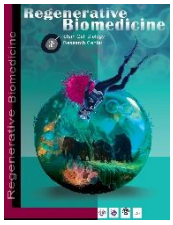

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Review Article

Extracellular Vesicles: An Emerging Tool in Inflammatory Bowel Disease

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Abstract

Inflammatory bowel disease is a group of chronic and recurrent autoimmune diseases that engage the gastrointestinal tract, including ulcerative colitis and Crohn's disease. Advances in understanding disease pathology have led to the development of new therapeutic approaches. For instance, extracellular vesicles have attracted clinicians due to their crucial roles in keeping intestinal epithelial barrier integrity and interactions with microbiota. Extracellular vesicles are potentially useful in drug delivery on account of their natural origin, low toxicity, stability in the bloodstream, limited immunogenicity, ability to breach biological barriers, and capability to target specific cells. In another aspect, extracellular vesicles as a therapeutic tool can reduce inflammation in colitis by decreasing pro-inflammatory, increasing anti-inflammatory cytokine levels, and inhibiting the NF-κB signaling pathway. Moreover, the interaction between gut microbiota and the intestine can affect the development of inflammatory bowel disease. Extracellular vesicles derived from probiotics, commensal bacteria, and parasites potentially treat inflammatory bowel disease. Despite their benefits, several challenges hinder exosome-based therapies, including low production yields, high costs of large-scale cell culture, limited cargo transport efficiency, and difficulties in quality control due to exosome heterogeneity. Further, evidence of the role of extracellular vesicles in the treatment of inflammatory bowel disease is confined to in vitro models and in vivo studies; thus, the research is at an early stage. The next significant step in extracellular vesicle research will be the translation of the approach into human clinical trials to confirm the findings and explore their therapeutic potential in inflammatory bowel disease.

Keywords: Crohn's disease, Exosome, Extracellular vesicle, Inflammatory bowel disease, Ulcerative colitis

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Introduction

Inflammatory bowel disease (IBD) refers to a set of chronic and recurrent autoimmune illnesses affecting the gastrointestinal system, including ulcerative colitis (UC) and Crohn's disease (CD) (1). IBD was once thought to be a Western disease, but it is now a worldwide disease, with an increasing prevalence in Westernized Asia, Africa, and South America (2). The increasing prevalence of IBD may be linked to industrialization and the adoption of Western lifestyles, as immigrants in developed nations have a higher risk of autoimmune illnesses than their homeland (3, 4).

IBD is characterized by inflammation of the mucosal layer of the colon (in UC) or all layers of any part of the gastrointestinal (GI) tract (in CD) which is accompanied by diarrhea, abdominal pain, bloody stools, weight loss, and vomiting (5, 6). The precise origins and pathogenesis of IBD remain unclear; nevertheless, various aspects have been substantiated, including genetic predisposition, immunological dysregulation, dysbiosis of the gut microbiota, and environmental influences (7-10). Furthermore, it is thought that loss of epithelial barrier function owing to unknown causes may expose gut microbiome components to the innate immune system. The exposure, together with immunological tolerance abnormalities and other predisposing variables, activates immune response pathways via immune mediator release, resulting in tissue inflammation and IBD clinical symptoms (6, 8, 11).

For years, the treatment of IBD was limited to symptom management by pharmacotherapy with aminoacylates, corticosteroids, immune modulators, and surgery if necessary.(14-12) Nevertheless, the progress made within IBD pathology has given way to the emergence of new therapeutic options that could change the disease progression. For instance, Anti-TNF-α drugs introduced in the last two decades offer significant benefits over traditional treatments (15, 16). Additionally, IBD medications play a crucial role in ensuring mucosal healing, avoiding hospital and surgical admissions, and improving the quality of life for IBD patients (17-19). However, findings suggest that approximately 30% of patients initially do not respond to anti-TNF- α agents and 23-46% lose their response in the first year of treatment (13, 20). In response to these constraints, the development of new biologics and small compounds has accelerated. Additional biologics and small molecules, such as anti-interleukin 12/23, anti-integrin medicines, and JAK inhibitors, are now in development to meet the needs of patients. Meanwhile, novel therapies such as apheresis, stem cell transplantation, and extracellular vesicles (EVs) have brought new hope in the treatment of IBD (1, 21). EVs are lipid bilayer-enclosed particles released by cells. The structures of EVs contain bioactive molecules such as proteins and nucleic acids, which play important roles in intercellular communication (22, 23).

