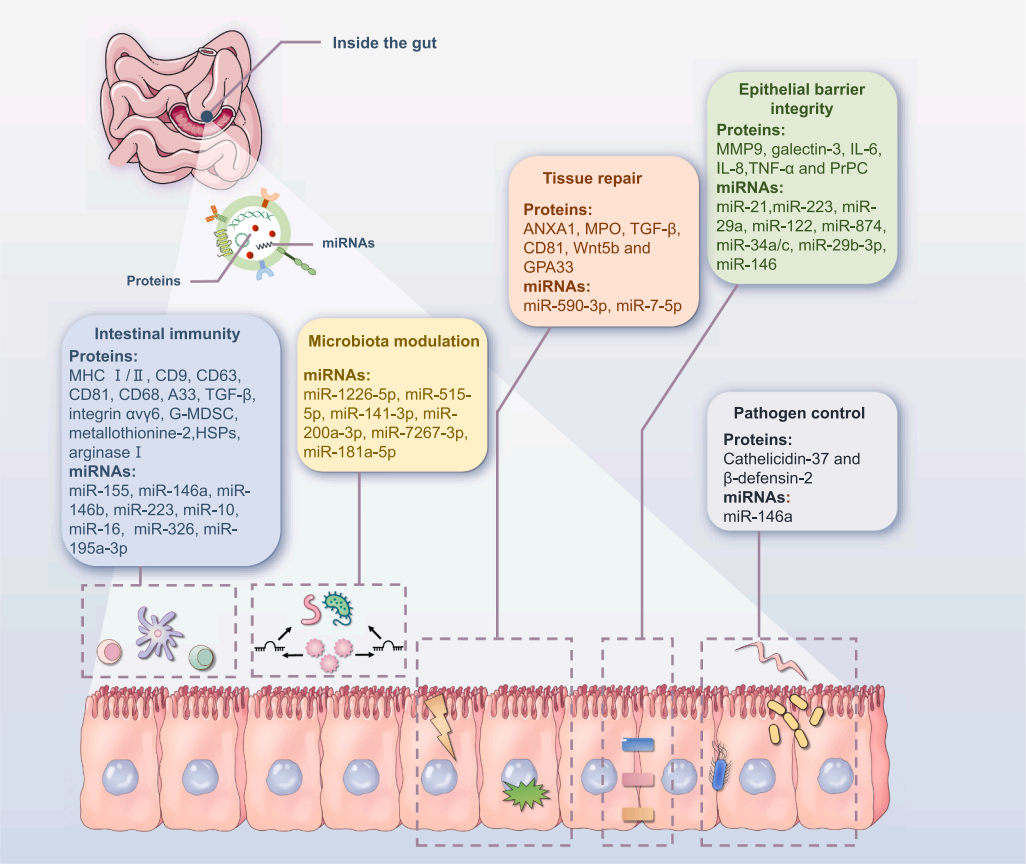

Fig. 2. Five main roles of EVs within the intestine environment via bioactive cargo like proteins and miRNAs.
The diverse bioactive components carried by EVs, such as proteins and miRNAs, have potential impacts within the intestine environment. These bioactive cargoes can influence various aspects including epithelial barrier function, tissue regeneration, immune responses, pathogen control, and microbiota modulation.

proper delivery to the GI tract is difficult, and developing efficient delivery systems is an ongoing challenge. Finally, regulatory approval for EV-based therapies in GI diseases is complex and time-consuming, requiring extensive research and clinical trials to demonstrate efficacy and safety.

As research progresses, it is anticipated that EVs will become integral components of treatment strategies for GI-related diseases, offering new hope for patients suffering from these debilitating conditions. The continued exploration of EVs in both preclinical and clinical settings will be crucial in overcoming current challenges and unlocking their full potential in GI medicine.

3.2. *Regulation of gut physiological processes*

3.2.1. *Modulation of immune responses*

EVs are derived from both microbes and host cells, significantly contributing to gut immunity (Díaz-Garrido et al., 2021; Macia et al., 2019). Microbial-derived EVs contain specific molecules, such as lipopolysaccharides (LPS) and peptidoglycans in bacterial EVs, which can activate innate immune signaling pathways through toll-like receptors (TLRs) on immune cells, inducing the formation of pro-inflammatory cytokines (Kawai et al., 2024). Fungal EVs contain components like β-glucan and chitin (Nimrichter et al., 2016), which regulate dendritic cell (DC) maturation and antigen-presenting capabilities via specific receptors, such as dectin-1, complement receptor 3, cluster of differentiation 5, lactosylceramide, and so on (Jin et al., 2018; Kim et al., 2010).

Host-derived EVs carry miRNAs and proteins that can modulate gene expression in immune cells and maintain gut immune homeostasis (Bui et al., 2018). Nucleic acids within EVs, such as miRNAs and RNAs, have diverse effects. For example, in patients with IBD, miR-155, derived from EVs released by DCs, is overexpressed (Alexander et al., 2015). This overexpression promotes inflammation by downregulating the suppressor of cytokine signaling 1 (SOCS-1), a feedback inhibitor of inflammation, and SH2-containing inositol 5-phosphatase-1 (SHIP-1), a negative regulator of the PI3K-Akt signaling pathway (Hu et al., 2021; Lu et al., 2017). The experimental models of colitis have shown that inhibiting miR-155 can reduce inflammation (Cao et al., 2021). In contrast, miR-223 exhibits a dual role. It targets NLRP3 inflammasome in monocytes to inhibit IL-1 production in murine colitis, reducing inflammation and improving colitis signs (Neudecker et al., 2017). However, it can also be secreted by mast cell-derived EVs and activates NF-κB, producing pro-inflammatory cytokines, which exacerbate the inflammatory response in colitis (Kim et al., 2016). Unlike miR-155, which mainly promotes inflammation by downregulating negative regulators, miR-223 has a dual role with an environment-dependent impact on inflammation. Additionally, small RNAs within EVs can transfer between cells, mediating the repression of target genes, and they are involved in cytokine-mediated signaling, which in turn, can affect the activity of immune cells in the gut and regulate the balance of pro-inflammatory and anti-inflammatory responses, thereby having a significant function on maintaining intestinal immune homeostasis. (Fernández Messina et al., 2015).

Heat shock proteins (HSPs) like HSP70 and HSP90 in EVs from most cell types bind to TLRs on immune cells in the intestine, triggering a signaling cascade that results in the activation of NF-κB. This subsequently regulates the expression of genes related to immune responses (Kowal et al., 2016). Moreover, EVs from IECs express major histocompatibility complex (MHC) I and MHC II, allowing them to sample and process antigens (Van Niel et al., 2001), which are then delivered to DCs to enhance antigen presentation and exert immunomodulatory

influences on $CD4^+$ or $CD8^+$ T cells, thereby prompting T-cell activation via T-cell receptor (Mallegol et al., 2007; Nolte-'t Hoen et al., 2009). IEC-derived EVs carry integrin αvβ6 and food antigens to promote DCs to generate active TGF-β and induce Tregs (Diaz Garrido et al., 2021).

Metabolites carried by EVs also have impacts on gut immunity. mEVs can modulate metabolites like SCFAs and regulate local intestinal immunity by increasing the levels of IgA and secretory IgA (sIgA) (Tan et al., 2022). EVs can also carry butyrate, an anti-inflammatory SCFA produced by commensal bacteria, which promotes Treg differentiation and maintains gut immune tolerance (Siddiqui and Cresci, 2021).

In conclusion, through the modulation of immune responses, EVs contribute significantly to maintaining intestinal immune homeostasis. The diverse molecules carried by microbial-derived and host-derived EVs, along with the metabolites they transport, work in concert to shape the immune environment of the gut. Understanding these mechanisms provides valuable insights for the establishment of innovative treatment strategies to address various gut disorders and promote overall gut health.

3.2.2. *Regulation of the intestinal barrier integrity*

The gut barrier is a complex and dynamic structure, and its integrity is strongly linked to the occurrence and progression of various diseases, including IBD, irritable bowel syndrome, and infectious diseases (Barbara et al., 2021; Bui et al., 2018). It could be affected by the gut microbiota, immune responses, and the bioactive cargo carried by EVs (Aarts et al., 2021; Chen et al., 2021c; Dmytriv et al., 2024; Natividad and Verdu, 2013; Takiishi et al., 2017). EVs carry miRNAs and proteins that adjust the expression and function of TJPs. Some EVs contain miRNAs that regulate Zonulin which controls the opening and closing of tight connections, affecting intestinal permeability *in vivo* and *in vitro* (Zhang et al., 2017). Moreover, miRNAs in EVs can also target TJP mRNAs and regulate their expression level (Cichon et al., 2014). Furthermore, EVs interact with intestinal immune cells to modulate the immune response and indirectly affect the integrity of the intestinal barrier (Rossaint et al., 2016). miR-21 is associated with negative impacts on barrier integrity. During inflammation, miR-21 is packaged in EVs released by colon epithelial cells, driven by the substance P neuropeptide signaling pathway (Bakirtzi et al., 2019). It disrupts intestinal barrier integrity by reducing TJPs like Occludin and E- cadherin, leading to increased permeability and promoting the release of pro-inflammatory cytokines (*e.g.*, prostaglandin E2, IL-6, IL-8), which exacerbates inflammation (Zhang et al., 2015). Similarly, miR-223, released in EVs by LPS-induced macrophages, targets transmembrane and immunoglobulin domains containing 1, a key protein for maintaining barrier function (Chang et al., 2023). Elevated levels of miR-223 downregulate Claudin-8, compromising barrier integrity and allowing harmful substances to penetrate the gut lining. Conversely, miR-146a contained in EVs derived from MSCs has been shown to inhibit the NF-κB signaling pathway, which reduces inflammation and enhances the stability of tight junctions between IECs. Additionally, it influences communication between epithelial cells and immune cells, fostering a balanced immune environment that supports barrier function. By responding to signals from the gut microbiota, miR-146 helps the intestinal barrier adapt to changes in the microbial landscape, preventing dysfunction and associated GI disorders (Wu et al., 2019).

Proteins carried by EVs can stabilize junctional complexes and promote cell adhesion, thereby enhancing the structural cohesion of the epithelial layer. Some proteins can degrade essential adhesion molecules, leading to increased permeability and barrier dysfunction during inflammatory states. For instance, galectin-3, present in EVs from macrophages, plays a protective role by stabilizing junctional components, including desmoglein-2 (DSG-2), within the intestinal epithelium. DSG-2 plays a role in regulating gut barrier function through p38 MAPK signaling, which is essential for the appropriate localization of ZO-1 (Ungewiß et al., 2017). By reinforcing DSG-2 at adhesion sites, galectin-3 enhances the integrity of IECs and promotes a more resilient epithelial barrier (Jiang et al., 2014). This stabilization counteracts the damaging effects of inflammatory mediators, supporting barrier function and recovery during inflammatory episodes. Additionally, prion protein (PrPC) released in EVs from activated platelets is vital for cell-to-cell communication within the intestinal epithelium. It interacts with desmosome proteins to maintain the structural cohesion of the epithelial barrier (Robertson et al., 2006). A deficiency in PrPC increases paracellular permeability and reduces critical adhesion proteins, such as E-cadherin, Desmoplakin, Plakoglobin, Claudin-4, Occludin, ZO-1, and Tricellulin at intercellular junctions. However, some proteins can undermine barrier integrity. Matrix metalloproteinase 9 (MMP-9), found in EVs from polymorphonuclear neutrophils (PMNs), negatively impacts IECs by cleaving DSG-2. In inflamed tissues, the degradation of DSG-2 by MMP-9 facilitates PMN migration across the barrier, exacerbating inflammation and compromising barrier integrity (Butin-Israeli et al., 2016).

Metabolites, such as SCFAs and amino acids, further contribute to the regulation of intestinal barrier integrity. Oral administration of EVs enhanced the abundance of beneficial bacteria in the gut, like *Ruminiclostridium* and *Ruminococcaceae* UCG-014, which are known to produce SCFAs that enhance the function of the epithelial barrier by modulating Treg cells, protecting the colon from inflammatory damage, and promoting colon homeostasis (Li et al., 2021; Smith et al., 2013). However, some metabolites, such as inflammatory cytokines, will lead to epithelial damage and barrier dysfunction. Granulocyte-derived EVs from intestinal aspirates from patients with IBD contain inflammatory cytokines, for example, IL-6, IL-8, and TNF-α, which increase epithelial permeability by down-regulated or mislocalized TJPs such as ZO-1, Claudins, Occludins, and junctional adhesion molecule A (Ma et al., 2004; Mazzon and Cuzzocrea, 2008; Mitsuhashi et al., 2016).

