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REVIEW

Mesenchymal Stem Cells Derived Extracellular Vesicles in Inflammatory Bowel Disease: Therapeutic Efficacy and Bioengineering Applications

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Abstract: Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) have emerged as a promising therapeutic approach for inflammatory bowel disease (IBD) due to their anti-inflammatory properties, immune modulation, and tissue regeneration potential. However, challenges in optimizing their production, efficacy, and understanding their therapeutic mechanisms remain. MSC-EVs, particularly those derived from bone marrow and umbilical mesenchymal stem cells, have shown significant therapeutic potential in both preclinical and clinical studies. Although the advantages of MSC-EVs over traditional therapies, such as low immunogenicity and non-invasive administration, limitations in their targeting capabilities and stability in fibrotic tissues impede full clinical translation. This review succinctly outlines a comparative analysis of MSC-EVs derived from various sources, such as bone marrow, adipose tissue, perinatal tissues, dental tissues, olfactory mucosa, and hair follicles in IBD treatment. Additionally, the applications of bioengineered MSC-EVs, including their use as nanodrug carriers and in targeted therapies, are discussed, with an emphasis on the future potential of integrating MSC-EVs with biomaterials like hydrogels. Finally, the current challenges and potential solutions for translating MSC-EVs from bench to bedside are discussed. This review aims to elucidate the therapeutic roles of MSC-EVs in IBD and inspire the development of innovative tissue-engineering materials.

Keywords: inflammatory bowel disease, stem cells, engineered extracellular vesicles, materials, nanotherapies, clinical trial

Introduction

Inflammatory bowel diseases (IBDs), encompassing Crohn's disease (CD) and ulcerative colitis (UC), are a group of autoimmune disorders characterized by persistent and relapsing intestinal inflammation.[1,2] UC is confined to the colon, progressively affecting the rectum to the proximal cecum, and is marked by superficial mucosal inflammation that can lead to ulceration, severe bleeding, toxic megacolon, and fulminant colitis.[3] In contrast, CD can affect any part of the gastrointestinal tract, from the mouth to the perianal region, in a discontinuous manner. It is characterized by transmural inflammation, leading to complications such as fibrotic stenosis, fistulas, and abscesses.[4] The prevalence of IBD exceeds 0.3% in North America, Oceania and many European countries, affecting over 1.5 million individuals in the United States and approximately 6.8 million globally.[5–7] Its incidence and prevalence have steadily increased with industrialization, imposing significant burdens on patients, healthcare systems, and society.

The exact etiology of IBD remains unclear, though it is generally hypothesized to result from an abnormal immune response to gut microbes, triggered by environmental factors in genetically predisposed individuals.[8] This process involves complex interactions among genetic, environmental, and microbial factors, leading to excessive activation of the mucosal immune system and dysregulated cytokine responses. The gut immune system consists of innate immunity, including neutrophils, macrophages, dendritic cells (DCs), and natural killer cells, as well as adaptive immunity

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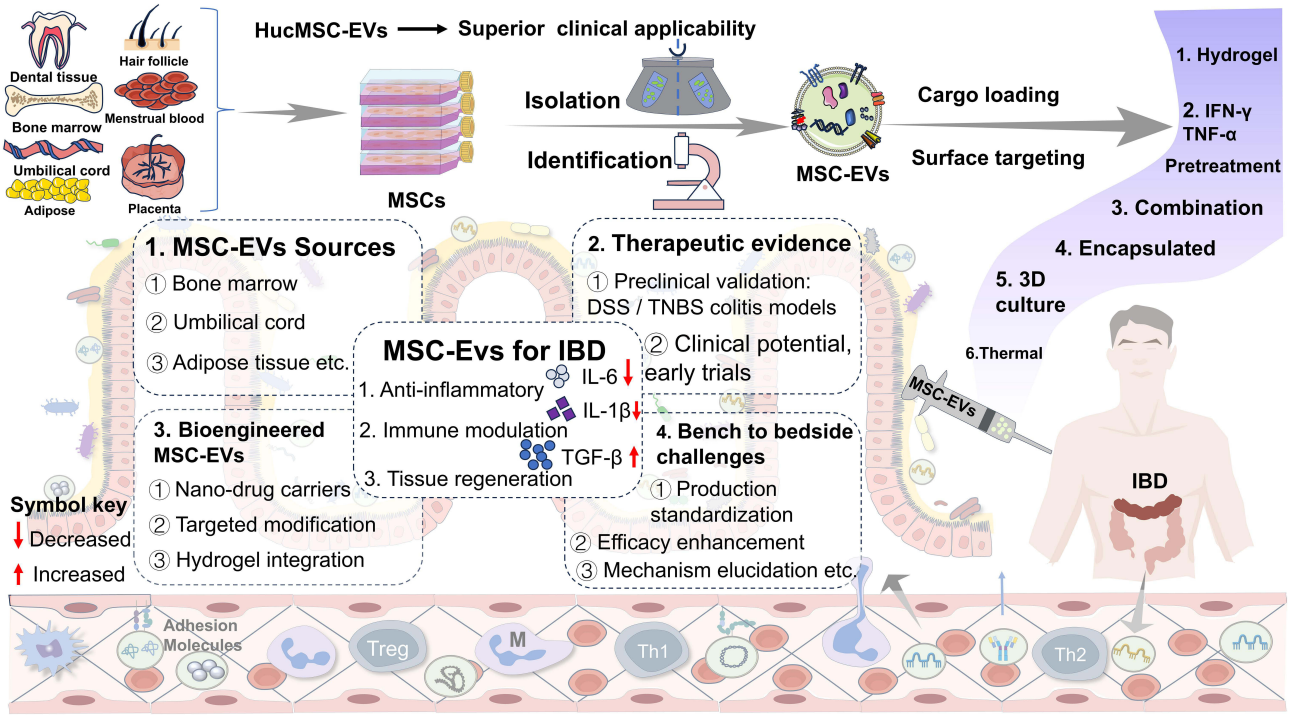
Graphical Abstract