More recently, EVs have attracted much attention in the treatment of IBDs because of their important role in the maintenance of intestinal epithelial barrier integrity and their interactions with the microbiota (Fig 1) (24). They may act as advanced drug delivery systems that are capable of encapsulating medications directly into inflamed tissues

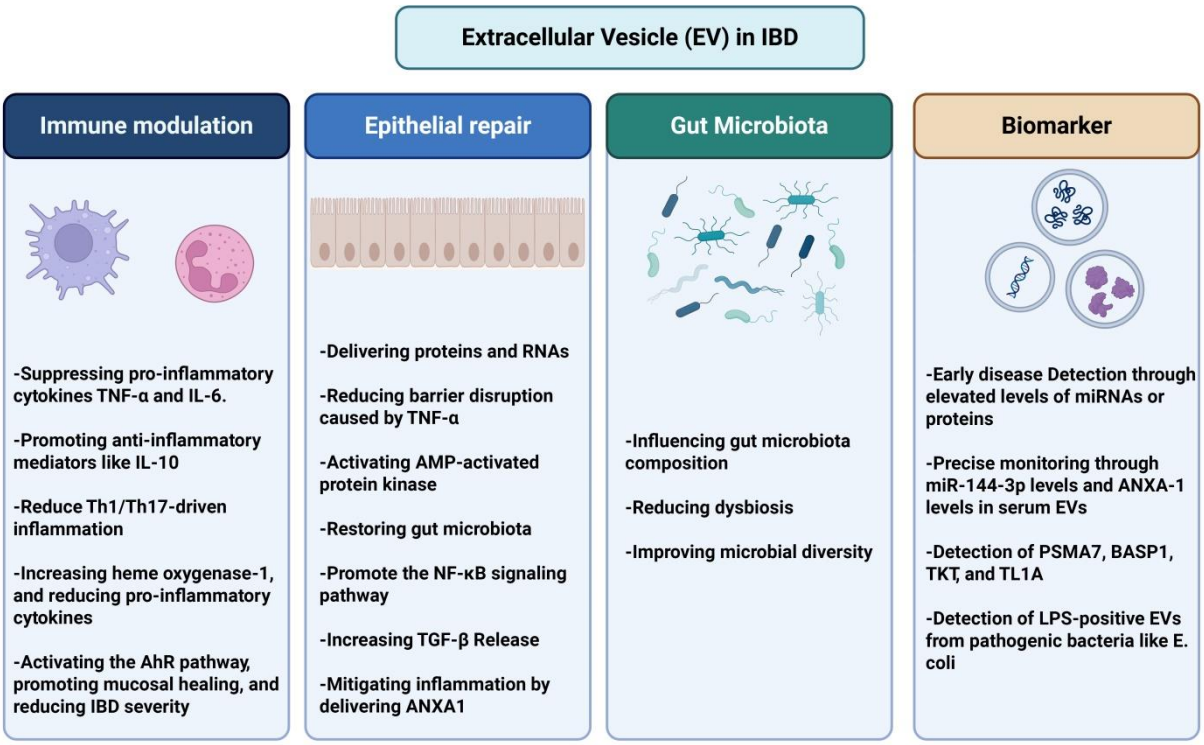

Figure 1. EVs applications in IBD. AhR, aryl hydrocarbon receptor; ANXA1, annexin-1; BASP1, brain acid soluble protein 1, IL, interleukin; miRNA, micro-RNA; PSMA7, prostate-specific membrane antigen 7; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TGF-β, transforming growth factor beta; Th, T-helper; TKT, transketolase; TL1A, tumor necrosis factor-like cytokine 1A; TNF-α, tumor necrosis factor-alpha.

with minimum systemic exposure and side effects. Their lower immunogenicity, compared to cell-based treatments, allows for multiple applications in chronic conditions such as IBD. They can also function as biomarkers for diagnosing the disease. Furthermore, EV-based therapies can provide the possibility of personalized treatment and reduce the trial-and-error approach in the management of IBD (25, 26). Considering the importance of understanding the pathogenesis of IBD and its clinical implications, the mechanisms of action of EVs as novel tools in the management of IBD will be discussed. In this regard, a targeted literature search was performed across reputable and core databases, utilizing a range of keywords pertinent to regenerative biomedicine and IBD.