Although we have discussed the effects of these bioactive molecules carried by EVs on intestinal barrier integrity separately, it should be noted that these factors do not act alone *in vivo*. They may interact with each other, either synergistically or antagonistically, to jointly influence the integrity of the intestinal barrier. Further research is needed to explore these complex interactions and their comprehensive impact on intestinal barrier health.

3.2.3. *Impact on tissue repair*

Damage in the gut can occur due to various factors such as inflammation, infection, and stress (Gareau et al., 2008; Patankar and Becker, 2020; Solaymani-Mohammadi and Singer, 2013). EVs are of crucial importance throughout this process by transferring bioactive molecules that can promote cell proliferation, migration, and differentiation (Pan et al., 2022; Quesenberry et al., 2015). For example, EVs can deliver growth factors and cytokines to damaged cells, stimulating their regeneration (Silva et al., 2017). They can also modulate the immune response to prevent excessive inflammation that could impede tissue repair (Hu et al., 2021).

When EVs from beneficial bacteria, such as *Escherichia coli* Nissle 1917, enhance the expression of Trefoil Factor 3 (TFF3) in goblet cells. This increase in TFF3 levels leads to the downregulation of miR-7-5p, which normally promotes the degradation of TFF3 mRNAs. As miR-7-5p levels decrease, TFF3 production rises, facilitating the strengthening of the intestinal epithelial barrier and promoting cell migration and proliferation during tissue repair. Conversely, when EVs from less beneficial bacteria, like ECOR12, are involved, they may not activate the necessary signaling pathways, resulting in the upregulation of miR-7-5p and subsequent inhibition of TFF3 production, thereby impairing tissue repair processes (Olivo Martínez et al., 2023). Another miRNA, miR-590-3p, derived from M2 macrophages, promotes the proliferation and wound healing of colonic epithelial cells by targeting the serine/threonine-protein kinase LATS1, thereby activating the transcription of yes-associated protein-regulated genes. Additionally, it inhibits the activation of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6, which further aids in the healing process (Deng et al., 2021).

Annexin A1(ANXA1), found in EVs derived from both IECs and PMNs, promotes the healing of intestinal mucosal wounds (Leoni et al., 2012). In a mouse model of colitis, the release of ANXA1 significantly increases during the wound closure process. ANXA1 achieves its effects by attaching to formyl peptide receptors present on responsive cells (Leoni et al., 2015), which subsequently activates epithelial NADPH oxidase NOX1. This activation leads to the formation of reactive oxygen species, crucial for regulating the activity of focal adhesion kinase (Leoni et al., 2012), an enzyme that participates in cell migration and adhesion. The role of ANXA1 is particularly important in inflammatory conditions, as its elevation in patients with acute inflammation and injury indicates its important part in the healing process (Shen et al., 2022).

Wnt5b is a signaling molecule found in EVs from IECs and macrophages. It can activate the WNT/β-catenin pathway, which is important for maintaining intestinal epithelial homeostasis and wound closure (Miyoshi, 2017). Wnt5b-containing EVs activate other cells to migrate and proliferate in a paracrine way, crucial for the regenerative response in the intestine.

In summary, the multifaceted roles of EVs in intestinal tissue repair underscore their significance in mediating various cellular processes and signaling pathways. Understanding these mechanisms is crucial for revealing their potential application in clinical therapy, opening up possibilities for innovative treatment strategies focused on enhancing gut tissue repair and regeneration.

3.2.4. Control of pathogen recognition and clearance

The gut is continuously exposed to a diverse range of pathogens such as bacteria, viruses, and parasites. The immune system in the gut has developed multiple mechanisms for pathogen recognition and clearance. Among these mechanisms, EVs play a significant role by delivering bioactive molecules that enhance pathogen recognition and clearance. In the gut environment, EVs can bind to and recognize pathogens through the receptors present on their surfaces, initiating immune responses and facilitating pathogen clearance by promoting phagocytosis or inducing the release of antimicrobial substances (Kuipers et al., 2018). For example, Probiotic and *Escherichia coli* Nissle 1917 EVs activate DCs to activate the Th1 response crucial for resisting pathogen infections (Diaz-Garrido et al., 2019). By delivering identical microbial-associated molecular patterns, including LPS (a ligand for TLR4), lipoproteins (a ligand for TLR2), peptidoglycan (an activator of NOD1/NOD2), DNA and RNA (ligands for TLR9 and TLR7), EVs trigger a consensual signaling cascade. This cascade results in the upregulation of antimicrobial peptides and inflammatory cytokines, indispensable for the body's defense against pathogens. Additionally, EVs can interact with gut microbiota, modulating their composition and activity to enhance pathogen resistance.

The *V. cholerae* V:5/04non-O1 non-O139 clinical isolate secretes gut BEVs. These EVs interact with T84 cells for 2 h and result in the upregulation of miR-146a. Studies have indicated that miR-146a acts as an inhibitor of the host's innate immune response. It achieves this through suppressing the expression of pro-inflammatory cytokines, for example, IL-8, TNF-α, and IL-1β, thus facilitating the survival and proliferation of pathogens in the host environment. This occurs by binding to specific sequences within the mRNA molecules of these cytokines, causing their degradation or inhibiting their translation. This suppression of pro-inflammatory gene expression creates a favorable environment for certain pathogens to evade detection and clearance (Bitar et al., 2019).

Two antimicrobial peptides, namely cathelicidin-37 and defensin-2, are synthesized and encapsulated within EVs of IECs. When *Cryptosporidium parvum* infects, IECs increase production and luminal secretion of EVs. This response is triggered by TLR-4 signaling that coordinates a Snap23-dependent vesicular exocytosis pathway. As a result, EVs are rich in epithelial-derived antimicrobial peptides that play a crucial role in fighting infection. When *Cryptosporidium parvum* encounters EVs of IECs, its infectiousness is weakened both *in vitro* and *in vivo*, indicating that EVs serve an important purpose in the body's antimicrobial defense against invading pathogens in the intestinal mucosa (Hu et al., 2013). Defensins carried by EVs are involved in maintaining a healthy gut and are relevant in IBD (Panigrahi et al., 2022; Wehkamp et al., 2005). Numerous *in vitro* studies have verified that defensins display remarkable antibacterial, antifungal, and antiviral activities towards a diverse range of microorganisms (Raj and Dentino, 2002; Risso, 2000; Selsted et al., 1984). Defensin itself also has direct antibacterial activity, and there is a bactericidal concentration, at which concentration can directly act on bacteria (Wehkamp et al., 2005; Yang et al., 2001).

In conclusion, the control of pathogen recognition and clearance in the gut is a complex and multi-faceted process. EVs, along with their carried miRNAs, proteins, and metabolites, work in concert to either enhance or suppress the immune response. This intricate network of interactions not only determines the outcome of pathogen-host battles but also has implications for maintaining gut health and treating related diseases. More research is necessary to thoroughly understand the potential of modulating these mechanisms for therapeutic purposes and to develop novel strategies for combating gut pathogens.

3.3. Shaping of gut microbiota function

EVs serve as key communicators between cells and the gut microbiome, orchestrating the complex interactions among microorganisms in the GI tract. By transporting miRNAs and metabolites, microbe-derived EVs can cross the intestinal barrier and modulate the intestinal microbiota (Park et al., 2021; Zhang et al., 2022), affecting its composition, structure, growth, activity, diversity, abundance, and metabolic pathways. miRNAs are important in shaping gut microbiota function. miR-1226-5p promotes the growth of *Escherichia coli*, while miR-515-5p stimulates the growth of *Fusobacterium nucleatum* (Liu et al., 2016). These fecal miRNAs are present in EVs and have been found they directly modulate the expression of bacterial genes and influence the growth of the gut microbiota. Since miRNAs can align to either the positive or negative target strand, they can operate at the DNA level to affect gene expression or directly on RNA. However, the specific mechanism underlying this phenomenon needs more investigation. Besides, miR-141-3p and miR-200a-3p are both transported through EVs shed host's IECs and +4 niche-derived Hopx-expressing cells, including Goblet and Paneth cells (Liu et al., 2016; Moloney et al., 2018). miR-141-3p has shown a notable correlation with the relative abundance of the phylum *Bacteroidetes* and *Firmicutes*. Similarly, various phyla including *Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes*, and *Proteobacteria* showed significant correlations with miR-200a-3p. miRNAs are involved in interactions with epithelial ion transporters, regulating host gene expression. They mainly exert this influence by inhibiting mRNA translation or causing mRNA degradation (Bartel, 2004). In the interaction between miRNAs and bacteria, they usually do not act directly on bacteria but indirectly affect the survival and activity of bacteria by influencing the structure and function of host epithelial cells. When miRNAs target and suppress genes related to epithelial cell functions, it can lead to alterations in cell properties that might facilitate bacterial penetration or disrupt the epithelium's defense mechanisms against bacteria (Goodrich et al., 2014). Additionally, plant-derived EVs modulated the composition and localization of gut microbiota and also strengthened the gut barrier function to mitigate colitis. mdo-miR7267-3p is derived from ginger EVs, specifically target genes in the monooxygenase ycnE of *Lactobacillus rhamnosus* (LGG), thereby affecting the metabolites of LGG. The production of indole-3-carboxaldehyde, which is a substance capable of activating aryl hydrocarbon receptors, while mdo-miR7267-3p or I3A itself can induce the production of IL-22. IL-22, an immune molecule associated with improved intestinal barrier function, could be raised by the regulation of GELN-RNAs, thereby helping to alleviate colitis in mice (Teng et al., 2018). Highly metastatic CRC cells release EVs enriched with miR-181a-5p, which are delivered to hepatic stellate cells (HSCs). Once inside the HSCs, miR-181a-5p targets the SOCS3 gene, activating the

IL6/STAT3 signaling pathway and leading to the enhanced expression of pro-inflammatory genes like IL-6 and IL-8. These changes in gene expression can alter the gut microbiota structure, potentially creating a tumor microenvironment that promotes the progression and metastasis of CRC (Zhao et al., 2022).

Abundant proteins and miRNAs in mEVs have been found to reshape the gut microbiota. Specifically, in DSS-induced colitis, gut microbial diversity decreases, but mEVs treatment moderately restores it. Fewer OTUs were detected in DSS-treated mice in comparison with control and mEVs-treated mice. And α-diversity indices, such as Chao 1 index, Shannon index, Observed species, and Faith_pd, which are markedly reduced in DSS-treated mice, are effectively restored with mEV treatment, indicating enhanced richness and diversity of the gut microbiota. At the phylum level, DSS treatment significantly decreases the relative abundance of *Bacteroidetes* and increases *Proteobacteria* and *Verrucomicrobia*, but mEV treatment nearly restores these to control levels. At the genus level, DSS-treated mice show reduced *Enterobacteriaceae* and *unclassified_Bacteroidia,* partially recovered by mEV treatment. Additionally, DSS-induced mice exhibit increased *Enterococcaceae* and *Desulfovibrionales-unclassified Desulfovibrionaceae*, which remain unchanged in the EV-treated group. Notably, the beneficial bacterium *Akkermansia* is significantly increased in EV-treated mice. The mechanisms by which mEVs influence gut microbiota are likely multifaceted, but further research is needed to elucidate the exact pathways involved (Tong et al., 2021).

The gut microbiota of mice given high-protein food produces considerably more EVs. These EVs activate TLR4, resulting in a rise in the expression of IgA-inducing cytokines APRIL, B cell chemokine CCL28, and the IgA transporter PIGR in the epithelial cells. As a result, this promotes the production of sIgA, an important factor in ensuring host-microbiota mutualism. Moreover, a high-protein diet causes changes in the gut microbiota composition. It may lead to alterations in the ratio of *Firmicutes/Bacteroidetes* and an increase in the representation of certain phyla like *Actinobacteria* in the case of high protein-fed mice. At the genus level, it results in an increased abundance of bacteria such as *Bifidobacterium* in high protein-fed mice. The altered microbiota composition due to the high-protein diet also continues to impact the production of succinic acid and the release of EVs, establishing a complex feedback cycle that affects the host's immune response and the connection between the host and the microbiota (Tan et al., 2022). Similarly, bile acids can activate the FXR, leading to the transcription of antimicrobial peptides like inducible nitric oxide synthase and IL-18, thus modulating the gut microbiota's composition. Bile acids also act on emulsifying dietary fats, aiding in their digestion and absorption, which can indirectly affect the microbial community (Inagaki et al., 2006).

Overall, EVs contribute to regulating gut health through various mechanisms and in-depth research into the functions and mechanisms of EVs is crucial for comprehending gut-related diseases and developing effective intervention strategies.

4. Challenges and perspectives

EVs hold significant potential for promoting gut health, however, their development in gut health applications is hindered by unresolved issues related to several major aspects, such as isolation and purification, size diversity, cargo loading and function, targeted delivery, stability, safety, and reproducibility (De Sousa et al., 2023; Nieuwland et al., 2022).