involving T and B lymphocytes. This system is shielded from intestinal microorganisms by a tightly connected monolayer of epithelial cells. In IBD, immune mechanisms that separate commensal microbes from the intestinal epithelium, eliminate penetrating microbes, and suppress inappropriate T-cell responses are disrupted, leading to dysregulated intestinal homeostasis.[9] Close interactions among the intestinal microbiota, epithelial cells, and immune cells are crucial for maintaining this balance. However, a defective intestinal epithelial barrier and bacterial dysbiosis result in the aggregation and localized activation of immune cells, amplifying pro-inflammatory signaling and ultimately causing chronic intestinal inflammation. Given the central role of immune abnormalities in IBD pathogenesis, current treatment strategies primarily include aminosalicylates, corticosteroids, immunomodulators, and biologic agents.[10] Despite their widespread clinical use, these therapies present notable limitations. Prolonged immunosuppressive treatment may increase vulnerability to infections, while biologic therapies are often associated with substantial economic burden. In addition, a considerable proportion of patients exhibit inadequate or loss of therapeutic response, which restricts long-term disease control and underscores the urgent need for novel therapeutic strategies with improved safety and sustained efficacy.

Of various clinical trials, stem cell–based therapy is regarded the most promising option in the field of regenerative medicine.[11] Adult stem cells, known as mesenchymal stem cells (MSCs), possess self-renewal capacity, multipotent differentiation potential, and immunosuppressive effects both in vitro and in vivo.[12] These functions are crucial for tissue regeneration and functional recovery by promoting cell migration, epithelialization, angiogenesis, and granulation tissue formation, thereby highlighting MSCs as potential therapeutic agents for IBD.[13] However, cell-based stem cell therapies still face practical hurdles. Allogeneic MSCs can trigger recipient immune responses due to donor-derived antigens.[14] The number of stem cells, along with their proliferative and differentiation capacities, shows a significant decline with age.[15] Moreover, MSC performance varies with donor health, tissue source, and culture conditions, producing substantial

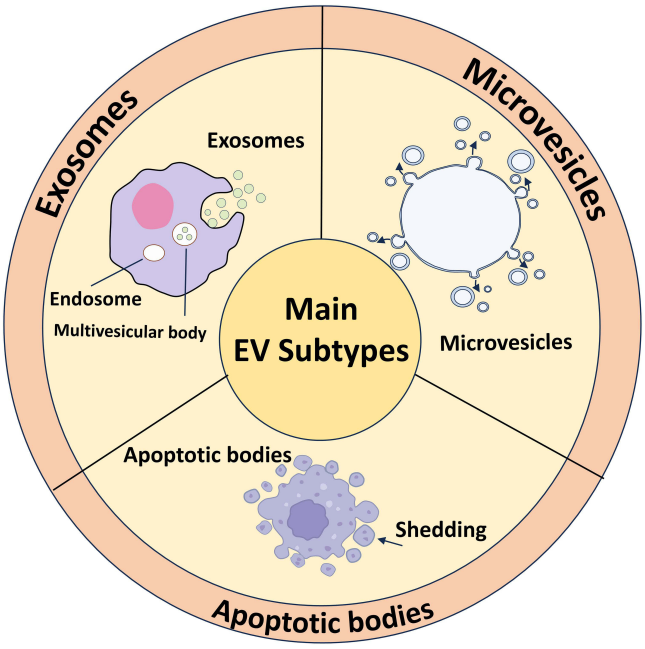


Figure 1 Main EV subtypes. Three main types of extracellular vesicles: exosomes, microvesicles, and apoptotic bodies.

heterogeneity and poor reproducibility of therapeutic effects and lineage potential. Consequently, recent research has shifted toward isolating and harnessing stem cell–derived bioactive components rather than administering live cells.

Emerging evidence shows that the therapeutic effects of MSCs largely depend on extracellular vesicles (EVs), a key paracrine mechanism exerting strong immunomodulatory effects.[16] EVs are membrane-bound vesicles released by various cell types, including MSCs, and are generally classified into exosomes, microvesicles, and apoptotic bodies according to their biogenesis and size (Figure 1).[17] Figure 1 depicts how exosomes originate from the endosomal system through multivesicular body formation, whereas microvesicles are generated by outward budding of the plasma membrane and apoptotic bodies are released during cell fragmentation. These vesicles mirror the biological characteristics of their donor cells by carrying diverse cargos, including RNA species (mRNA, miRNA, lncRNA, and circRNA), DNA, lipids, and metabolites.[18] Among EVs, exosomes represent the most critical subtype in mediating cell-to-cell communication. Physiologically, exosome biogenesis is defined by a characteristic double invagination of the plasma membrane.[19]

Compared with viable stem cells, stem cell–derived EVs, as non-living biomaterials, offer a longer storage lifespan and lower immunogenicity, and are largely unaffected by issues such as reduced cell viability or tumorigenicity that limit current stem cell therapies.[20] Owing to these advantages, stem cell–derived EVs have emerged over the past decade as a safer and more prominent alternative to stem cell therapy for IBD.[21] Relative to conventional therapies, MSC-EVs may also provide several theoretical benefits in IBD, including potential efficacy through anti-inflammatory, immunomodulatory, and mucosal repair effects,[21] improved safety as a cell-free therapeutic platform,[22] and better patient convenience owing to their off-the-shelf potential and easier storage.[23] However, the manufacturing cost of pharmaceutical-grade MSC-EVs remains high, and direct clinical evidence against standard therapies is still limited.[24] Given the growing diversity of recent studies, this review summarizes the therapeutic effects of MSC-derived EVs from different sources, as well as their various applications in the treatment of IBD.