An overview of extracellular vehicles (EVs)

EVs are defined as lipid bilayer-enclosed vesicles, which are secreted by a variety of cells ranging from human to plant cells. Traditionally, based on size and biogenesis, EVs can be classified into three major types: exosomes, microvesicles (MVs), and apoptotic bodies (ApoBDs). Exosomes are the

smallest EVs (from 30 to 150 nm). They originate from within the cell in multivesicular bodies (MVBs) and are released into the extracellular space by the fusion of MVBs with the cell membrane. Exosomes perform a variety of functions, including intercellular signaling, serving as biomarkers, and acting as vectors for cell therapy (27, 28). Microvesicles (MVs) are larger, ranging in size from 150 nm to 1 μm (27). MVs are important in various physiological and pathological processes, such as cancer progression, neuroinflammation, and tissue repair (29-31). ApoBDs are the largest EVs, which are characterized by their irregular shape and formed during the process of apoptosis (27). ApoBDs are loaded by cellular debris and are essential for the clearance of apoptotic cells by phagocytes (32). At first, EV's secretion was viewed as a mechanism for removing unwanted substances from cells, but now they are recognized to play a crucial role in a variety of intercellular communication (33). EVs can carry a wide range of biomolecules, including nucleic acids, proteins, lipids, and carbohydrates, shielding their cargo from degrading enzymes like nucleases and proteases during transport. The lipid bilayer maintains stability for the EVs and their associated cargoes under adverse physicochemical conditions such as long-term storage, multiple freeze-thaw cycles, and extreme pH levels (34).

EVs are mediators in a wide range of physiological and pathological processes, including intercellular component exchange. They also act as a communication bridge between the donor and recipient cells. Upon reaching EVs to recipient cells, through various mechanisms such as direct membrane fusion, receptor-ligand interaction, endocytosis, and phagocytosis which enables them to alter the cellular functions of recipient cells. Notably, EVs can cross biological barriers, such as the blood-brain barrier (BBB), while maintaining low immunogenicity and toxicity during transportation (33). Various pathological conditions, such as oxidative stress, cellular transformation, apoptosis, and ethanol-induced cell injury, can also provoke the release of EVs. Since EVs are present in some of the body fluids, they can be utilized as biomarkers for addressing dysfunctional cells in liquid biopsies (33, 34). Finally, their natural origin, low toxicity, stability in the bloodstream, limited immunogenicity, ability to overcome biological barriers, and capacity for cell targeting indicate EVs as a promising tool in drug delivery (27, 35).

Potential role of EVS in IBD pathogenesis and clinical applications

EVs have been used as a therapeutic tool in IBD treatment due to their unique characteristics and their potential advantages (36, 37). As in IBD, there is an interaction between genetic predisposition, environmental factors, and gut microbiota dysbiosis, IBD disrupts the mucosal homeostasis involved in epithelial barrier dysfunction, dysregulation in innate immunity, and disturbed T cell response (25, 38). EVs maintain intestinal homeostasis and alleviate inflammation. They play a role in anti-inflammatory reactions, restoration of vascular and epithelial barrier function, immune cell recruitment, microbiota composition reconstitution, and cellular

metabolite export. Therefore, they are significant for IBD diagnosis and therapy. They are also safe and feasible alternatives to cell therapy. Additionally, they are permeable and stable and have a low immunogenicity and cytotoxicity rate. Moreover, they have a potential for clinical use as biomarkers, therapeutics, and prognosis estimation of IBD (38, 39).

Immune modulation by EVs

EVs are involved in regulating the immune responses by modulating cytokine production, suppressing pro-inflammatory cytokines such as TNF-α and IL-6, and promoting anti-inflammatory mediators such as IL-10 (25, 40, 41). However, EVs are found to trigger inflammation, depending on their cargo. For instance, exosomes from visceral adipose tissue induce M1 polarization and aggravate the symptoms of IBD, while MSC-EVs promote the M2b phenotype and alleviate colitis by up-regulating regulatory T cells and down-regulating pro-inflammatory cytokines including IL-1β, IL-6, and IL-17A (42). Additionally, mesenchymal stem cells (MSCs)-EVs suppress Th1/Th17-driven inflammation by delivering anti-inflammatory cytokines and miRNAs targeting pro-inflammatory pathways. For instance, NF-κB, a well-known transcription factor involved in inflammation, is inhibited by EV-derived miRNAs such as miR-146a and miR-155, resulting in decreased production of inflammatory cytokines like IL-1β and TNF-α (43). Similarly, JAK-STAT signaling, which is one of the major players in immune cell activation, is modulated by EV-associated proteins and miRNAs, contributing to reduced Th1/Th17-mediated inflammation, enhanced Treg responses, and inhibited DC maturation (44-46). Activated Treg cells secret exosomes that contain miR-195a-3p. These molecules impair pro-apoptotic caspase 12 function and finally, result in IBD alleviation (47). Moreover, intestinal epithelial cells (IECs) secrete exosomes with increased levels of TGF-β that contribute to immune balance and decrease IBD severity (24).