4.1. Isolation and purification of high-purity EVs

Achieving high-purity isolation and purification of EVs is a challenging assignment, mainly because of the existence of diverse components and various subtypes of EVs (Doyle and Wang, 2019). The absence of standardized procedures for isolating, purifying, and characterizing EVs has resulted in issues such as contamination, low yield, and challenges in distinguishing EVs from other extracellular particles. This has ultimately hindered the reproducibility and comparability of research findings (De Sousa et al., 2023). Moreover, the absence of standardized procedures presents challenges in determining the most suitable approach for specific research purposes.

For instance, theoretical investigations into the types, amounts, and functions of BEVs from human feces play a vital role in comprehending their secretion and function within the gut microbiota. However, the complex composition of human feces, which includes water, bacteria, lipids, proteins, undigested food residues, and exfoliated epithelial cells, presents difficulties in the isolation and purification of BEVs. To address these issues, researchers are developing and optimizing methods specifically tailored for gut samples. Current isolation strategies for BEVs primarily utilize ultra-high-speed centrifugation, density gradient centrifugation, and size exclusion chromatography (Coumans et al., 2017; Kang et al., 2013; Northrop Albrecht et al., 2022; Park et al., 2022; Tulkens, De Wever, et al., 2020; Tulkens, Vergauwen, et al., 2020). However, studies have demonstrated that single-approach strategies are insufficient for effectively isolating EVs from soluble proteins in body fluids, including lipoproteins in the blood (Simonsen, 2017) and Tamm-Horsfall protein in the urine (Correll et al., 2022). Besides, the size range of eukaryotic EVs often overlaps with that of BEVs, thus requiring further methodological advancements to enhance the yield of BEVs. Combining multi-step centrifugation with density gradient centrifugation can improve the purity and yield of EVs (Liangsupree et al., 2021). Moreover, using immunocapture techniques with specific antibodies or affinity tags can more effectively isolate particular types of EVs from complex samples (Brambilla et al., 2021).

Standardizing these methods is crucial for enhancing the reproducibility and comparability of gut health research. By establishing consistent standards for EV isolation and purification, researchers can more accurately compare results across different studies, thereby advancing scientific progress in the field of gut health.

4.2. Functional significance of EV size diversity

The size diversity of EVs presents a profound challenge and holds great significance in the field of EV biology. Different-sized EV subtypes possess distinct functions as they can carry different cargo. For example, exosomes, which are relatively small EVs with diameters spanning from about 30 to 150 nm, are formed through the endosomal pathway. They are abundant in proteins, nucleic acids, and lipids and carry signaling molecules from their cell of origin. This enables them to perform a significant part in cell-to-cell communication and signaling (Ludwig and Giebel, 2012). Microvesicles, ranging from about 100 to 1000 nm and formed by budding directly from the plasma membrane, have different cargo and membrane antigens compared to exosomes. They facilitate cellular interactions in a manner distinct from exosomes (Ratajczak and Ratajczak, 2017). Apoptotic bodies, the largest EV subtype with a diameter ranging from about 500 to 4000 nm, are produced during programmed cell death and often contain organelles or nuclear contents. They are involved in the removal of damaged cells and the regulation of immune responses. Different-sized EVs carry unique molecules that play roles in the physiological and pathological processes of the gut (Dixson et al., 2023). Besides, the wide range of sizes and heterogeneity of EVs greatly influence their targeting abilities (Ferguson and Nguyen, 2016). This complexity in cell-to-cell communication mediated by EVs necessitates further investigation to understand the implications of size diversity and the functional significance of EVs in intercellular communication (Mathieu et al., 2019). An important aspect to consider is how the biogenesis pathway and the composition of EVs contribute to their diverse functions and the complexity of intercellular communication. The distinct formation processes of different-sized EVs lead to variations in their cargo and membrane characteristics, which in turn determine their functions. By understanding these mechanisms, we can

gain a deeper understanding of why different-sized EVs may have different functions and how they contribute to biological processes.

4.3. Cargo loading and function

Numerous complexities and obstacles persist regarding cargo loading and function for targeted delivery systems to treat gut-related diseases (Meng et al., 2020). Understanding how EVs function in both gut health and diseased states is crucial for developing effective treatments. While EVs have been demonstrated to be involved in diverse physiological processes such as immune regulation, tissue repair, and cell signaling, it is essential to differentiate between the effects directly mediated by EVs and those induced by their cargo upon recipient cells, to elucidate the mechanisms underlying EVs-mediated biological responses (Wiklander et al., 2019). Understanding how specific cargo molecules carried by EVs regulate the physiology and function of target cells remains challenging. The challenge of cargo loading in EVs lies in understanding the selective packaging mechanism for specific substances including proteins, RNAs, and DNAs in EVs (Chiou et al., 2018). While certain molecules exhibit cell-specific signatures in EVs, the mechanism behind this selectivity remains unknown. However, instances of nonselective packaging, as seen with miRNAs, have been observed. The existence of RNA-processing enzymes in EVs indicates that cargo molecules are being processed within the EVs (Esmaeili et al., 2022; Jeppesen et al., 2023). Identifying redundancies and relationships between EV cargo components might necessitate the use of advanced single-vesicle analysis tools (Bordanaba Florit et al., 2021). These tools can help identify redundancies and relationships between EV cargo components to better understand the role of EVs in gut health. By delving deeper into the cargo loading mechanism and function of EVs, we can develop more effective treatments to improve gut health and treat gut-related diseases.

4.4. Targeted delivery

The interactions of EVs with the immune system and intracellular trafficking pathways determine their ability to reach and deliver cargo to specific cells or organs. The mechanisms by which bioactive cargo carried by EVs is efficiently delivered and processed inside target cells remain unresolved. While abundant EVs released to nearby cells may not require precise targeting mechanisms, reaching distant targets requires a unique bar code on EV surfaces for recognition by receptor molecules on recipient cells (Ginini et al., 2022). The gut is a complex microenvironment and cytokines present on EV surfaces may act as part of this bar code, engaging with cell-specific receptors, thereby influencing immune responses and inflammatory states in the gut (Li et al., 2024a; Santos et al., 2021). For example, EVs in the gut can carry anti-inflammatory cytokines that modulate local immune responses and reduce inflammation by interacting with specific receptors on gut epithelial cells or immune cells. Conversely, EVs released by pathogens might carry pro-inflammatory cytokines, activating gut immune cells and leading to inflammation and compromised gut barrier function.

A comprehensive understanding of the cargo content and interactions of EVs with target cells is essential for elucidating their biological effects and designing strategies to modulate EV targeting mechanisms.

4.5. EVs stability

The challenges surrounding EV stability are multifaceted and crucial for advancing the field of EV-based gut health. Preservation during transportation and storage is a key consideration to ensure the maintenance of the integrity and function of EVs (Kanojia et al., 2018). Factors like temperature fluctuations, pH variations, and physical pressure during handling can all impact the stability of EVs. *In vivo,* stability presents another set of challenges, including protecting EVs from enzymatic degradation in gut fluids and enhancing their circulation time in the bloodstream (Raise et al., 2020). The hostile gut environment poses a significant barrier to oral EV delivery, necessitating the development of innovative encapsulation technologies that shield EVs from digestive enzymes and acidic conditions (Lopes et al., 2023). Strategies to prolong EV circulation time, such as surface modifications with polymers like polyethylene glycol or cell-specific targeting ligands, can enhance EV's biodistribution and increase their gut-healthy efficacy.

4.6. Safety profile of EVs

One significant challenge in the application of EVs is ensuring their safety for gut health. While EVs are generally considered safe, comprehensive evaluations are necessary to assess their safety and potential risks (Xie et al., 2022). For instance, in the case of functional foods enriched with EVs derived from probiotics, it is important to take into account the influence of these EVs on gut health (Raise et al., 2020; Xie et al., 2023). Comprehensive assessments should investigate how EVs are absorbed, distributed, metabolized, and excreted within the body to provide insights into the safety profile of EVs (Domínguez Rubio et al., 2022; Molina Tijeras et al., 2019).

4.7. EVs reproducibility

Although fermentation vessels allow for the large-scale cultivation of bacteria, the output of EVs from bacterial cultures may fall short of meeting the requirements for cost-effective, large-scale manufacturing. Studies have demonstrated that employing different culture systems and genetic alterations can result in enhanced secretion of EVs (Bost et al., 2022; Syromiatnikova et al., 2022). Besides, data reproducibility in EV research remains a significant challenge. Several initiatives, such as the EVs-TRACK platform (http://evtrack.org), the massive open online course on the detection and isolation of EVs, the minimal information for studies on EVs guidelines, the standardization of EVs concentration measurements, and the compendium on EVs flow cytometry (Nieuwland et al., 2020; 2022; Van Deun et al., 2017), aim to address this challenge by promoting transparent reporting and centralizing knowledge in EV research. Furthermore, the establishment of the Rigor and Standardization Subcommittee, as well as the encouragement of task force activities, education, and transparent reporting, have raised awareness among EV researchers about the importance of producing and reporting traceable and reproducible results. Collaborative efforts, technique standardization, statistical analysis, and open data sharing are crucial for improving data reproducibility within the scientific community, ultimately contributing to the advancement of EVs and gut health research (Nieuwland et al., 2022).

5. Conclusions

EVs, sourced from plants, animals, and microbiota, are essential in the complex regulation of gut health through their dynamic interactions within the GI system. The bioactive cargo within EVs exhibits a myriad of functions, including maintaining epithelial barrier integrity, modulating immune responses, promoting tissue regeneration, preventing pathogen invasion, and shaping the gut microbiota. This review also highlights the outstanding challenges in EV research for gut health, encompassing aspects such as isolation and purification methods, biogenesis mechanisms, cargo loading and function, targeted delivery, stability, safety, and reproducibility. Addressing these challenges will propel the field of EVs in gut health forward, potentially leading to efficacious interventions for improved gut health. The synergy of interdisciplinary collaboration among biology, microbiology, immunology, and engineering will deepen our understanding of EVs' critical role in gut health. Future endeavors will concentrate on elucidating EV mechanisms and overcoming obstacles for optimal applications in gut health.

CRediT authorship contribution statement

Qiming Wu: Visualization, Writing – review & editing. **Juntao Kan:** Methodology, Writing – review & editing. **Caili Fu:** Methodology, Writing – review & editing. **Xin Liu:** Data curation, Writing – original draft, Writing – review & editing. **Zhengying Cui:** Data curation, Writing – original draft, Writing – review & editing. **Sixu Wang:** Data curation, Writing – original draft, Writing – review & editing. **Yi Le:** Data curation, Writing – original draft, Writing – review & editing. **Zhanming Li:** Data curation, Writing – original draft, Writing – review & editing. **Qin Liu:** Visualization, Writing – review & editing. **Yuyu Zhang:** Visualization, Writing – review & editing. **Jun Du:** Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jun Du reports financial support was provided by Amway China Co., Limited. Yuyu Zhang reports financial support was provided by National Natural Science Foundation of China. Caili Fu reports financial support was provided by Science and Technology Project of Jiangsu Province. Caili Fu reports financial support was provided by Key Research and Development Project of Hainan Province. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

References

Aarts, J., Boleij, A., Pieters, B.C.H., Feitsma, A.L., van Neerven, R.J.J., Ten Klooster, J.P, M'Rabet, L., Arntz, O.J., Koenders, M.I., van de Loo, F.A.J., 2021. Flood control: how milk-derived extracellular vesicles can help to improve the intestinal barrier function and break the gut-joint axis in rheumatoid arthritis. Front. Immunol. 12, 703277. https://doi.org/10.3389/fimmu.2021.703277.

Alexander, M., Hu, R., Runtsch, M.C., Kagele, D.A., Mosbruger, T.L., Tolmachova, T., Seabra, M.C., Round, J.L., Ward, D.M., O'Connell, R.M, 2015. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. Nat. Commun. 6 (1), 7321. https://doi.org/10.1038/ncomms8321.

Alvarez, C.S., Badia, J., Bosch, M., Giménez, R., Baldomà, L., 2016. Outer membrane vesicles and soluble factors released by probiotic *Escherichia coli* Nissle 1917 and commensal ECOR63 enhance barrier function by regulating expression of tight junction proteins in intestinal epithelial cells. Front. Microbiol. 7, 1981. https://doi.org/10.3389/fmicb.2016.01981.

Alvarez, C.S., Giménez, R., Cañas, M.A., Vera, R., Díaz Garrido, N., Badia, J., Baldomà, L., 2019. Extracellular vesicles and soluble factors secreted by *Escherichia coli* Nissle 1917 and ECOR63 protect against enteropathogenic *E. coli*-induced intestinal epithelial barrier dysfunction. BMC Microbiol. 19 (1), 1–12. https://doi.org/10.1186/s12866-019-1534-3.