Overview of MSC-EVs

MSC-EVs are lipid bilayer-enclosed vesicles secreted by MSC from diverse tissue sources, including bone marrow, adipose tissue, umbilical cord, placenta, dental tissues, hair follicles, and olfactory mucosa. Similar to other EV subtypes, MSC-EVs carry a variety of bioactive cargos, such as proteins, lipids, DNA, and multiple RNA species, which enable

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them to mediate intercellular communication and regulate recipient cell functions.[25] Importantly, the composition and biological activity of MSC-EVs are closely influenced by the origin and physiological status of the parent MSCs, as well as by culture and preconditioning conditions.[26] Therefore, MSC-EVs from different sources may exhibit distinct immunomodulatory, reparative, and anti-inflammatory properties in IBD, making source-based comparison particularly important for understanding their therapeutic heterogeneity and translational potential.

Therapeutic Efficiency of MSC-EVs in IBD

The characterization of EVs depends on the status of their originating MSCs. Accordingly, the therapeutic effects of EVs from different MSC types on IBD are summarized in Table 1.

BMSC-EVs

Bone marrow-derived MSCs (BMSCs), located in the bone marrow stroma, are easily accessible, abundantly available, and possess strong immunomodulatory and differentiation potential, making them and their EVs widely utilized in clinical applications.[41] BMSC-derived EVs (BMSC-EVs) alleviate IBD by suppressing the NF-κB signaling pathway to reduce colonic inflammation, regulating the oxidant/antioxidant balance by inhibiting oxidant levels, and preventing apoptosis through both exogenous death receptor and endogenous mitochondrial signaling pathways.[31] Meanwhile, BMSC-EVs not only promote epithelial regeneration and reduce epithelial cell apoptosis, but also exert potent anti-fibrotic effects by targeting inhibition of the CCN2–TGF-β axis to suppress fibroblast activation and migration and reduce extracellular matrix deposition, ultimately alleviating colitis.[28,32] Metallothionein-2 contained in BMSC-EVs is essential for suppressing inflammatory responses in UC, likely by inhibiting NF-κB activation through MZF1 via enhanced IκBα transcription and reduced IκBα phosphorylation.[27] Cellular pyroptosis, a key regulator of inflammation-associated cell death, plays a crucial role in IBD progression. MiR-539-5p from BMSC-EVs inhibits LPS-induced pyroptosis in murine small intestinal epithelial cells via the NLRP3/caspase-1 signaling pathway.[29] Furthermore, BMSC-EVs carry miR-378a-3p, which suppresses GATA-binding protein 2, downregulates aquaporin-4 expression, and blocks the peroxisome proliferator-activated receptor α signaling pathway, aiding in the treatment of IBD.[30] In addition, mouse BMSC-EVs promote macrophage proliferation and induce M1 to M2 macrophage polarization via the JAK1/STAT1/STAT6 signaling pathway.[42] As summarized in Figure 2, MSC-EVs modulate several representative pathways involved in IBD treatment, including inhibition of NF-κB signaling, suppression of pyroptosis through the NLRP3/caspase-1 pathway, activation of Wnt/β-catenin signaling to promote epithelial regeneration, and regulation of macrophage polarization through the JAK1/STAT1/STAT6 axis. Specifically, MSC-EVs may inhibit NF-κB activation through cargos such as MT-2 and miR-326,[38] suppress NLRP3/caspase-1-mediated pyroptosis via miR-539-5p,[29] and promote epithelial repair by activating Wnt signaling.[43] Some pathways involved in MSC-EVs-mediated treatment of IBD are illustrated in Figure 2.

Oxygen tension in the bone marrow lumen ranges from 1% to 7%,[44] making BMSCs cultured under hypoxic conditions more effective for treating hypoxic inflammatory tissues. Hypoxia-cultured BMSC-EVs improve IBD by regulating reactive oxygen species accumulation, DNA damage, and immune homeostasis in intestinal epithelial cells via hypoxia-inducible factor 1-alpha (HIF-1α).[45] Moreover, EVs from IFN-γ-pretreated BMSCs contain elevated levels of miR-125a and miR-125b, which target the 3′-UTR of Stat3, inhibiting Th17 cell differentiation and inflammation while promoting Treg cell differentiation, thereby alleviating IBD symptoms.[46]

Beyond the effects of BMSC-EVs and their pretreatment, their enriched miRNA and protein cargoes exhibit significant therapeutic potential. EphB2-overexpressing BMSC-EVs enhance homing to damaged colon, reduce mucosal inflammation by modulating inflammatory factors, alleviate oxidative stress to restore the intestinal barrier, and maintain immune homeostasis through STAT3 inhibition.[47] Additionally, PD-L1, a critical regulator of autoimmunity and immune tolerance, enhances the therapeutic efficacy of BMSC-EVs. PD-L1-overexpressing BMSC-EVs attenuate oxidative stress, inflammation, and apoptosis in IBD by blocking the PI3K/Akt/mTOR pathway and regulating the Th17/Treg balance.[48] Wu et al depleted TSG-6—an anti-inflammatory and tissue-protective immunomodulatory molecule secreted by MSCs in response to inflammation—from BMSC-EVs. This depletion abolished their protective effects, eliminating the inhibition of NLRP3/caspase-1/GSDMD- mediated pyroptosis in IECs and increasing IL-1β/IL-18 release, thereby