In addition to human-derived EVs, particles from other sources like plants and bacteria have been used in IBD treatment. In this regard, ginger-derived EVs carry miRNAs that target gut bacteria such as *Lactobacillus rhamnosus* which can regulate genes (e.g., monooxygenase ycnE) and increase levels of indole-3-carboxaldehyde (AhR ligand). Activation of the AhR pathway results in increased production of IL-22, which promotes mucosal healing, modulates inflammatory responses, and reduces IBD severity. Grapefruit-derived EVs target intestinal macrophages, increase heme oxygenase-1, and reduce pro-inflammatory cytokine (36, 48, 49). On the other hand, lipopolysaccharide (LPS)-positive EVs which are derived from pathogenic bacteria such as *E.coli* can trigger inflammation by affecting Toll-like receptors (TLRs). Studies showed that the aforementioned bacteria and their EVs are increased in patients with IBD and can be used as biomarkers for disease diagnosis or a target for therapeutic agents (50). However, investigations continue to better understand the diverse capabilities of EVs in modulating immunity and disease pathogenesis, providing valuable tools for various therapeutic and diagnostic purposes.

EVs and epithelial repair

Beyond immune modulation, EVs promote epithelial repair by delivering proteins and RNAs that enhance tight junction integrity. They also reduce barrier disruption caused by TNF-α and other inflammatory mediators. For instance, EVs derived from broccoli activate AMP-activated protein kinase that promotes intestinal healing. Ginger-derived EVs protect intestinal barriers and restore gut microbiota which may support IBD management. Moreover, human-derived EVs contain diverse cargo that actively participates in epithelial healing. For instance, miR-146b-containing EVs derived from dendritic cells (DC) promote the NF-κB signaling pathway and consequently enhance the barrier integrity and alleviate the inflammation in dextran sulfate sodium (DSS)-induced colitis. Additionally, long non-coding RNA NEAT1, delivered by EVs, promotes intestinal epithelial barrier repair and reduces inflammation by reducing TNF-α and increasing the production of CD206, IL-10, and arginase-1. On the other hand, EVs containing miR-223 disrupt the barrier by inhibiting claudin-8 expression, thus enhancing epithelial proteins and lipids which are involved in repairing the intestinal barrier are also found in EVs. For instance, EGF (Epidermal Growth Factor) which may be presented in EVs promotes epithelial regeneration by enhancing cell survival, proliferation, and differentiation. Additionally, lipids enhance cellular membrane repair by providing structural components necessary for cell membrane integrity (51). Furthermore, EVs derived from MSCs have been shown to contain bioactive molecules like Hepatocyte Growth Factor (HGF) and WNT proteins, both of which support epithelial proliferation and differentiation (52, 53). This multi-faceted cargo delivery further solidifies EVs as a promising regenerative therapy for IBD.

Besides, in the pathophysiological process of IBD, different cells such as neutrophils and IEC release EVs that contain annexin A1(ANXA1). ANXA1 binds to formyl peptide receptors (FPRs) on responsive cells, such as phagocytes and epithelial cells, to decrease inflammation and promote wound healing. FRPs comprise three subtypes FPR1, FPR2/ALX, and FPR3 with FRP1 being in charge of wound healing. Notably, the Ac2-26 peptide was identified as a functional mimetic of ANXA1. Delivering Ac2-26 peptide through EVs to the inflamed area of IBD can promote wound healing (54). Furthermore, in IBD, the body starts to release EVs with transforming growth factor beta (TGF-β) as a compensatory reaction intended to inhibit CD4+ and modulate inflammation. It is worth noting that in several cases the level of TGF-β was stabile which may be due to the threshold of tissue damage necessary to activate this response (55). Collectively, these findings highlight the role of EVs in promoting epithelial repair in the context of IBD (55).