Alzahrani, F.A., Khan, M.I., Kameli, N., Alsahafi, E., Riza, Y.M., 2023. Plant-derived extracellular vesicles and their exciting potential as the future of next-generation drug delivery. Biomolecules 13 (5), 839. https://doi.org/10.3390/biom13050839.

Bakirtzi, K., Man Law, I.K., Fang, K., Iliopoulos, D., Pothoulakis, C., 2019. MiR-21 in substance P-induced exosomes promotes cell proliferation and migration in human colonic epithelial cells. Am. J. Physiol. Gastrointest. Liver 317 (6), G802–G810. https://doi.org/10.1152/ajpgi.00043.2019.

Barbara, G., Barbaro, M.R., Fuschi, D., Palombo, M., Falangone, F., Cremon, C., Marasco, G., Stanghellini, V., 2021. Inflammatory and microbiota-related regulation of the intestinal epithelial barrier. Front. Nutr. 8, 718356. https://doi.org/10.3389/fnut.2021.718356.

Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116 (2), 281–297. https://doi.org/10.1016/s0092-8674(04)00045-5.

Bauer, K.M., Nelson, M.C., Tang, W.W., Chiaro, T.R., Brown, D.G., Ghazaryan, A., Lee, S. H., Weis, A.M., Hill, J.H., Klag, K.A., 2022. CD11c+ myeloid cell exosomes reduce intestinal inflammation during colitis. JCI Insight 7 (19). https://doi.org/10.1172/jci.insight.159469.

Bischoff, S.C., 2011. 'Gut health': a new objective in medicine? BMC Med. 9, 1–14. https://doi.org/10.1186/1741-7015-9-24.

Bitar, A., Aung, K.M., Wai, S.N., Hammarström, M.L., 2019. *Vibrio cholerae* derived outer membrane vesicles modulate the inflammatory response of human intestinal epithelial cells by inducing microRNA-146a. Sci. Rep. 9 (1), 7212. https://doi.org/10.1038/s41598-019-43691-9.

Bordanaba Florit, G., Royo, F., Kruglik, S.G., Falcón-Pérez, J.M., 2021. Using single-vesicle technologies to unravel the heterogeneity of extracellular vesicles. Nat. Protoc. 16 (7), 3163–3185. https://doi.org/10.1038/s41596-021-00551-z.

Bost, J.P., Saher, O., Hagey, D., Mamand, D.R., Liang, X., Zheng, W., Corso, G., Gustafsson, O., Görgens, A., Smith, C.E., 2022. Growth media conditions influence the secretion route and release levels of engineered extracellular vesicles. Adv. Healthc. Mater. 11 (5), 2101658. https://doi.org/10.1002/adhm.202101658.

Brambilla, D., Sola, L., Ferretti, A.M., Chiodi, E., Zarovni, N., Fortunato, D., Criscuoli, M., Dolo, V., Giusti, I., Murdica, V., 2021. EV separation: release of intact extracellular vesicles immunocaptured on magnetic particles. Anal. Chem. 93 (13), 5476–5483. https://doi.org/10.1021/acs.analchem.0c05194.

Brown, L., Wolf, J.M., Prados Rosales, R, Casadevall, A, 2015. Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. Nat. Rev. Microbiol. 13 (10), 620–630. https://doi.org/10.1038/nrmicro3480.

Bui, T.M., Mascarenhas, L.A., Sumagin, R., 2018. Extracellular vesicles regulate immune responses and cellular function in intestinal inflammation and repair. Tissue Barriers 6 (2), e1431038. https://doi.org/10.1080/21688370.2018.1431038.

Butin-Israeli, V., Houser, M.C., Feng, M., Thorp, E.B., Nusrat, A., Parkos, C.A., Sumagin, R., 2016. Deposition of microparticles by neutrophils onto inflamed epithelium: a new mechanism to disrupt epithelial intercellular adhesions and promote transepithelial migration. FASEB J. 30 (12), 4007. https://doi.org/10.1096/fj.201600734R.

Cao, Y.Y., Wang, Z., Wang, Z.H., Jiang, X.G., Lu, W.H., 2021. Inhibition of miR-155 alleviates sepsis-induced inflammation and intestinal barrier dysfunction by inactivating NF-kappaB signaling. Int. Immunopharmacol. 90, 107218. https://doi.org/10.1016/j.intimp.2020.107218.

Chang, L., Chiu, H., Wu, M., Shen, T., 2022. The role of small extracellular vesicles in the progression of colorectal cancer and its clinical applications. Int. J. Mol. Sci. 23 (3), 1379. https://doi.org/10.3390/ijms23031379.

Chang, X., Song, Y., Xia, T., He, Z.x., Zhao, S., Wang, Z., Gu, L., Li, Z., Xu, C., Wang, S., 2023. Macrophage-derived exosomes promote intestinal mucosal barrier dysfunction in inflammatory bowel disease by regulating TMIGD1 via mircroRNA-223. Int. Immunopharmacol. 121, 110447. https://doi.org/10.1016/j.intimp.2023.110447.

Chen, Q., Zu, M., Gong, H., Ma, Y., Sun, J., Ran, S., Shi, X., Zhang, J., Xiao, B., 2023. Tea leaf-derived exosome-like nanotherapeutics retard breast tumor growth by pro-apoptosis and microbiota modulation. J. Nanobiotechnol. 21 (1), 6. https://doi.org/10.1186/s12951-022-01755-5.

Chen, S., Yong, Y., Ju, X., 2021a. Effect of heat stress on growth and production performance of livestock and poultry: mechanism to prevention. J. Therm. Biol. 99, 103019. https://doi.org/10.1016/j.jtherbio.2021.103019.

Chen, X., Liu, B., Li, X., An, T.T., Zhou, Y., Li, G., Wu Smart, J., Alvarez, S., Naldrett, M.J., Eudy, J., 2021b. Identification of anti-inflammatory vesicle-like nanoparticles in honey. J. Extracell. Vesicles 10 (4), e12069. https://doi.org/10.1002/jev2.12069.

Chen, Y., Cui, W., Li, X., Yang, H., 2021c. Interaction between commensal bacteria, immune response and the intestinal barrier in inflammatory bowel disease. Front. Immunol. 12, 761981. https://doi.org/10.3389/fimmu.2021.761981.

Cheng, J., Zhang, Y., Ma, L., Du, W., Zhang, Q., Gao, R., Zhao, X., Chen, Y., Jiang, L., Li, X., 2023. Macrophage-derived extracellular vesicles-coated palladium nanoformulations modulate inflammatory and immune homeostasis for targeting therapy of ulcerative colitis. Adv. Sci. 10 (33), 2304002. https://doi.org/10.1002/advs.202304002.

Chiou, N., Kageyama, R., Ansel, K.M., 2018. Selective export into extracellular vesicles and function of tRNA fragments during T cell activation. Cell Rep. 25 (12), 3356–3370. https://doi.org/10.1016/j.celrep.2018.11.073 e3354.

Cichon, C., Sabharwal, H., Rüter, C., Schmidt, M.A., 2014. MicroRNAs regulate tight junction proteins and modulate epithelial/endothelial barrier functions. Tissue Barriers 2 (4), e944446. https://doi.org/10.4161/21688362.2014.944446.

Correll, V.L., Otto, J.J., Risi, C.M., Main, B.P., Boutros, P.C., Kislinger, T., Galkin, V.E., Nyalwidhe, J.O., Semmes, O.J., Yang, L., 2022. Optimization of small extracellular vesicle isolation from expressed prostatic secretions in urine for in-depth proteomic analysis. J. Extracell. Vesicles 11 (2), e12184. https://doi.org/10.1002/jev2.12184.

Coumans, F.A., Brisson, A.R., Buzas, E.I., Dignat-George, F., Drees, E.E., El-Andaloussi, S., Emanueli, C., Gasecka, A., Hendrix, A., Hill, A.F., 2017. Methodological guidelines to study extracellular vesicles. Circ. Res. 120 (10), 1632–1648. https://doi.org/10.1161/CIRCRESAHA.117.309417.

Cuesta, C.M., Guerri, C., Ureña, J., Pascual, M., 2021. Role of microbiota-derived extracellular vesicles in gut-brain communication. Int. J. Mol. Sci. 22 (8), 4235. https://doi.org/10.3390/ijms22084235.

Dadgar, N., Altemus, J., Li, Y., Lightner, A.L., 2022. Effect of crohn's disease mesenteric mesenchymal stem cells and their extracellular vesicles on T-cell immunosuppressive