Table 1 Effects of MSC-EVs on IBD

MSCs Sources	Extracellular Vesicles Isolation Methods	In Vitro	In Vitro Effects	In Vivo	Animal Models of IBD	Infusion Methods	Dose of Injections	Extracellular Vesicles Reactive Molecules	In Vivo Effects	Underline Mechanisms	References
Human bone marrow	Ultracentrifugation	√	Contributed to the maintenance of gut homeostasis	√	DSS and TNBS induced mouse model	Tail vein injection	/	Metallothionein-2	Down-regulated inflammatory responses, maintained intestinal barrier integrity, and polarized M2b macrophages	Suppressed NF-κB activation via myeloid zinc finger 1 by enhancing IκBα transcription and inhibiting the phosphorylation of IκBα	[27]
Mice bone marrow	Ultracentrifugation	√	Stimulated epithelial regeneration	√	DSS-induced mouse model	Situ injection	20 μg	/	Decreased epithelial apoptosis and alleviated experimental colitis	/	[28]
Mice bone marrow	ExoQuick-TC (System Bioscience)	/	/	√	DSS-induced mouse model	Intraperitoneal injection	2×10^{10}	MiR-539-5p	Suppressed pyroptosis to inhibit IBD progression	NLRP3/caspase-1 signalling pathway	[29]
Mice bone marrow	Ultracentrifugation	√	Reduced LPS-induced apoptosis of M064 cells	√	TNBS-induced mouse model	Intraperitoneal injection	200 μg	MiR-378a-3p	Reduced the GATA2 expression, and downregulated AQP4 to suppressed the occurrence of IBD	Blocked the PPAR-α signalling pathway	[30]
Rat bone marrow	Ultracentrifugation	/	/	√	TNBS-induced rat model	Intraperitoneal injection	50 μg, 100 μg and 200 μg	/	Down-regulated pro-inflammatory cytokines, modulated anti-oxidant /oxidant balance, and moderated apoptosis	Inhibited NF-κBp65 signal transduction pathways, extrinsic death receptor signal pathway and the intrinsic mitochondrial signal pathway	[31]
Rat bone marrow	Ultracentrifugation	√	Inhibited fibroblast activation and migration, downregulating α-SMA, collagen I, and N-cadherin in TGF-β1–stimulated intestinal fibroblasts	√	DSS-induced mouse model	Intraperitoneal injection	200 μg	/	Decreased collagen deposition and α-SMA, and downregulated fibrotic genes (COL1α1, TIMP1, fibronectin), attenuating intestinal fibrosis	Suppressed the CCN2-TGF-β signaling axis	[32]
Human umbilical cord	Ultracentrifugation	√	Alleviated inflammatory responses and promoted proliferation of mouse intestinal epithelial cells	√	DSS-induced mouse model	Tail vein injection	100 μL	/	Reversed epithelial-mesenchymal transition and effectively alleviated IBD symptoms	By upregulating the O-GlcNAc glycosylation modification level of RACK1 molecules in intestinal epithelial cells	[33]
Human umbilical cord	Ultracentrifugation and ultrafiltration	√	Activated SIRT1 and FXR while inhibited FXR acetylation and NLRP3 inflammasomes in macrophages	√	DSS-induced mouse model	Tail vein injection	1 mg	/	Activated SIRT1 and FXR while inhibited FXR acetylation and NLRP3 inflammasomes in mice colons	Activating the SIRT1-FXR pathway	[34]
Human umbilical cord	Ultracentrifugation	/	/	√	DSS-induced mouse model	Tail vein injection	/	/	Inhibited the infiltration of Macrophages and relieved inflammatory responses	Inhibited the expression of IL-7 in macrophages	[35]
Human umbilical cord	Ultrafiltration and ultracentrifugation	√	Inhibited lipid peroxidation and alleviated ferroptosis in intestinal epithelial cells		DSS-induced mouse model	Tail vein injection	1 mg/20 g	MiR-129-5p	Inhibited lipid peroxidation and ferroptosis, reduced intestinal inflammation and repaired damages	Derived miR-129-5p targets ACSL4 to inhibit lipid peroxidation and repair IBD	[36]
Human umbilical cord	Density gradient centrifugation	/	/	√	DSS/ azoxymethane induced mouse model	Tail vein injection	200 μg	MiR-146a	Alleviated malignant transformation of colitis in Mice	MiR-146a inhibited SUMO1 and alleviated the deterioration of inflammation in fetal human cells	[37]
Human umbilical cord	Exosome extraction reagent (SBI)	√	Inhibited neddylation process of fetal human cells in an inflammatory environment	√	DSS-induced mouse model	Tail vein injection	1 mg	MiR-326	Inhibited neddylation and the activation of NF-Kb signaling pathway to relieve IBD	MiR-326 targeted the expression of NEDD8 to inhibit the neddylation process and achieved the effect of relieving IBD	[38]
Human placenta	Ultracentrifugation	/	/	√	TNBS-induced mouse model	Situ injection	200 μg	/	Reduced the expression of apoptotic proteins and promoted mucosal healing and oxidative stress	/	[39]
Canine adipose tissue	Ultracentrifugation	√	Increased IL-10 expression and increase Tregs and M2 type polarization		DSS-induced mouse model	Intraperitoneal injection	100 ug	TSG-6	Modulated pro- and anti- inflammatory cytokines	/	[40]

Note: Different sources of MSC-EVs for the treatment of IBD were summarized in Table 1, including MSCs Sources, Extracellular Vesicles Isolation Methods, In Vitro, In Vitro Effects, In Vivo, Animal Models of IBD, Infusion Methods, Dose of Injections, Extracellular Vesicles Reactive Molecules, In Vivo Effects, Underline Mechanisms, and References. The tick symbol (√) indicates that the corresponding experiment/assessment was performed or data were reported; "/" indicates not reported/not applicable for that item.