Impact of EVs on gut microbiota

Gut microbiota can be altered by several factors such as genetic, environmental, dietary, microbial, and chemical, resulting in dysbiosis as is seen in IBD (56). EVs can influence gut microbiota composition, reduce dysbiosis, and improve microbial diversity, a critical factor in IBD pathogenesis. Studies indicated that EVs from different sources affect the shape of the gut microbiome. For instance, milk-derived EVs can decrease

deleterious bacteria such as *Enterococcus*, *Turicibacter*,*Helicobacter*,*Desulfovibrionaceae, unclassified Desulfovibrionaceae,* and *Mogibacteriaceae* by inhibiting proinflammatory cytokines secretion while they increase beneficial bacteria (e.g., *Akkermansia, S24_7, Paraprevotella,* and *Verrucomicrobiaceae*) (56). Additionally, milk-derived EVs can restore the decline in the diversity of gut microbiota in DSS-induced colitis (56). Furthermore, breast milk-derived EVs contain proteins such as AnnexinA5, Flotilin-1, and CD9 that are involved in gut microbiota modulation. Besides milk in sources of EVs, bacterial EVs have also been shown to play a key role in modulating gut microbiota populations (57). In this regard, Hao et al. suggested that *Lactobacillus plantarum* Q7-derived extracellular vesicles alleviate DSS-induced colitis in mice by increasing anti-inflammatory bacteria (*Bifidobacteria* and *Muribaculaceae*) (58). Due to limited studies on the impact of EVs on gut microbiota, the mechanisms behind the scene are unclear in comparison with intestinal barrier and immunomodulation. Further research is needed to understand the role of EVs in gut microbiota modulation and its correlation with IBD pathogenesis to develop novel therapeutics.

EVs as biomarkers for IBD diagnosis

EVs may provide an early sign of disease even before clinical symptoms are apparent. For instance, the levels of certain miRNAs or proteins in EVs may be elevated by active disease or disease progression, thus enabling earlier detection and more precise monitoring. The concentration of specific EV markers in the blood or other fluids has also correlated with the severity of disease, treatment response, or recurrence. For instance, the levels of miR-144-3p in EVs correlated with the endoscopic score of CD and therefore may provide a more accurate marker of disease progression than more traditional markers, such as C-reactive protein. Moreover, Annexin-1 is a protein that is highly present in the serum EVs of active IBD patients. Its concentration parallels the degree of inflammation and, hence, may act as a diagnostic marker. MicroRNA (miR-144-3p) is highly expressed in the serum of patients with Crohn's Disease. Therefore, it is positively correlated with the endoscopic score of disease activity and has the potential to help follow-up recurrence after surgery. Moreover, some proteins were found to be exclusively present in the EVs of IBD patients, including PSMA7, BASP1, TKT, and TLN1, which may indicate their use as biomarkers for diagnosis and disease monitoring (41, 59). Overall, such therapeutic effects, coupled with their capabilities for selective targeting of inflamed tissues, minimal immunogenicity, and adaptability for personalized medicine, render EVs a powerful tool in IBD treatment. Indeed, the challenges are how to optimize isolation, standardization, and large-scale production processes to enable translation into the clinics. Studies should also focus more on high-purity, cost-effective EV isolation and processing techniques (25, 60, 61).

Clinical implementation

The clinical translation of EV-based therapies in IBD is advancing rapidly, with both

preclinical and clinical studies demonstrating promising results. EVs derived from diverse sources, including bacterial outer membrane vesicles (OMVs), plant-derived nanovesicles, and stem cells, have shown potential in modulating immune responses, restoring intestinal barrier integrity, and influencing gut microbiota composition (62-64). However, translating these findings into clinical practice involves overcoming challenges related to large-scale production, standardization, and regulatory approval (26, 65). The following table summarizes key studies exploring EV-based approaches in IBD management (Table. 1).

EV therapies vs. existing treatments

The IBD treatment has evolved from conventional small-molecule drugs (e.g., aminosalicylates, corticosteroids) to biologics targeting specific inflammatory pathways (e.g., anti-TNFα) and oral small-molecule inhibitors (e.g., JAK/STAT blockers)(73, 74). These approaches have improved clinical outcomes of patients, but several limitations, such as systemic side effects, infections, and loss of response, disrupt their efficacy (1, 75). EV-based therapies emerged as a novel approach in IBD management. EVs by modulating immune responses, repairing mucosal barriers, and delivering bioactive high specific cargo (e.g., miRNAs, proteins), capable of overcoming challenges of current treatments (42, 49). The supplementary table contrasts the mechanisms, advantages, and limitations of EV therapies against established IBD therapies (Supplementary 1).