capacity. J. Cell. Mol. Med. 26 (19), 4924–4939. https://doi.org/10.1111/jcmm.17483.
De Robertis, M., Sarra, A., D'Oria, V., Mura, F., Bordi, F., Postorino, P., Fratantonio, D, 2020. Blueberry-derived exosome-like nanoparticles counter the response to TNF-α-induced change on gene expression in EA. hy926 cells. Biomolecules 10 (5), 742. https://doi.org/10.3390/biom10050742.
De Sousa, K.P., Rossi, I., Abdullahi, M., Ramirez, M.I., Stratton, D., Inal, J.M., 2023. Isolation and characterization of extracellular vesicles and future directions in diagnosis and therapy. WIREs Nanomed. Nanobiotechnol. 15 (1), e1835. https://doi.org/10.1002/wnan.1835.
Deng, F., Yan, J., Lu, J., Luo, M., Xia, P., Liu, S., Wang, X., Zhi, F., Liu, D., 2021. M2 macrophage-derived exosomal miR-590-3p attenuates DSS-induced mucosal damage and promotes epithelial repair via the LATS1/YAP/β-catenin signalling axis. J. Crohns Colitis 15 (4), 665–677. https://doi.org/10.1093/ecco-jcc/jjaa214.
Deng, Z., Mu, J., Tseng, M., Wattenberg, B., Zhuang, X., Egilmez, N.K., Wang, Q., Zhang, L., Norris, J., Guo, H., Yan, J., Haribabu, B., Miller, D., Zhang, H.G., 2015. Enterobacteria-secreted particles induce production of exosome-like S1P-containing particles by intestinal epithelium to drive Th17-mediated tumorigenesis. Nat. Commun. 6 (1), 6956. https://doi.org/10.1038/ncomms7956.
Diaz-Garrido, N., Fábrega, M.J., Vera, R., Giménez, R., Badia, J., Baldomà, L., 2019. Membrane vesicles from the probiotic Nissle 1917 and gut resident *Escherichia coli* strains distinctly modulate human dendritic cells and subsequent T cell responses. J. Funct. Foods 61, 103495. https://doi.org/10.1016/j.jff.2019.103495.
Díaz-Garrido, N., Badia, J., Baldomà, L., 2021. Microbiota-derived extracellular vesicles in interkingdom communication in the gut. J. Extracell. Vesicles 10 (13), e12161. https://doi.org/10.1002/jev2.12161.
Diaz Garrido, N., Cordero, C., Olivo Martinez, Y., Badia, J., Baldomà, L., 2021. Cell-to-cell communication by host-released extracellular vesicles in the gut: implications in health and disease. Int. J. Mol. Sci. 22 (4), 2213. https://doi.org/10.3390/ijms22042213.
Dixson, A.C., Dawson, T.R., Di Vizio, D., Weaver, A.M., 2023. Context-specific regulation of extracellular vesicle biogenesis and cargo selection. Nat. Rev. Mol. Cell Biol. 24 (7), 454–476. https://doi.org/10.1038/s41580-023-00576-0.
Djouad, F., Bouffi, C., Ghannam, S., Noël, D., Jorgensen, C., 2009. Mesenchymal stem cells: innovative therapeutic tools for rheumatic diseases. Nat. Rev. Rheumatol. 5 (7), 392–399. https://doi.org/10.1038/nrrheum.2009.104.
Dmytriv, T.R., Storey, K.B., Lushchak, V.I., 2024. Intestinal barrier permeability: the influence of gut microbiota, nutrition, and exercise. Front. Physiol. 15, 1380713. https://doi.org/10.3389/fphys.2024.1380713.
Domínguez Rubio, A.P, D'Antoni, C.L., Piuri, M., Pérez, O.E, 2022. Probiotics, their extracellular vesicles and infectious diseases. Front. Microbiol. 13, 864720. https://doi.org/10.3389/fmicb.2022.864720.
Doyle, L.M., Wang, M.Z., 2019. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. Cells 8 (7), 727. https://doi.org/10.3390/cells8070727.
Du, C., Quan, S., Nan, X., Zhao, Y., Shi, F., Luo, Q., Xiong, B., 2021. Effects of oral milk extracellular vesicles on the gut microbiome and serum metabolome in mice. Food Funct. 12 (21), 10938–10949. https://doi.org/10.1039/d1fo02255e.
Eom, J.Y., Choi, S.H., Kim, H.J., Kim, D.h., Bae, J.H., Kwon, G.S., Lee, D.h., Hwang, J.H., Kim, D.K., Baek, M.C., 2022. Hemp-derived nanovesicles protect leaky gut and liver injury in dextran sodium sulfate-induced colitis. Int. J. Mol. Sci. 23 (17), 9955. https://doi.org/10.3390/ijms23179955.
Esmaeili, A., Alini, M., Baghaban Eslaminejad, M., Hosseini, S., 2022. Engineering strategies for customizing extracellular vesicle uptake in a therapeutic context. Stem Cell Res. Ther. 13 (1), 129. https://doi.org/10.1186/s13287-022-02806-2.
Ferguson, S.W., Nguyen, J., 2016. Exosomes as therapeutics: the implications of molecular composition and exosomal heterogeneity. J. Control. Release 228, 179–190. https://doi.org/10.1016/j.jconrel.2016.02.037.
Fernández Messina, L, Gutiérrez Vázquez, C., Rivas García, E., Sánchez Madrid, F., de la Fuente, H, 2015. Immunomodulatory role of microRNAs transferred by extracellular vesicles. Biol. Cell 107 (3), 61–77. https://doi.org/10.1111/boc.201400081.
Festi, D., Schiumerini, R., Eusebi, L.H., Marasco, G., Taddia, M., Colecchia, A., 2014. Gut microbiota and metabolic syndrome. World J. Gastroenterol. 20 (43), 16079. https://doi.org/10.3748/wjg.v20.i43.16079.
Fujita, D., Arai, T., Komori, H., Shirasaki, Y., Wakayama, T., Nakanishi, T., Tamai, I., 2018. Apple-derived nanoparticles modulate expression of organic-anion-transporting polypeptide (OATP) 2B1 in Caco-2 cells. Mol. Pharm. 15 (12), 5772–5780. https://doi.org/10.1021/acs.molpharmaceut.8b00921.
Gandek, T.B., van der Koog, L., Nagelkerke, A., 2023. A comparison of cellular uptake mechanisms, delivery efficacy, and intracellular fate between liposomes and extracellular vesicles. Adv. Healthc. Mater. 12 (25), 2300319. https://doi.org/10.1002/adhm.202300319.
Gareau, M.G., Silva, M.A., Perdue, M.H., 2008. Pathophysiological mechanisms of stress-induced intestina damage. Curr. Mol. Med. 8 (4), 274–281. https://doi.org/10.2174/156652408784533760.
Ghannam, S., Bouffi, C., Djouad, F., Jorgensen, C., Noël, D., 2010. Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. Stem Cell Res. Ther. 1 (1), 1–7. https://doi.org/10.1186/scrt2.
Ginini, L., Billan, S., Fridman, E., Gil, Z., 2022. Insight into extracellular vesicle-cell communication: from cell recognition to intracellular fate. Cells 11 (9), 1375. https://doi.org/10.3390/cells11091375.
Goodrich, J.K., Waters, J.L., Poole, A.C., Sutter, J.L., Koren, O., Blekhman, R., Beaumont, M., Van Treuren, W., Knight, R., Bell, J.T., 2014. Human genetics shape the gut microbiome. Cell 159 (4), 789–799. https://doi.org/10.1016/j.cell.2014.09.053.
Guo, M., Zhang, K., Zhang, J., 2022a. Human breast milk–derived exosomal miR-148a-3p protects against necrotizing enterocolitis by regulating p53 and sirtuin 1. Inflammation 45 (3), 1254–1268. https://doi.org/10.1007/s10753-021-01618-5.
Guo, Y., Xu, C., Gong, R., Hu, T., Zhang, X., Xie, X., Chi, J., Li, H., Xia, X., Liu, X., 2022b. Exosomal CagA from *Helicobacter pylori* aggravates intestinal epithelium barrier dysfunction in chronic colitis by facilitating Claudin-2 expression. Gut Pathog. 14 (1), 13. https://doi.org/10.1186/s13099-022-00486-0.
Hao, H., Zhang, X., Tong, L., Liu, Q., Liang, X., Bu, Y., Gong, P., Liu, T., Zhang, L., Xia, Y., 2021. Effect of extracellular vesicles derived from *Lactobacillus plantarum* Q7 on gut microbiota and ulcerative colitis in mice. Front. Immunol. 12, 777147. https://doi.org/10.3389/fimmu.2021.777147.
He, S., Liu, G., Zhu, X., 2021. Human breast milk-derived exosomes may help maintain intestinal epithelial barrier integrity. Proc. Nutr. Soc. 90 (2), 366–372. https://doi.org/10.1038/s41390-021-01449-y.
Hiippala, K., Barreto, G., Burrello, C., Diaz-Basabe, A., Suutarinen, M., Kainulainen, V., Bowers, J.R., Lemmer, D., Engelthaler, D.M., Eklund, K.K., Facciotti, F., Satokari, R., 2020. Novel odoribacter splanchnicus strain and its outer membrane vesicles exert immunoregulatory effects *in vitro*. Front. Microbiol. 11, 575455. https://doi.org/10.3389/fmicb.2020.575455.
Hu, G., Gong, A.Y., Roth, A.L., Huang, B.Q., Ward, H.D., Zhu, G., LaRusso, N.F., Hanson, N.D., Chen, X.M., 2013. Release of luminal exosomes contributes to TLR4-mediated epithelial antimicrobial defense. PLoS Pathog. 9 (4), e1003261. https://doi.org/10.1371/journal.ppat.1003261.
Hu, Q., Lyon, C.J., Fletcher, J.K., Tang, W., Wan, M., Hu, T.Y., 2021. Extracellular vesicle activities regulating macrophage-and tissue-mediated injury and repair responses. Acta Pharm. Sin. B 11 (6), 1493–1512. https://doi.org/10.1016/j.apsb.2020.12.014.
Huang, J., Wang, X., Wang, Z., Deng, L., Wang, Y., Tang, Y., Luo, L., Leung, E.L.H., 2023. Extracellular vesicles as a novel mediator of interkingdom communication. Cytokine Growth Factor Rev. https://doi.org/10.1016/j.cytogfr.2023.08.005.
Iacob, S., Iacob, D.G., Luminos, L.M., 2019. Intestinal microbiota as a host defense mechanism to infectious threats. Front. Microbiol. 9, 3328. https://doi.org/10.3389/fmicb.2018.03328.
Inagaki, T., Moschetta, A., Lee, Y.K., Peng, L., Zhao, G., Downes, M., Yu, R.T., Shelton, J. M., Richardson, J.A., Repa, J.J., 2006. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc. Natl. Acad. Sci. U. S. A. 103 (10), 3920–3925. https://doi.org/10.1073/pnas.0509592103.
Jackson, A.D., McLaughlin, J., 2009. Digestion and absorption. Surgery 27 (6), 231–236. https://doi.org/10.1016/j.mpsur.2009.03.003.
Jeppesen, D.K., Zhang, Q., Franklin, J.L., Coffey, R.J., 2023. Extracellular vesicles and nanoparticles: emerging complexities. Trends Cell Biol. 33 (8), 667–681. https://doi.org/10.1016/j.tcb.2023.01.002.
Jiang, J., Mei, J., Ma, Y., Jiang, S., Zhang, J., Yi, S., Feng, C., Liu, Y., Liu, Y., 2022. Tumor hijacks macrophages and microbiota through extracellular vesicles. Exploration 2 (1), 20210144. https://doi.org/10.1002/EXP.20210144.
Jiang, K., Rankin, C.R., Nava, P., Sumagin, R., Kamekura, R., Stowell, S.R., Feng, M., Parkos, C.A., Nusrat, A., 2014. Galectin-3 regulates desmoglein-2 and intestinal epithelial intercellular adhesion. J. Biol. Chem. 289 (15), 10510–10517. https://doi.org/10.1074/jbc.M113.538538.
Jiang, M., Li, F., Liu, Y., Gu, Z., Zhang, L., Lee, J., He, L., Vatsalya, V., Zhang, H.G., Deng, Z., 2023. Probiotic-derived nanoparticles inhibit ALD through intestinal miR194 suppression and subsequent FXR activation. Hepatology 77 (4), 1164–1180. https://doi.org/10.1002/hep.32608.
Jin, Y., Li, P., Wang, F., 2018. β-glucans as potential immunoadjuvants: a review on the adjuvanticity, structure-activity relationship and receptor recognition properties. Vaccine 36 (35), 5235–5244. https://doi.org/10.1016/j.vaccine.2018.07.038.
Ju, S., Mu, J., Dokland, T., Zhuang, X., Wang, Q., Jiang, H., Xiang, X., Deng, Z.B., Wang, B., Zhang, L., 2013. Grape exosome-like nanoparticles induce intestinal stem cells and protect mice from DSS-induced colitis. Mol. Ther. 21 (7), 1345–1357. https://doi.org/10.1038/mt.2013.64.
Kang, C.S., Ban, M., Choi, E.J., Moon, H.G., Jeon, J.S., Kim, D.K., Park, S.K., Jeon, S.G., Roh, T.Y., Myung, S.J., 2013. Extracellular vesicles derived from gut microbiota, especially *Akkermansia muciniphila*, protect the progression of dextran sulfate sodium-induced colitis. PLoS One 8 (10), e76520. https://doi.org/10.1371/journal.pone.0076520.
Kanojia, G., Raeven, R.H., van der Maas, L., Bindels, T.H., van Riet, E., Metz, B., Soema, P.C., Ten Have, R., Frijlink, H.W., Amorij, J.P., 2018. Development of a thermostable spray dried outer membrane vesicle pertussis vaccine for pulmonary immunization. J. Control. Release 286, 167–178. https://doi.org/10.1016/j.jconrel.2018.07.035.
Kawai, T., Ikegawa, M., Ori, D., Akira, S., 2024. Decoding toll-like receptors: recent insights and perspectives in innate immunity. Immunity 57 (4), 649–673. https://doi.org/10.1016/j.immuni.2024.03.004.
Keshavarz Azizi Raftar, S., Ashrafian, F., Yadegar, A., Lari, A., Moradi, H.R., Shahriary, A., Azimirad, M., Alavifard, H., Mohsenifar, Z., Davari, M., 2021. The protective effects of live and pasteurized *Akkermansia muciniphila* and its extracellular vesicles against HFD/CCl4-induced liver injury. Microbiol. Spectr. 9 (2). https://doi.org/10.1128/Spectrum.00484-21 e00484-00421.
Kim, H.S., Kim, J.Y., Ryu, H.S., Park, H.G., Kim, Y.O., Kang, J.S., Kim, H.M., Hong, J.T., Kim, Y., Han, S.B., 2010. Induction of dendritic cell maturation by β-glucan isolated from sparassis crispa. Int. Immunopharmacol. 10 (10), 1284–1294. https://doi.org/10.1016/j.intimp.2010.07.012.
Kim, H.Y., Kwon, H.Y., Ha Thi, H.T., Lee, H.J., Kim, G.I., Hahm, K.B., Hong, S., 2016. MicroRNA-132 and microRNA-223 control positive feedback circuit by regulating FOXO3a in inflammatory bowel disease. J. Gastroenterol. Hepatol. 31 (10), 1727–1735. https://doi.org/10.1111/jgh.13321.

Kim, M.H., van Noort, D., Sung, J.H., Park, S., 2021. Organ-on-a-chip for studying gut-brain interaction mediated by extracellular vesicles in the gut microenvironment. Int. J. Mol. Sci. 22 (24), 13513. https://doi.org/10.3390/ijms222413513.

Kowal, J., Arras, G., Colombo, M., Jouve, M., Morath, J.P., Primdal-Bengtson, B., Dingli, F., Loew, D., Tkach, M., Théry, C., 2016. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc. Natl. Acad. Sci. U. S. A. 113 (8), E968–E977. https://doi.org/10.1073/pnas.1521230113.

Kozieł, M.J., Ziaja, M., Piastowska Ciesielska, A.W., 2021. Intestinal barrier, claudins and mycotoxins. Toxins 13 (11), 758. https://doi.org/10.3390/toxins13110758.

Kuipers, M.E., Hokke, C.H., Smits, H.H., Nolte-'t Hoen, E.N., 2018. Pathogen-derived extracellular vesicle-associated molecules that affect the host immune system: an overview. Front. Microbiol. 9, 2182. https://doi.org/10.3389/fmicb.2018.02182.

Kumar, A., Sundaram, K., Teng, Y., Mu, J., Sriwastva, M.K., Zhang, L., Hood, J.L., Yan, J., Zhang, X., Park, J.W., 2022. Ginger nanoparticles mediated induction of Foxa2 prevents high-fat diet-induced insulin resistance. Theranostics 12 (3), 1388. https://doi.org/10.7150/thno.62514.