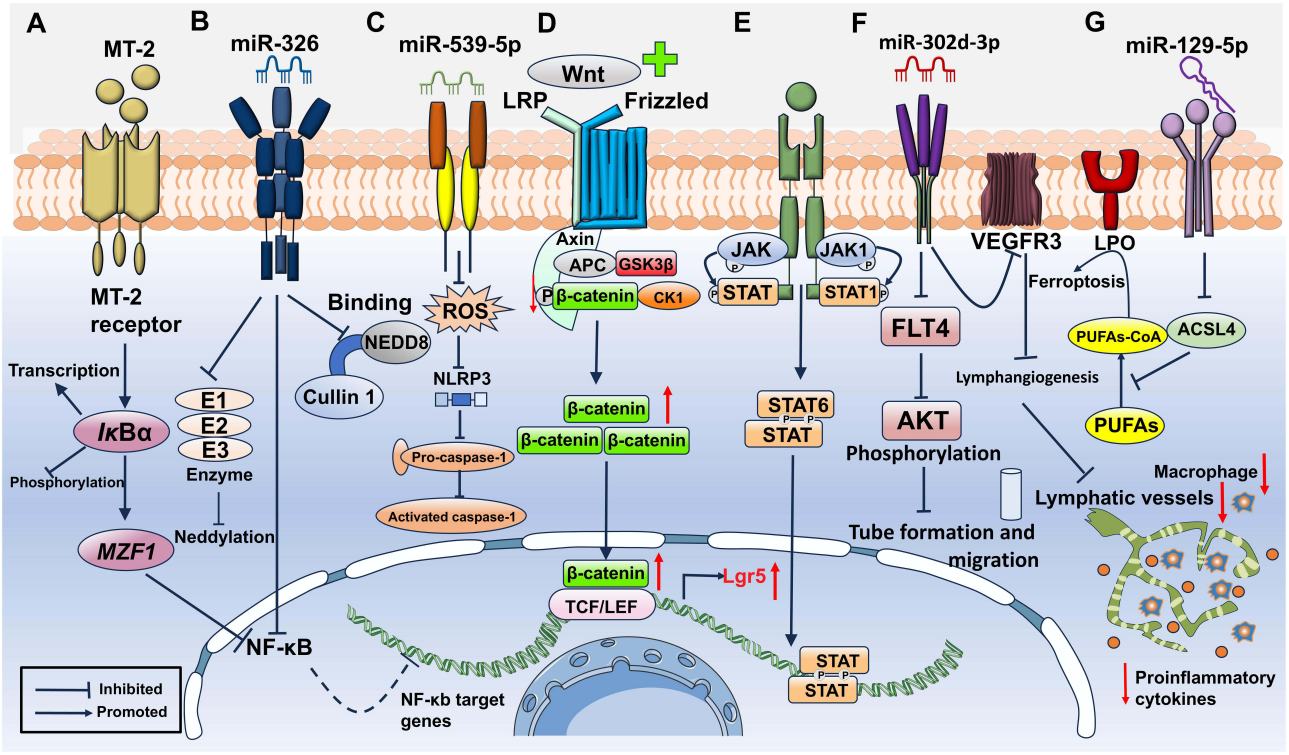

Figure 2 Some of the major pathways involved in the treatment of IBD with MSC-EVs. (**A**) MSC-EVs are shown to deliver MT-2, which inhibits NF-κB activation by enhancing IκBα transcription and inhibiting its phosphorylation through MZF1. (**B**) MiR-326 within MSC-EVs targets NEDD8, inhibiting the neddylation of the NF-κB pathway and its associated E1, E2, and E3 enzymes, thereby alleviating IBD. (**C**) MSC-EVs enriched with miR-539-5p mitigate IBD by inhibiting pyroptosis via the NLRP3/caspase-1 signaling pathway. (**D**) MSC-EVs promote intestinal stem cell and epithelial regeneration through activation of the Wnt signaling pathway. (**E**) The positive effect of MSC-EVs in treating UC is linked to the JAK1/STAT1/STAT6 signaling pathway. (**F**) MSC-EVs regulate lymphangiogenesis through the miR-302d-3p/VEGFR3/AKT axis to treat IBD. (**G**) MSC-EVs carry miR-129-5p to alleviate intestinal inflammation by reducing ACSL4 expression and inhibiting LPO and ferroptosis. Symbols and annotations: "↑/↓" indicate increased/decreased levels or activity relative to the inflamed/untreated condition; the green "+" denotes activation of Wnt signaling; T-bar lines indicate inhibition and arrowed lines indicate promotion, as shown in the schematic key; red arrows highlight the direction of change for the indicated biological outcomes (eg., macrophages and pro-inflammatory cytokines).

Abbreviations: ACSL4, acyl-CoA synthetase long-chain family member 4; CoA, coenzyme A; FLT4, Fms-related receptor tyrosine kinase 4; IBD, inflammatory bowel disease; LPO, lipid peroxidation; MSC-EVs, mesenchymal stem cell-derived extracellular vesicles; MT-2, Metallothionein-2; MZF1, myeloid zinc finger 1; NEDD8, neural precursor cell expressed developmentally down-regulated 8; PUFAs, polyunsaturated fatty acids; VEGFR, Vascular endothelial growth factor receptor.

confirming that the therapeutic efficacy of BMSC-EVs is TSG-6–dependent.[49] Apoptotic BMSC-EVs induced by the Fas death receptor suppress T-cell activation and proliferation while attracting monocytes through the cyclooxygenase 2/prostaglandin E2 (PGE2) axis, mediating immunosuppression and monocyte chemotaxis.[50] Furthermore, BMSC-EVs combined with green tea polyphenols demonstrate superior efficacy in colitis treatments compared to BMSC-EVs alone, preserving innate immunity integrity through enhanced anti-inflammatory and regenerative effects.[51]

HucMSC-EVs

Perinatal tissue-derived MSCs, including human umbilical cord MSCs (hucMSCs) and human placenta MSCs (hP-MSCs), along with their EVs, have been extensively utilized in autoimmune disease research due to their non-invasive sourcing, high proliferation, and differentiation potential.[52] HucMSC-derived EVs (HucMSC-EVs) mitigate colitis through multiple mechanisms, including modulation of the Treg/Th17 balance,[53] suppression of IL-7 expression in macrophages,[35] and promotion of M2 macrophage polarization via the METTL3-Slc37a2-YTHDF1 axis.[54] By modulating O-GlcNAc glycosylation of RACK1, hucMSC-EVs reverse epithelial-mesenchymal transition, suppress NLRP3 inflammasome–mediated inflammation, reduce epithelial apoptosis, promote epithelial regeneration, and ultimately alleviate IBD both in vitro and in vivo.[33] HucMSC-EVs stimulated by the ER stress inducer TSG further ameliorate IBD by enhancing the production of immunomodulatory factors, promoting M2 macrophage polarization, and inducing Treg differentiation.[55] Additionally, bile acids and their receptor, farnesoid X receptor, mediate chemical exchanges

between gut microbes and the host immune system. HucMSC-EVs significantly alleviate macroscopic and microscopic inflammation, modulate intestinal metagenomic and metabolomic profiles, and upregulate colonic farnesoid X receptor expression, thereby attenuating colitis.[56]