Challenges and limitations

Growing investigations on EV research havearticulated interest in their application to clinical practice. Despite their great potential, the application of EVs is confronted with several challenges (100). For instance, EVs are classified into different subtypes, which vary in size, composition, and function. Besides the diversity, complications in separating EVs from non-EV components have put special challenges on their isolation and concentration process. Advanced isolation methods must prioritize both purity and scalability to ensure clinical relevance. Therefore, selecting newer techniques such as tangential flow filtration, field-flow fractionation, asymmetric flow field-flow fractionation, and novel immunization technologies intend to better isolation and concentration of EVs (101, 102). Another consideration is the cellular origin of EVs' molecular composition and their therapeutic effectiveness and dose-response consistency. Standardizing cell culture conditions and donor sourcing can mitigate variability in EV production. The heterogeneity of source cells can affect the reproducibility and efficiency of EV-based therapies and thus presents a challenge toward standardization (103, 104). Moreover, storage conditions of EVs and their source matrices, including biofluids, tissues, or conditioned media, are essential to maintain the stability, particle number, aggregation, and functional properties of EVs. Recent studies highlight that improper storage can break EV membrane integrity and reduce bioactivity. Storage containers, temperature and duration, EV processing and storage, buffers and cryoprotectants, and alternate storage techniques are some of the important factors in the storage process (101, 105).

Table 1. Preclinical and Clinical Studies on Extracellular Vesicles in Inflammatory Bowel Disease (**Abbreviations:** EVs: Extracellular Vesicles, OMVs: Outer Membrane Vesicles, PSC-IBD: Primary Sclerosing Cholangitis-Inflammatory Bowel Disease, TNVs: Turmeric-Derived Nanovesicles, MSC: Mesenchymal Stem Cells, mEVs: Milk-Derived Extracellular Vesicles, SCFAs: Short-Chain Fatty Acids, BDNs: Broccoli-Derived Nanoparticles, DC: Dendritic Cells, EpCAM: Epithelial Cell Adhesion Molecule, GDNPs: Ginger-Derived Nanoparticles, IBD: Inflammatory Bowel Disease)

Study	EV source	Developmental Stage	Primary Focus	References
Dorner et al.,2024	Bacterial OMVs	Preclinical & Clinical	Role of gut pathobiont-derived OMVs in promoting liver inflammation and fibrosis in PSC-IBD	66
Gao et al., 2022	Turmeric	Preclinical	Development of TNVs to alleviate ulcerative colitis through intestinal barrier restoration, microbiota regulation, and macrophage reshaping	67
Alberti et al., 2021	Mesenchymal stem cells	Preclinical	Pathogenesis and therapeutic potential of EVs in IBD and perianal fistulizing disease	68
Tong et al., 2020	mEVs	Preclinical	Investigating the effects of mEVs on gut microbiota composition, SCFAs, and intestinal immunity	69
Deng et al., 2017	BDNs	Preclinical	Investigating the effects of edible nanoparticles (BDNs) on gut immune homeostasis and DC activation for tolerogenic responses	70
Jiang et al., 2016	Intestinal epithelial cells	Preclinical	Impact of EpCAM on EV localization and protective effects against IBD	71
Zhang et al., 2016	Edible plant ginger (GDNPs 1, GDNPs 2, GDNPs 3)	Preclinical	Development of nanoparticle drug carriers for targeted delivery in IBD treatment, targeting colon inflammation, and improving treatment outcomes by utilizing ginger-derived nanoparticles	72

Furthermore, the therapeutic application of EVs is not without limitations. For instance, fast immune system clearance, nontargeted specificity, and low efficiency of cytoplasmic delivery can limit EV's therapeutic efficacy (106). However, engineering EVs with targeting ligands or stealth coatings may enhance their pharmacokinetics (107). Moreover, regulatory challenges are also a significant obstacle, since the evolving regulatory environment, huge amounts of data requirements, and lack of EV-specific regulations create complexities in market approval and clinical utilization (108, 109). Therefore, the challenges and limitations of EVs necessitate developing clear guidelines to ensure safety and efficacy in clinical applications (Table 2) (110).