Kurata, A., Kiyohara, S., Imai, T., Yamasaki Yashiki, S., Zaima, N., Moriyama, T., Kishimoto, N., Uegaki, K., 2022. Characterization of extracellular vesicles from *Lactiplantibacillus plantarum*. Sci. Rep. 12 (1), 13330. https://doi.org/10.1038/s41598-022-17629-7.

Lei, C., Mu, J., Teng, Y., He, L., Xu, F., Zhang, X., Sundaram, K., Kumar, A., Sriwastva, M. K., Lawrenz, M.B., 2020. Lemon exosome-like nanoparticles-manipulated probiotics protect mice from *C. diff* infection. iScience 23 (10). https://doi.org/10.1016/j.isci.2020.101571.

Lei, C., Teng, Y., He, L., Sayed, M., Mu, J., Xu, F., Zhang, X., Kumar, A., Sundaram, K., Sriwastva, M.K., 2021. Lemon exosome-like nanoparticles enhance stress survival of gut bacteria by RNase P-mediated specific tRNA decay. iScience 24 (6), 102511. https://doi.org/10.1016/j.isci.2021.102511.

Leoni, G., Alam, A., Neumann, P.A., Lambeth, J.D., Cheng, G., McCoy, J., Hilgarth, R.S., Kundu, K., Murthy, N., Kusters, D., 2012. Annexin A1, formyl peptide receptor, and NOX1 orchestrate epithelial repair. Eur. J. Clin. Investig. 123 (1), 443–454. https://doi.org/10.1172/JCI65831.

Leoni, G., Neumann, P.A., Kamaly, N., Quiros, M., Nishio, H., Jones, H.R., Sumagin, R., Hilgarth, R.S., Alam, A., Fredman, G., 2015. Annexin A1–containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair. Eur. J. Clin. Investig. 125 (3), 1215–1227. https://doi.org/10.1172/JCI76693.

Li, A., Yang, Y., Qin, S., Lv, S., Jin, T., Li, K., Han, Z., Li, Y., 2021. Microbiome analysis reveals gut microbiota alteration of early-weaned Yimeng black goats with the effect of milk replacer and age. Microb. Cell Factories 20, 1–14. https://doi.org/10.1186/s12934-021-01568-5.

Li, S., Li, W., Wu, X., Zhang, B., Liu, L., Yin, L., 2024a. Immune cell-derived extracellular vesicles for precision therapy of inflammatory-related diseases. J. Control. Release 368, 533–547. https://doi.org/10.1016/j.jconrel.2024.03.007.

Li, Y., Yu, Z., Zhang, D., Zhang, F., Zhang, D., Chen, M., 2024b. Extracellular vesicles for the treatment of ulcerative colitis: a systematic review and meta-analysis of animal studies. Heliyon 10 (17), e36890. https://doi.org/10.1016/j.heliyon.2024.e36890.

Liang, L., Yang, C., Liu, L., Mai, G., Li, H., Wu, L., Jin, M., Chen, Y., 2022. Commensal bacteria-derived extracellular vesicles suppress ulcerative colitis through regulating the macrophages polarization and remodeling the gut microbiota. Microb. Cell Factories 21 (1), 88. https://doi.org/10.1186/s12934-022-01812-6.

Liangsupree, T., Multia, E., Riekkola, M.L., 2021. Modern isolation and separation techniques for extracellular vesicles. J. Chromatogr. A 1636, 461773. https://doi.org/10.1016/j.chroma.2020.461773.

Liu, S., Da Cunha, A.P., Rezende, R.M., Cialic, R., Wei, Z., Bry, L., Comstock, L.E., Gandhi, R., Weiner, H.L., 2016. The host shapes the gut microbiota via fecal microRNA. Cell Host Microbe 19 (1), 32–43. https://doi.org/10.1016/j.chom.2015.12.005.

Liu, Y., Tan, M., Zhu, W., Cao, Y., Peng, L., Yan, Z., Zhao, G., 2022. *In vitro* effects of Tartary buckwheat-derived nanovesicles on gut microbiota. J. Agric. Food Chem. 70 (8), 2616–2629. https://doi.org/10.1021/acs.jafc.1c07658.

Lopes, S.A., Roque Borda, C.A., Duarte, J.L., Di Filippo, L.D., Borges Cardoso, V.M., Pavan, F.R., Chorilli, M., Meneguin, A.B., 2023. Delivery strategies of probiotics from nano-and microparticles: trends in the treatment of inflammatory bowel disease—an overview. Pharmaceutics 15 (11), 2600. https://doi.org/10.3390/pharmaceutics15112600.

Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K., Knight, R., 2012. Diversity, stability and resilience of the human gut microbiota. Nature 489 (7415), 220–230. https://doi.org/10.1038/nature11550.

Lu, S., Xu, J., Zhao, Z., Guo, Y., Zhang, H., Jurutka, P.W., Huang, D., Cao, C., Cheng, S., 2023. Dietary Lactobacillus rhamnosus GG extracellular vesicles enhance antiprogrammed cell death 1 (anti-PD-1) immunotherapy efficacy against colorectal cancer. Food Funct. 14 (23), 10314–10328. https://doi.org/10.1039/d3fo02018e.

Lu, Z., Wu, J., Jiang, W., Xiao, J., Tao, K., Ma, L., Zheng, P., Wan, R., Wang, X., 2017. MicroRNA-155 promotes the pathogenesis of experimental colitis by repressing SHIP-1 expression. World J. Gastroenterol. 23 (6), 976. https://doi.org/10.3748/wjg.v23.i6.976.

Ludwig, A.K., Giebel, B., 2012. Exosomes: small vesicles participating in intercellular communication. Int. J. Biochem. Cell Biol. 44 (1), 11–15. https://doi.org/10.1016/j.biocel.2011.10.005.

Ma, L., Lyu, W., Song, Y., Chen, K., Lv, L., Yang, H., Wang, W., Xiao, Y., 2023. Anti-inflammatory effect of *Clostridium butyricum*-derived extracellular vesicles in ulcerative colitis: impact on host microRNAs expressions and gut microbiome profiles. Mol. Nutr. Food Res. 67 (13), 2200884. https://doi.org/10.1002/mnfr.202200884.

Ma, T.Y., Iwamoto, G.K., Hoa, N.T., Akotia, V., Pedram, A., Boivin, M.A., Said, H.M., 2004. TNF-α-induced increase in intestinal epithelial tight junction permeability requires NF-κB activation. Am. J. Physiol. Gastrointest. Liver 286 (3), G367–G376. https://doi.org/10.1152/ajpgi.00173.2003.

Macia, L., Nanan, R., Hosseini Beheshti, E., Grau, G.E., 2019. Host-and microbiota-derived extracellular vesicles, immune function, and disease development. Int. J. Mol. Sci. 21 (1), 107. https://doi.org/10.3390/ijms21010107.

Malan Muller, S, Valles Colomer, M., Raes, J., Lowry, C.A., Seedat, S., Hemmings, S.M, 2018. The gut microbiome and mental health: implications for anxiety-and trauma-related disorders. Omics 22 (2), 90–107. https://doi.org/10.1089/omi.2017.0077.

Mallegol, J., Van Niel, G., Lebreton, C., Lepelletier, Y., Candalh, C., Dugave, C., Heath, J. K., Raposo, G., Cerf–Bensussan, N., Heyman, M, 2007. T84-intestinal epithelial exosomes bear MHC class II/peptide complexes potentiating antigen presentation by dendritic cells. Gastroenterology 132 (5), 1866–1876. https://doi.org/10.1053/j.gastro.2007.02.043.

Martin, A.M., Sun, E.W., Keating, D.J., 2020. Mechanisms controlling hormone secretion in human gut and its relevance to metabolism. J. Endocrinol. 244 (1), R1–R15. https://doi.org/10.1530/JOE-19-0399.

Mathieu, M., Martin Jaular, L., Lavieu, G., Théry, C., 2019. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat. Cell Biol. 21 (1), 9–17. https://doi.org/10.1038/s41556-018-0250-9.

Mayer, E.A., Nance, K., Chen, S., 2022. The gut–brain axis. Annu. Rev. Med. 73 (1), 439–453. https://doi.org/10.1146/annurev-med-042320-014032.

Mazzon, E., Cuzzocrea, S., 2008. Role of TNF-α in ileum tight junction alteration in mouse model of restraint stress. Am. J. Physiol. Gastrointest. Liver 294 (5), G1268–G1280. https://doi.org/10.1152/ajpgi.00014.2008.

Meng, W., He, C., Hao, Y., Wang, L., Li, L., Zhu, G., 2020. Prospects and challenges of extracellular vesicle-based drug delivery system: considering cell source. Drug Deliv. 27 (1), 585–598. https://doi.org/10.1080/10717544.2020.1748758.

Mitsuhashi, S., Feldbrügge, L., Csizmadia, E., Mitsuhashi, M., Robson, S.C., Moss, A.C., 2016. Luminal extracellular vesicles (EVs) in inflammatory bowel disease (IBD) exhibit proinflammatory effects on epithelial cells and macrophages. Inflamm. Bowel Dis. 22 (7), 1587–1595. https://doi.org/10.1097/MIB.0000000000000840.

Miyoshi, H., 2017. Wnt-expressing cells in the intestines: guides for tissue remodeling. J. Biochem. 161 (1), 19–25. https://doi.org/10.1093/jb/mvw070.

Molina Tijeras, J.A, Gálvez, J., Rodríguez Cabezas, M.E., 2019. The immunomodulatory properties of extracellular vesicles derived from probiotics: a novel approach for the management of gastrointestinal diseases. Nutrients 11 (5), 1038. https://doi.org/10.3390/nu11051038.

Moloney, G.M., Viola, M.F., Hoban, A.E., Dinan, T.G., Cryan, J.F., 2018. Faecal microRNAs: indicators of imbalance at the host-microbe interface? Benef. Microbes 9 (2), 175–183. https://doi.org/10.3920/BM2017.0013.

Moosavi, S.M., Akhavan Sepahi, A., Mousavi, S.F., Vaziri, F., Siadat, S.D., 2020. The effect of *Faecalibacterium prausnitzii* and its extracellular vesicles on the permeability of intestinal epithelial cells and expression of PPARs and ANGPTL4 in the Caco-2 cell culture model. J. Diabetes Metab. Disord. 19 (2), 1061–1069. https://doi.org/10.1007/s40200-020-00605-1.

Mu, J., Zhuang, X., Wang, Q., Jiang, H., Deng, Z.B., Wang, B., Zhang, L., Kakar, S., Jun, Y., Miller, D., 2014. Interspecies communication between plant and mouse gut host cells through edible plant derived exosome-like nanoparticles. Mol. Nutr. Food Res. 58 (7), 1561–1573. https://doi.org/10.1002/mnfr.201300729.

Natividad, J.M., Verdu, E.F., 2013. Modulation of intestinal barrier by intestinal microbiota: pathological and therapeutic implications. Pharmacol. Res. 69 (1), 42–51. https://doi.org/10.1016/j.phrs.2012.10.007.

Neudecker, V., Haneklaus, M., Jensen, O., Khailova, L., Masterson, J.C., Tye, H., Biette, K., Jedlicka, P., Brodsky, K.S., Gerich, M.E., 2017. Myeloid-derived miR-223 regulates intestinal inflammation via repression of the NLRP3 inflammasome. J. Exp. Med. 214 (6), 1737–1752. https://doi.org/10.1084/jem.20160462.

Nieuwland, R., Falcón Pérez, J.M., Théry, C., Witwer, K.W., 2020. Rigor and standardization of extracellular vesicle research: paving the road towards robustness. J. Extracell. Vesicles 10 (2), e12037. https://doi.org/10.1002/jev2.12037.

Nieuwland, R., Siljander, P.R.M, Falcón Pérez, J.M, Witwer, K.W, 2022. Reproducibility of extracellular vesicle research. Eur. J. Cell Biol. 101 (3), 151226. https://doi.org/10.1016/j.ejcb.2022.151226.

Nimrichter, L., De Souza, M.M., Del Poeta, M., Nosanchuk, J.D., Joffe, L., Tavares, P.d. M., Rodrigues, M.L., 2016. Extracellular vesicle-associated transitory cell wall components and their impact on the interaction of fungi with host cells. Front. Microbiol. 7, 1034. https://doi.org/10.3389/fmicb.2016.01034.

Nolte-'t Hoen, E.N., Buschow, S.I., Anderton, S.M., Stoorvogel, W., Wauben, M.H., 2009. Activated T cells recruit exosomes secreted by dendritic cells via LFA-1. Blood 113 (9), 1977–1981. https://doi.org/10.1182/blood-2008-08-174094.