The hyperactivation of NLRP3 inflammasomes exacerbates the onset and progression of inflammatory diseases, including IBD. HucMSC-EVs attenuate colitis by delivering miR-378a-5p, which suppresses NLRP3 activation in colonocytes, including macrophages, thereby preventing cellular pyroptosis.[57] Zhou et al demonstrated that HucMSC-EVs can upregulate SIRT1 and FXR expression in macrophages and mice colons, thereby activating the SIRT1–FXR pathway to reduce FXR acetylation, directly downregulate NLRP3 inflammasome activation, suppress the inflammatory response, and ultimately alleviate IBD.[34] Meanwhile, hucMSC-EVs inhibit lipid peroxidation and ferroptosis in intestinal epithelial cells, reduce inflammation, and target ACSL4 via miR-129-5p delivery.[36] TSG-6 is delivered by hucMSC-EVs to repair the intestinal mucosal barrier and restore Th2/Th17 balance when administered intraperitoneally.[58] Furthermore, hucMSC-EVs enhance intestinal lymphatic drainage, reduce lymphangiogenesis and macrophage infiltration, and exert IBD-attenuating effects through the miR-302d-3p/VEGFR3/AKT axis.[59] SUMO1-mediated SUMOylation, a critical post-translational modification regulating IBD progression, is suppressed by hucMSC-EVs via their encapsulated miR-146a.[37] Upon report, the IκB protein inactivates the pro-inflammatory transcription factor NF-κB, but its degradation is primarily dependent on neddylation. HucMSC-EVs carrying miR-326 attenuate intestinal inflammatory injury by suppressing neddylation in intestinal mucosal cells.[38] Notably, Duan et al showed that hP-MSC-derived EVs alleviated colitis by modulating inflammatory factor balance, reducing oxidative stress, downregulating apoptotic protein expression, and preserving epithelial cell integrity.[60]

AMSC-EVs

Due to their accessibility, low invasiveness, high yield from adipose tissue, and pro-angiogenic, immunomodulatory, and anti-inflammatory properties, adipose-derived MSCs (AMSCs) are the most commonly used MSC type in clinical trials.[61] AMSC-derived EVs (AMSC-EVs) have been found to mitigate IBD damage by suppressing apoptosis, promoting epithelial regeneration, reducing inflammatory responses, and preserving intestinal barrier integrity.[62] They also block the metastasis of DNA-damaged cells and ameliorate experimental acute colitis by inducing Treg cells and reducing inflammatory cytokines.[63] Xiong et al identified MFGE8, a highly expressed component in AMSC-EVs, as a key bioactive compound mediating the alleviation of intestinal fibrosis. AMSC-EVs mitigate CD-associated intestinal fibrosis, at least partially, by downregulating the FAK/Akt signaling pathway.[64] Furthermore, creeping fat, characterized by hypertrophic mesenteric adipose tissue encasing inflamed intestinal segments, is a distinct yet poorly understood hallmark of CD. EVs from creeping fat-derived adipose stem cells carry elevated levels of miR-132-3p, which improve lymphatic function through the RASA1/ERK1/2 signaling pathway, effectively alleviating chronic mesenteric inflammation and colitis in CD.[65]

The functions of EVs secreted by stem cells can be modulated by adjusting culture conditions, such as the addition of inflammatory factors.[66] For instance, canine AMSC-EVs triggered by TNF-α and INF-γ alleviate experimental murine colitis by enhancing Treg populations in the inflamed colon and regulating the M1/M2 macrophage balance.[67] Hypoxia-induced AMSC-EVs enriched with miR-216a-5p regulate HMGB1/TLR4/NF-κB signaling to promote M2 macrophage polarization, thereby attenuating UC.[68] Various MSC-EV pretreatment methods for IBD treatment are summarized in Table 2.

Studies suggest that AMSC-EVs offer therapeutic potential for IBD, with enhanced efficacy when combined with anti-IL-12 p40, significantly reducing Disease Activity Index scores.[72] Optimizing the administration route, injection frequency, and dosage is essential for maximizing therapeutic outcomes. Notably, the combination of melatonin and AMSC-EVs has been investigated to reduce inflammation, oxidative stress, apoptosis, and fibrosis markers in DSS-induced acute colitis.[73] Multiple combination therapies utilizing MSC-EVs for IBD treatment are summarized in Table 3.

The source of MSCs significantly influences their phenotype and function, with adipose tissues derived from various sites, including diseased mesentery, subcutaneous fat, omentum, and normal mesentery. AMSCs and their EVs from healthy individuals drive M2 macrophage polarization, effectively reversing the inflammatory phenotype. In contrast, those from Crohn's diseased intestinal mesentery induce M1 macrophage polarization and sustain inflammation,[74]