Discussion and conclusion

IBD is a multifactorial, chronic condition, and its potential effects on the quality of life of affected patients (112, 113). Although pharmacotherapies and biologics have been developed limitations such as non-responsiveness, loss of efficacy, and systemic side effects have led to an interest in novel treatment approaches (114). Recently, emerging evidence has underlined the value of EVs as novel approaches to overcome such obstacles in IBD interventions (115). EVs such as exosomes, microvesicles, and apoptotic bodies, can be involved in cell-to-cell signaling, immunomodulation, epithelial repair, and regulation of gut microbiota (116-118). They are fit for therapeutic delivery because of the potential for loading several bioactive molecules like proteins, lipids, and nucleic acids which maintain their stability even in adverse conditions (119). Besides, EVs derived from cow's milk and plant-based sources had been demonstrated to resist enzymatic digestion along the gastrointestinal tract. Hence, they can be known as one of the most appropriate vesicles for oral drug administration. As an example, in nude mice, Cow's milk-derived EVs loaded with paclitaxel (PAC) demonstrated improved pharmacokinetic profiles. In addition, orally administered PAC-loaded EVs reduced systemic and immunological toxicity compared to intravenous delivery at equivalent doses. Moreover, Casein-coated and PEGylated EVs (derived from milk) have been developed to resist degradation in the stomach and enhance permeability through intestinal mucin layers (36). Furthermore, low immunogenicity and the capability to cross biological barriers such as the intestinal mucosa and blood-brain barrier can also enhance their therapeutic potential (120).

Particularly in IBD, EVs are drawing interest because of their role in modulating immune response, restoring intestinal barrier function, and gut microbiome reconstitution (64). For instance, MSC, ginger, and milk EVs demonstrated anti-inflammatory properties that enhanced epithelial integrity while reducing oxidative stress. These observations are encouraging because disruption of immune homeostasis and epithelial barriers has been implicated in the pathogenesis of IBD (121). Furthermore, EVs are a natural and biocompatible vehicle for drug delivery and can load therapeutic agents to be selectively delivered to inflamed tissues, hence limiting systemic exposure and side effects (122).

Table 2. Challenges and Limitations in the Therapeutic Application of Extracellular Vesicles (EVs)

Aspect	Challenges	Reference
Isolation and Concentration	Unachievable absolute isolation and concentration due to EV heterogeneity (different subtypes vary in size, composition, and function) and separation from non-EV components.	(101)
Storage and Retrieval Conditions	EV impact on storage and retrieval conditions stability, particle number, aggregation, and function.	(101)
Biological Variability	Originating from various cell sources and its affecting on molecular composition, therapeutic potential, and dosing consistency.	(103)
Safety Concerns	Potential for immunogenicity or adverse reactions Need for thorough safety assessments before clinical use	(111)
Functional Assays	Lack of reliable assays to assess therapeutic potential Challenges in confirming cargo functionality after engineering	(111)
Targeting and Biodistribution	Difficulty in achieving specific targeting to desired tissues or cells Variability in biodistribution patterns in vivo Lack of efficacy by rapid clearance of the immune system	(106, 111)
Engineering Limitations	Modification may alter natural targeting capabilities Risk of increasing size, which can affect circulation and delivery	(111)
Commercialization	Difficulty in scaling up production Regulatory hurdles for clinical application	(111)

EVs can also act as biomarkers in IBD. Some of the proteins and microRNAs expressed in EVs, such as miR-144-3p and annexin A1, are closely related to disease activity and its further process. EVs can also be helpful in early diagnosis, assessment of disease activity, and even in predicting the treatment response. Therefore, EVs can remarkably limit the "trial and error" methods so common in IBD management, with personalized and much more effective therapy (41, 59). Despite the potential of EVs in the treatment of diseases, large-scale manufacturing of EVs and following regulations towards clinical application have 124). (123, 124). This manufacturing yields originates from the limited secretory capacity of cells, the technical and financial challenges of scaling up cultures, and lengthy production timelines, which hinders the industrial ability of EVs (42). To address these challenges, more collaborative research, technological innovation, and policy development are needed. Further studies are required to optimize the isolation techniques of EVs, functional assays, and engineering strategies to enhance therapeutic efficacy and specificity. Therefore, though challenging, the complete realization of the potential of EV-based therapies will point toward a way to

safer, more effective, personalized treatment options for IBD (125).

Conflict of interest

The authors have no conflict of interest to declare.

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