Northrop Albrecht, E.J., Taylor, W.R., Huang, B.Q., Kisiel, J.B., Lucien, F, 2022. Assessment of extracellular vesicle isolation methods from human stool supernatant. J. Extracell. Vesicles 11 (4), e12208. https://doi.org/10.1002/jev2.12208.

Ocansey, D.K., Zhang, L., Wang, Y., Yan, Y., Qian, H., Zhang, X., Xu, W., Mao, F., 2020. Exosome-mediated effects and applications in inflammatory bowel disease. Biol. Rev. 95 (5), 1287–1307. https://doi.org/10.1111/brv.12608.

Ocansey, D.K.W., Zhang, Z., Xu, X., Liu, L., Amoah, S., Chen, X., Wang, B., Zhang, X., Mao, F., 2022. Mesenchymal stem cell-derived exosome mitigates colitis via the modulation of the gut metagenomics–metabolomics–farnesoid X receptor axis. Biomater. Sci. 10 (17), 4822–4836. https://doi.org/10.1039/d2bm00559j.

Olivo Martínez, Y., Bosch, M., Badia, J., Baldomà, L., 2023. Modulation of the intestinal barrier integrity and repair by microbiota extracellular vesicles through the differential regulation of Trefoil Factor 3 in LS174T goblet cells. Nutrients 15 (11), 2437. https://doi.org/10.3390/nu15112437.

Olivo Martínez, Y., Martínez Ruiz, S., Cordero, C., Badia, J., Baldoma, L., 2024. Extracellular vesicles of the probiotic *Escherichia coli* Nissle 1917 reduce PepT1 levels in IL-1β-treated Caco-2 cells via upregulation of miR-193a-3p. Nutrients 16 (16), 2719. https://doi.org/10.3390/nu16162719.

Pabst, R., 1987. The anatomical basis for the immune function of the gut. Ital. J. Anat. Embryol. 176 (2), 135–144. https://doi.org/10.1007/BF00310046. Italian.

Pan, J., Sheng, S., Ye, L., Xu, X., Ma, Y., Feng, X., Qiu, L., Fan, Z., Wang, Y., Xia, X., 2022. Extracellular vesicles derived from glioblastoma promote proliferation and migration of neural progenitor cells via PI3K-Akt pathway. Cell Commun. Signal. 20 (1), 7. https://doi.org/10.1186/s12964-021-00760-9.

Panigrahi, S., Ghosh, S.K., Ferrari, B., Wyrick, J.M., Podrez, E.A., Weinberg, A., Sieg, S.F., 2022. Human β-defensin-3 is associated with platelet-derived extracellular vesicles and is a potential contributor to endothelial dysfunction. Front. Mol. Biosci. 9, 824954. https://doi.org/10.3389/fmolb.2022.824954.

Park, J., Kim, N.E., Yoon, H., Shin, C.M., Kim, N., Lee, D.H., Park, J.Y., Choi, C.H., Kim, J. G., Kim, Y.K., Shin, T.S., Yang, J., Park, Y.S., 2021. Fecal microbiota and gut microbe-derived extracellular vesicles in colorectal cancer. Front. Oncol. 11, 650026. https://doi.org/10.3389/fonc.2021.650026.

Park, Y.E., Moon, H.S., Yong, D., Seo, H., Yang, J., Shin, T.S., Kim, Y.K., Kim, J.R., Lee, Y. N., Kim, Y.H., 2022. Microbial changes in stool, saliva, serum, and urine before and after anti-TNF-α therapy in patients with inflammatory bowel diseases. Sci. Rep. 12 (1), 6359. https://doi.org/10.1038/s41598-022-10450-2.

Patankar, J.V., Becker, C., 2020. Cell death in the gut epithelium and implications for chronic inflammation. Nat. Rev. Gastroenterol. Hepatol. 17 (9), 543–556. https://doi.org/10.1038/s41575-020-0326-4.

Pérez-Escamilla, R., Tomori, C., Hernández-Cordero, S., Baker, P., Barros, A.J., Bégin, F., Chapman, D.J., Grummer-Strawn, L.M., McCoy, D., Menon, P., 2023. Breastfeeding: crucially important, but increasingly challenged in a market-driven world. Lancet 401 (10375), 472–485. https://doi.org/10.1016/S0140-6736(22)01932-8.

Pitt, J.M., Kroemer, G., Zitvogel, L., 2016. Extracellular vesicles: masters of intercellular communication and potential clinical interventions. Eur. J. Clin. Investig. 126 (4), 1139–1143. https://doi.org/10.1172/JCI87316.

Quesenberry, P.J., Aliotta, J., Deregibus, M.C., Camussi, G., 2015. Role of extracellular RNA-carrying vesicles in cell differentiation and reprogramming. Stem Cell Res. Ther. 6, 1–10. https://doi.org/10.1186/s13287-015-0150-x.

Raimondo, S., Naselli, F., Fontana, S., Monteleone, F., Dico, A.L., Saieva, L., Zito, G., Flugy, A., Manno, M., Di Bella, M.A., 2015. Citrus limon-derived nanovesicles inhibit cancer cell proliferation and suppress CML xenograft growth by inducing TRAIL-mediated cell death. Oncotarget 6 (23), 19514. https://doi.org/10.18632/oncotarget.4004.

Raise, A., Dupont, S., Iaconelli, C., Caliri, C., Charriau, A., Gervais, P., Chambin, O., Beney, L., 2020. Comparison of two encapsulation processes to protect the commensal gut probiotic bacterium *Faecalibacterium prausnitzii* from the digestive tract. J. Drug Deliv. Sci. Technol. 56, 101608. https://doi.org/10.1016/j.jddst.2020.101608.

Raj, P.A., Dentino, A.R., 2002. Current status of defensins and their role in innate and adaptive immunity. FEMS Microbiol. Lett. 206 (1), 9–18. https://doi.org/10.1111/j.1574-6968.2002.tb10979.x.

Rasoamanana, R., Darcel, N., Fromentin, G., Tomé, D., 2012. Nutrient sensing and signalling by the gut. Proc. Nutr. Soc. 71 (4), 446–455. https://doi.org/10.1017/S0029665112000110.

Ratajczak, M.Z., Ratajczak, J., 2017. Extracellular microvesicles as game changers in better understanding the complexity of cellular interactions—From bench to clinical applications. Am. J. Med. Sci. 354 (5), 449–452. https://doi.org/10.1016/j.amjms.2017.06.001.

Risso, A., 2000. Leukocyte antimicrobial peptides: multifunctional effector molecules of innate immunity. J. Leukoc. Biol. 68 (6), 785–792. https://doi.org/10.1189/jlb.68.6.785.

Robertson, C., Booth, S.A., Beniac, D.R., Coulthart, M.B., Booth, T.F., McNicol, A., 2006. Cellular prion protein is released on exosomes from activated platelets. Blood 107 (10), 3907–3911. https://doi.org/10.1182/blood-2005-02-0802.

Rome, S., 2019. Biological properties of plant-derived extracellular vesicles. Food Funct. 10 (2), 529–538. https://doi.org/10.1039/c8fo02295j.

Rossaint, J., Kühne, K., Skupski, J., Van Aken, H., Looney, M.R., Hidalgo, A., Zarbock, A., 2016. Directed transport of neutrophil-derived extracellular vesicles enables platelet-mediated innate immune response. Nat. Commun. 7 (1), 13464. https://doi.org/10.1038/ncomms13464.

Santos, J., Dalla, P.V., Milthorpe, B.K., 2021. A molecular analysis of cytokine content across extracellular vesicles, secretions, and intracellular space from different site-specific adipose-derived stem cells. Int. J. Mol. Sci. 23 (1), 397. https://doi.org/10.3390/ijms23010397.

Selsted, M., Szklarek, D., Lehrer, R., 1984. Purification and antibacterial activity of antimicrobial peptides of rabbit granulocytes. Infect. Immun. 45 (1), 150–154. https://doi.org/10.1128/iai.45.1.150-154.1984.

Shao, M., Jin, X., Chen, S., Yang, N., Feng, G., 2023. Plant-derived extracellular vesicles-a novel clinical anti-inflammatory drug carrier worthy of investigation. Biomed. Pharmacother. 169, 115904. https://doi.org/10.1016/j.biopha.2023.115904.

Shen, Q., Huang, Z., Yao, J., Jin, Y., 2022. Extracellular vesicles-mediated interaction within intestinal microenvironment in inflammatory bowel disease. J. Adv. Res. 37, 221–233. https://doi.org/10.1016/j.jare.2021.07.002.

Siddiqui, M.T., Cresci, G.A., 2021. The immunomodulatory functions of butyrate. J. Inflamm. Res. 14, 6025–6041. https://doi.org/10.2147/JIR.S300989.

Silva, A.M., Teixeira, J.H., Almeida, M.I., Goncalves, R.M., Barbosa, M.A., Santos, S.G., 2017. Extracellular vesicles: immunomodulatory messengers in the context of tissue repair/regeneration. Eur. J. Pharm. Sci. 98, 86–95. https://doi.org/10.1016/j.ejps.2016.09.017.

Simonsen, J.B., 2017. What are we looking at? Extracellular vesicles, lipoproteins, or both? Circ. Res. 121 (8), 920–922. https://doi.org/10.1161/CIRCRESAHA.117.311767.

Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly y, M., Glickman, J.N., Garrett, W.S., 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 341 (6145), 569–573. https://doi.org/10.1126/science.1241165.

Solaymani-Mohammadi, S., Singer, S.M., 2013. Regulation of intestinal epithelial cell cytoskeletal remodeling by cellular immunity following gut infection. Mucosal Immunol. 6 (2), 369–378. https://doi.org/10.1038/mi.2012.80.

Sriwastva, M.K., Deng, Z.B., Wang, B., Teng, Y., Kumar, A., Sundaram, K., Mu, J., Lei, C., Dryden, G.W., Xu, F., 2022. Exosome-like nanoparticles from mulberry bark prevent DSS-induced colitis via the AhR/COPS8 pathway. EMBO Rep. 23 (3), e53365. https://doi.org/10.15252/embr.202153365.

Syromiatnikova, V., Prokopeva, A., Gomzikova, M., 2022. Methods of the large-scale production of extracellular vesicles. Int. Immunopharmacol. 23 (18), 10522. https://doi.org/10.3390/ijms231810522.

Takiishi, T., Fenero, C.I.M., Câmara, N.O.S., 2017. Intestinal barrier and gut microbiota: shaping our immune responses throughout life. Tissue Barriers 5 (4), e1373208. https://doi.org/10.1080/21688370.2017.1373208.

Tan, J., Ni, D., Taitz, J., Pinget, G.V., Read, M., Senior, A., Wali, J.A., Elnour, R., Shanahan, E., Wu, H., 2022. Dietary protein increases T-cell-independent sIgA production through changes in gut microbiota-derived extracellular vesicles. Nat. Commun. 13 (1), 4336. https://doi.org/10.1038/s41467-022-31761-y.

Teng, Y., Ren, Y., Sayed, M., Hu, X., Lei, C., Kumar, A., Hutchins, E., Mu, J., Deng, Z., Luo, C., 2018. Plant-derived exosomal microRNAs shape the gut microbiota. Cell Host Microbe 24 (5), 637–652. https://doi.org/10.1016/j.chom.2018.10.001 e638.

Tong, L., Hao, H., Zhang, X., Zhang, Z., Lv, Y., Zhang, L., Yi, H., 2020. Oral administration of bovine milk-derived extracellular vesicles alters the gut microbiota and enhances intestinal immunity in mice. Mol. Nutr. Food Res. 64 (8), 1901251. https://doi.org/10.1002/mnfr.201901251.

Tong, L., Hao, H., Zhang, Z., Lv, Y., Liang, X., Liu, Q., Liu, T., Gong, P., Zhang, L., Cao, F., 2021. Milk-derived extracellular vesicles alleviate ulcerative colitis by regulating the gut immunity and reshaping the gut microbiota. Theranostics 11 (17), 8570. https://doi.org/10.7150/thno.62046.

Tong, L., Zhang, S., Liu, Q., Huang, C., Hao, H., Tan, M.S., Yu, X., Lou, C.K.L., Huang, R., Zhang, Z., 2023. Milk-derived extracellular vesicles protect intestinal barrier integrity in the gut-liver axis. Sci. Adv. 9 (15), eade5041. https://doi.org/10.1126/sciadv.ade5041.

Tremaroli, V., Bäckhed, F., 2012. Functional interactions between the gut microbiota and host metabolism. Nature 489 (7415), 242–249. https://doi.org/10.1038/nature11552.