Table 2 Pretreated MSC-EVs for the Treatment of IBD

Pretreatment Methods	Pretreatment Effects	MSCs Sources	Extracellular Vesicles Isolation Methods	In Vitro	In Vitro Effects	In Vivo	Animal Models of IBD	Infusion Methods	Dose of Injections	Extracellular Vesicles Reactive Molecules	In Vivo Effects	Underline Mechanisms	References
IFN-γ	Increased the level of miR-125a and miR-125b	Mouse bone marrow	Ultracentrifugation	√	Promoted Treg cells differentiation and repressed Th17 cell	√	DSS-induced mouse model	Tail vein injection	200 μg	MiR-125a and miR-125b	Attenuated colitis in mice	MiR-125a and miR-125b repress Th17 cell differentiation via targeting Stat3	[46]
TNF-α and IFN-γ	Enhanced Treg cells and regulated the M1/M2 balance	Canine adipose tissue	Ultracentrifugation	√	Enhanced Tregs and induction of M2-type macrophage polarization	√	DSS-induced mouse model	Intraperitoneal injection	100 μg	/	Induced M2-macrophage polarization, enhanced Tregs, and regulated the production of pro- and anti- inflammatory cytokines	/	[67]
Hypoxia	Enriched miR-216a-5p and promoted macrophage M2	Mice adipose tissue	Ultracentrifugation	√	Promoted macrophage M2 polarization	√	DSS-induced mouse model	Tail vein injection	200 μg	MiR-216a-5p	Regulated macrophage M2 polarization and have higher therapeutic efficiency than normoxic conditions	Regulate HMGB1 and modulate HMGB1/TLR4/NF-κB axis	[68]
Hypoxia	Increased HIF-1α production	Rat bone marrow	Isolation reagent (LifeTechnology)	/	/	√	DSS-induced rat model	Tail vein injection	/	HIF-1α	Reduced ROS production, DNA damage and apoptosis in intestinal epithelial cells	Regulate UC intestinal epithelial cell function through HIF-1α	[45]
Thapsigargin	Increased yield and expression of immunomodulatory factors	Human Wharton's jelly	Ultracentrifugation	√	Inhibited T cell proliferation and enhanced Treg cells and M2-type macrophage polarization	√	DSS-induced mouse model	Intraperitoneal injection	200 μg	/	Increased anti-inflammatory cytokines in the inflamed colon and enhanced Treg cells and M2 macrophage polarization	/	[55]
TNF-α	Upregulated miR-24-3p	Human menstrual blood	Ultracentrifugation	√	Increased the proportion of M2 phenotype of macrophages	√	DSS-induced mouse model	Intraperitoneal injection	200 μg	MiR-24-3p	TNF-α-pretreated MenSC-EVs relieved colonic inflammation	TNF-α, and the microRNA transform macrophages to M2 to relieve inflammation by binding to downstream IRF1	[69]
Hypoxia	Increased the level of miR-214-3p	Mice hair follicle	Ultracentrifugation	√	Promoted LPS-induced MODE-K cells recovery by maintaining mitochondrial dynamics, alleviating mitochondrial dysfunction, and enhancing both autophagy and mitophagy.	√	DSS-induced mouse model	Tail vein injection	100 μg	MiR-214-3p	Promoted colonic tight junction proteins expression, suppressed the oxidative stress response, and reduced UC-related inflammatory injury.	MiR-214-3p-mediated inhibition of the PI3K/AKT/mTOR signaling pathway, alleviation of mitochondrial dysfunction	[70]
LPS	Enhanced the anti-inflammatory efficacy	Human periodontal ligament tissue	Ultracentrifugation	/	/	√	DSS-induced mouse model	Tail vein injection	200 μL	/	Modulated intestinal macrophage polarization, maintained intestinal epithelial barrier function, improved inflammatory status, and influenced the diversity and composition of the gut microbiota	Activation of the PI3K/AKT signaling pathway	[71]

Note: MSC-EVs pretreated by different methods for the treatment of IBD were summarized in Table 2, including Pretreatment Methods, Pretreatment Effects, MSCs Sources, Extracellular Vesicles Isolation Methods, In Vitro, In Vitro Effects, In Vivo, Animal Models of IBD, Infusion Methods, Dose of Injections, Extracellular Vesicles Reactive Molecules, In Vivo Effects, Underline Mechanisms, and References. The tick symbol (√) indicates that the corresponding experiment/assessment was performed or data were reported; "/" indicates not reported/not applicable for that item.

Table 3 Combination Therapies of MSC-EVs on IBD

Agents Used in Combination	MSCs Sources	Extracellular Vesicles Isolation Methods	Animal Models of IBD	Infusion Methods	Dose of Injections	In Vivo Effects	References
Anti-IL-12 p40	Human adipose tissue	Tangential flow filtration	DSS-induced mouse model	Tail vein and Intraperitoneal injection	1×10^{10}	Downregulated mRNA expression levels of genes encoding IL-6, TNF-α, and iNOS	[72]
Melatonin	Rat adipose tissue	Ultracentrifugation	DSS-induced mouse model	Tail vein injection	1 μg, 2 μg, 10 μg, and 50 μg	Alleviated the expression of markers for inflammation, oxidative stress, apoptosis, and fibrosis	[73]
Green tea polyphenols	Rat bone marrow	Ultracentrifugation	Acetic acid induced mouse model	Tail vein injection	100 mg	Keeped integrity of innate immunity through regenerative and anti-inflammatory effects	[51]

Note: Different combination therapies of MSC-EVs for the treatment of IBD were summarized in Table 3, including Agents Used In Combination, MSCs Sources, Extracellular Vesicles Isolation Methods, Animal Models of IBD, Infusion Methods, Dose of Injections, In Vivo Effects, and References.

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exhibiting diminished immunosuppressive capacity in colitis treatment. This is potentially due to altered regulation of pathogenic T-cell responses or T-cell infiltration.[75] Therefore, when applying AMSC-EVs to treat IBD, the cell source and preparation methods must be carefully considered, as variations can lead to different therapeutic outcomes.[76]

Dental MSC-EVs

Mesenchymal stem cells derived from dental tissues have gained significant attention in recent decades due to their unique developmental origins, rapid proliferation rates, and immunomodulatory properties. To date, various dental MSC populations, including dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), and gingival MSCs (GMSCs), have been isolated and characterized.[77] These cells are easily obtained from the oral cavity or discarded tissues during dental procedures and can be expanded in vitro while maintaining genomic stability over extended periods. Functionally, dental MSCs and their EVs exhibit immune cell-like properties, producing anti-inflammatory cytokines and anti-apoptotic molecules in response to tissue injury and inflammation.