Tulkens, J., De Wever, O., Hendrix, A., 2020a. Analyzing bacterial extracellular vesicles in human body fluids by orthogonal biophysical separation and biochemical characterization. Nat. Protoc. 15 (1), 40–67. https://doi.org/10.1038/s41596-019-0236-5.

Tulkens, J., Vergauwen, G., Van Deun, J., Geeurickx, E., Dhondt, B., Lippens, L., De Scheerder, M.A., Miinalainen, I., Rappu, P., De Geest, B.G., 2020b. Increased levels of systemic LPS-positive bacterial extracellular vesicles in patients with intestinal barrier dysfunction. Gut 69 (1), 191–193. https://doi.org/10.1136/gutjnl-2018-317726.

Ungewiß, H., Vielmuth, F., Suzuki, S.T., Maiser, A., Harz, H., Leonhardt, H., Kugelmann, D., Schlegel, N., Waschke, J., 2017. Desmoglein 2 regulates the intestinal epithelial barrier via p38 mitogen-activated protein kinase. Sci. Rep. 7 (1), 6329. https://doi.org/10.1038/s41598-017-06713-y.

Valter, M., Verstockt, S., Finalet Ferreiro, J., Cleynen, I., 2021. Extracellular vesicles in inflammatory bowel disease: small particles, big players. J. Crohns Colitis 15 (3), 499–510. https://doi.org/10.1093/ecco-jcc/jjaa179.

Van Deun, J., Mestdagh, P., Agostinis, P., Akay, Ö., Anand, S., Anckaert, J., Martinez, Z. A., Baetens, T., Beghein, E., Bertier, L., 2017. EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. Nat. Methods 14 (3), 228–232. https://doi.org/10.1038/nmeth.4185.

Van Niel, G., d'Angelo, G., Raposo, G., 2018. Shedding light on the cell biology of extracellular vesicles. Nat. Rev. Mol. Cell Biol. 19 (4), 213–228. https://doi.org/10.1038/nrm.2017.125.

Van Niel, G., Mallegol, J., Bevilacqua, C., Candalh, C., Brugiere, S., Tomaskovic-Crook, E., Heath, J.K., Cerf-Bensussan, N., Heyman, M., 2003. Intestinal epithelial exosomes carry MHC class II/peptides able to inform the immune system in mice. Gut 52 (12), 1690–1697. https://doi.org/10.1136/gut.52.12.1690.

Van Niel, G., Raposo, G., Candalh, C., Boussac, M., Hershberg, R., Cerf–Bensussan, N., Heyman, M., 2001. Intestinal epithelial cells secrete exosome–like vesicles. Gastroenterology 121 (2), 337–349. https://doi.org/10.1053/gast.2001.26263.

Vancamelbeke, M., Vermeire, S., 2017. The intestinal barrier: a fundamental role in health and disease. Expert Rev. Gastroenterol. 11 (9), 821–834. https://doi.org/10.1080/17474124.2017.1343143.

Wang, B., Zhuang, X., Deng, Z.B., Jiang, H., Mu, J., Wang, Q., Xiang, X., Guo, H., Zhang, L., Dryden, G., 2014. Targeted drug delivery to intestinal macrophages by bioactive nanovesicles released from grapefruit. Mol. Ther. 22 (3), 522–534. https://doi.org/10.1038/mt.2013.190.

Wang, L., Gao, R., Li, B., Alganabi, M., He, W., Shen, C., Zhu, H., Pierro, A., 2022a. Human breast milk-derived exosomes protect against intestinal ischemia and reperfusion injury in neonatal rats. J. Pediatr. Surg. 57 (7), 1264–1268. https://doi.org/10.1016/j.jpedsurg.2022.02.029.

Wang, X., Lin, S., Wang, L., Cao, Z., Zhang, M., Zhang, Y., Liu, R., Liu, J., 2023. Versatility of bacterial outer membrane vesicles in regulating intestinal homeostasis. Sci. Adv. 9 (11), eade5079. https://doi.org/10.1126/sciadv.ade5079.

Wang, Y., Wang, J., Ma, J., Zhou, Y., Lu, R., 2022b. Focusing on future applications and current challenges of plant derived extracellular vesicles. Pharmaceuticals 15 (6), 708. https://doi.org/10.3390/ph15060708.

Wehkamp, J., Fellermann, K., Herrlinger, K.R., Bevins, C.L., Stange, E.F., 2005. Mechanisms of disease: defensins in gastrointestinal diseases. Nat. Clin. Pract. Gastroenterol. 2 (9), 406–415. https://doi.org/10.1038/ncpgasthep0265.

West, C.L., Stanisz, A.M., Mao, Y.K., Champagne-Jorgensen, K., Bienenstock, J., Kunze, W.A., 2020. Microvesicles from *Lactobacillus reuteri* (DSM-17938) completely reproduce modulation of gut motility by bacteria in mice. PLoS One 15 (1), e0225481. https://doi.org/10.1371/journal.pone.0225481.

Wiklander, O.P., Brennan, M.Á., Lötvall, J., Breakefield, X.O., El Andaloussi, S, 2019. Advances in therapeutic applications of extracellular vesicles. Sci. Transl. Med. 11 (492), eaav8521. https://doi.org/10.1126/scitranslmed.aav8521.

Williams, T., Salmanian, G., Burns, M., Maldonado, V., Smith, E., Porter, R.M., Song, Y. H., Samsonraj, R.M., 2023. Versatility of mesenchymal stem cell-derived extracellular vesicles in tissue repair and regenerative applications. Biochimie 207, 33–48. https://doi.org/10.1016/j.biochi.2022.11.011.

Wu, H., Fan, H., Shou, Z., Xu, M., Chen, Q., Ai, C., Dong, Y., Liu, Y., Nan, Z., Wang, Y., 2019. Extracellular vesicles containing miR-146a attenuate experimental colitis by targeting TRAF6 and IRAK1. Int. Immunopharmacol. 68, 204–212. https://doi.org/10.1016/j.intimp.2018.12.043.

Wu, K., Xing, F., Wu, S., Watabe, K., 2017. Extracellular vesicles as emerging targets in cancer: recent development from bench to bedside. Biochim. Biophys. Acta Rev. Cancer 1868 (2), 538–563. https://doi.org/10.1016/j.bbcan.2017.10.001.

Wu, R., Fan, X., Wang, Y., Shen, M., Zheng, Y., Zhao, S., Yang, L., 2022. Mesenchymal stem cell-derived extracellular vesicles in liver immunity and therapy. Front. Immunol. 13, 833878. https://doi.org/10.3389/fimmu.2022.833878.

Xi, S., Wang, Y., Wu, C., Peng, W., Zhu, Y., Hu, W., 2022. Intestinal epithelial cell exosome launches IL-1β-mediated neuron injury in sepsis-associated encephalopathy. Front. Cell. Infect. Microbiol. 11, 783049. https://doi.org/10.3389/fcimb.2021.783049.

Xie, J., Haesebrouck, F., Van Hoecke, L., Vandenbroucke, R.E., 2023. Bacterial extracellular vesicles: an emerging avenue to tackle diseases. Trends Microbiol. 31 (12), 1206–1224. https://doi.org/10.1016/j.tim.2023.05.010.

Xie, J., Li, Q., Haesebrouck, F., Van Hoecke, L., Vandenbroucke, R.E., 2022. The tremendous biomedical potential of bacterial extracellular vesicles. Trends Biotechnol. 40 (10), 1173–1194. https://doi.org/10.1016/j.tibtech.2022.03.005.

Xie, M., Hou, L., Sun, J., Zeng, B., Xi, Q., Luo, J., Chen, T., Zhang, Y., 2019. Porcine milk exosome miRNAs attenuate LPS-induced apoptosis through inhibiting TLR4/NF-κB and p53 pathways in intestinal epithelial cells. J. Agric. Food Chem. 67 (34), 9477–9491. https://doi.org/10.1021/acs.jafc.9b02925.

Yang, D., Chertov, O., Oppenheim, J., 2001. The role of mammalian antimicrobial peptides and proteins in awakening of innate host defenses and adaptive immunity. Cell. Mol. Life Sci. 58 (7), 978–989. https://doi.org/10.1007/PL00000914.

Yang, J., Zheng, X.G., Wu, Y.L., Wang, A.P., Wang, C.H., Chen, W.X., Zhong, S., Yang, H., 2022a. Intestinal epithelial cell-derived exosomes package microRNA-23a-3p alleviate gut damage after ischemia/reperfusion via targeting MAP4K4. Biomed. Pharmacother. 149, 112810. https://doi.org/10.1016/j.biopha.2022.112810.

Yang, L., Wang, T., Zhang, X., Zhang, H., Yan, N., Zhang, G., Yan, R., Li, Y., Yu, J., He, J., 2022b. Exosomes derived from human placental mesenchymal stem cells ameliorate myocardial infarction via anti-inflammation and restoring gut dysbiosis. BMC Cardiovasc. Disord. 22 (1), 61. https://doi.org/10.1186/s12872-022-02508-w.

Yu, S., Zhao, Z., Xu, X., Li, M., Li, P., 2019. Characterization of three different types of extracellular vesicles and their impact on bacterial growth. Food Chem. 272, 372–378. https://doi.org/10.1016/j.foodchem.2018.08.059.

Zeng, B., Chen, T., Luo, J., Zhang, L., Xi, Q., Jiang, Q., Sun, J., Zhang, Y., 2021. Biological characteristics and roles of noncoding RNAs in milk-derived extracellular vesicles. Adv. Nutr. 12 (3), 1006–1019. https://doi.org/10.1093/advances/nmaa124.

Zhang, B., Tian, Y., Jiang, P., Jiang, Y., Li, C., Liu, T., Zhou, R., Yang, N., Zhou, X., Liu, Z., 2017. MicroRNA-122a regulates zonulin by targeting EGFR in intestinal epithelial dysfunction. Cell. Physiol. Biochem. 42 (2), 848–858. https://doi.org/10.1159/000478629.

Zhang, B., Zhao, J., Jiang, M., Peng, D., Dou, X., Song, Y., Shi, J., 2022. The potential role of gut microbial-derived exosomes in metabolic-associated fatty liver disease: implications for treatment. Front. Immunol. 13, 893617. https://doi.org/10.3389/fimmu.2022.893617.

Zhang, L., Shen, J., Cheng, J., Fan, X., 2015. MicroRNA-21 regulates intestinal epithelial tight junction permeability. Cell Biochem. Funct. 33 (4), 235–240. https://doi.org/10.1002/cbf.3109.

Zhang, M., Viennois, E., Prasad, M., Zhang, Y., Wang, L., Zhang, Z., Han, M.K., Xiao, B., Xu, C., Srinivasan, S., 2016a. Edible ginger-derived nanoparticles: a novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. Biomaterials 101, 321–340. https://doi.org/10.1016/j.biomaterials.2016.06.018.

Zhang, M., Xiao, B., Wang, H., Han, M.K., Zhang, Z., Viennois, E., Xu, C., Merlin, D., 2016b. Edible ginger-derived nano-lipids loaded with doxorubicin as a novel drug-delivery approach for colon cancer therapy. Mol. Ther. 24 (10), 1783–1796. https://doi.org/10.1038/mt.2016.159.

Zhang, S., Wang, Q., Tan, D.E.L., Sikka, V., Ng, C.H., Xian, Y., Li, D., Muthiah, M., Chew, N.W., Storm, G., 2024. Gut-liver axis: potential mechanisms of action of food-derived extracellular vesicles. J. Extracell. Vesicles 13 (6), e12466. https://doi.org/10.1002/jev2.12466.

Zhao, S., Mi, Y., Zheng, B., Wei, P., Gu, Y., Zhang, Z., Xu, Y., Cai, S., Li, X., Li, D., 2022. Highly-metastatic colorectal cancer cell released miR-181a-5p-rich extracellular vesicles promote liver metastasis by activating hepatic stellate cells and remodelling the tumour microenvironment. J. Extracell. Vesicles 11 (1), e12186. https://doi.org/10.1002/jev2.12186.

Zhou, F., Paz, H.A., Sadri, M., Cui, J., Kachman, S.D., Fernando, S.C., Zempleni, J., 2019. Dietary bovine milk exosomes elicit changes in bacterial communities in C57BL/6 mice. Am. J. Physiol. Gastrointest. Liver 317 (5), G618–G624. https://doi.org/10.1152/ajpgi.00160.2019.

Zu, M., Xie, D., Canup, B.S., Chen, N., Wang, Y., Sun, R., Zhang, Z., Fu, Y., Dai, F., Xiao, B., 2021. 'Green'nanotherapeutics from tea leaves for orally targeted prevention and alleviation of colon diseases. Biomaterials 279, 121178. https://doi.org/10.1016/j.biomaterials.2021.121178.