GMSCs have been shown to alleviate experimental colitis by inducing immunosuppressive factors, suppressing inflammatory cytokines, promoting Treg cell infiltration in the colon,[77] and modulating inflammatory immune cells via IL-10 signaling.[78] Additionally, hepatocyte growth factor-transduced DPSCs attenuate intestinal mucosal injury by differentiating into intestinal stem cell-like cells, promoting their proliferation, suppressing inflammation, and mitigating oxidative stress-induced injury.[39] Furthermore, DPSCs modified to overexpress HIF-1α and telomerase, and conditioned by pro-inflammatory stimuli, release EVs with potent immunomodulatory properties. These EVs induce M2 macrophage polarization, suppress inflammatory responses in the intestinal endothelium, and modulate intestinal fibrosis, offering effective treatment for IBD.[79]

Ten-eleven translocation proteins 1 (Tet1) and Tet2, DNA demethylases, regulate MSC functions and modulate Treg cells to maintain immune homeostasis. PDLSCs pretreated with Tet1/Tet2 small interfering RNA enhance IBD therapy by increasing Treg cell populations, reducing Disease Activity Index scores, lowering inflammatory cell infiltration, and restoring epithelial structure.[80] Our study highlights the multifaceted actions of LPS-preconditioned PDLSC-EVs in intestinal protection. These vesicles direct macrophage polarization, strengthen epithelial barrier integrity, and alleviate inflammatory responses. In parallel, they contribute to microbial balance by enriching beneficial taxa, exemplified by *Clostridia_UCG-014*, while limiting the expansion of opportunistic genera such as *Streptococcus* and *Aeromonas*. Additionally, LPS-preconditioned PDLSC-EVs also reshape intestinal protein expression and activate the PI3K/AKT signaling pathway.[71] Although dental MSC-EVs show great promise for IBD treatment, related studies remain in their infancy and require further investigation.

MenSCs-EVs

Menstrual blood serves as a non-invasive and abundant source of menstrual blood-derived mesenchymal stem cells (MenSCs), characterized by high proliferative capacity, low immunogenicity, and absence of ethical concerns.[81,82] These cells can differentiate into multiple lineages and have been increasingly explored for in vitro and in vivo therapies.[83–85] Existing studies have demonstrated that MenSC derived extracellular vesicles (MenSC-EVs) exert notable therapeutic effects on gynecological disorders such as endometrial fibrosis,[86] premature ovarian failure,[87] and intrauterine adhesions,[88] while also alleviating lung inflammation,[89] attenuating fulminant hepatic failure,[90] and promoting diabetic wound healing.[91] Moreover, Xu et al reported that TNF-α preconditioning significantly increased the level of miR-24-3p in MenSC-EVs, which targets downstream IRF1 to drive M1-to-M2 macrophage polarization, thereby ameliorating UC.[69] Nevertheless, research on MenSC-EVs in IBD remains limited, though their clinical potential is promising. Future investigations may focus on elucidating the roles of other non-coding RNAs or proteins carried by MenSC-EVs in the immunoregulation of IBD.

HF-MSC-EVs

Hair follicle-derived MSC (HF-MSCs), obtained from hair follicles, are more accessible than other MSC sources and offer minimal invasiveness, cost-effectiveness, and low tissue damage during extraction.[92] These cells demonstrate strong differentiation potential and robust in vivo expansion, making them suitable for clinical applications. Prior

investigations have revealed that EVs derived from HF-MSCs (HF-MSC-EVs) can attenuate UVB-induced photoaging and ameliorate androgenetic alopecia.[93,94] Moreover, Li et al demonstrate that hypoxia-preconditioned HF-MSC-EVs promote the expression of colonic tight junction proteins, suppress oxidative stress, and reduce inflammation-associated damage in UC.[70] These effects may be mediated by miR-214-3p, which inhibits the PI3K/AKT/mTOR signaling pathway, maintains mitochondrial dynamics and stability, alleviates mitochondrial dysfunction, and enhances mitophagy.

OE-MSC-EVs

Olfactory ecto-derived MSCs (OE-MSCs), also known as olfactory mucosa-derived MSCs, isolated from the lamina propria of the human nasal cavity, exhibit higher proliferative, clonogenic, and immunosuppressive capacities than BMSCs.[95] Their extracellular vesicles (OE-MSC-EVs) exert potent immunomodulatory effects, showing promise in Alzheimer's disease and Sjögren's syndrome.[96,97] In IBD treatment, OE-MSC-EVs inhibit $CD4^+$ T cell proliferation, reduce IL-17 and IFN-γ release, and enhance TGF-β and IL-10 secretion. Additionally, they significantly alleviate colitis by suppressing Th1/Th17 subsets and promoting Treg cell populations.[98] However, the mechanism underlying OE-MSC-EV-mediated IBD treatment remain to be fully elucidated, particularly the molecules and pathways involved in T cell regulation and their impact on intestinal mucosa and microbiota.

Application of MSC-EVS in IBD

MSC-EVs as Therapeutic Agents

MSC-EVs can serve as drug carriers or enhance receptor targeting through bioengineering and surface modification, thereby improving their therapeutic potential for IBD (Figure 3). Figure 3 summarizes the major engineering strategies for MSC-EVs, including cargo loading, surface modification, and pretreatment-based optimization to enhance EV yield and function. The therapeutic effects of MSCs are primarily attributed to three components: homing to damaged sites, differentiate to replace injured cells, and paracrine secretion of bioactive factors.[99] However, studies indicate that less than 1% of intravenously administered MSC homed to damaged intestinal tissue.[77] In addition, conditioned media (CM) from amniotic fluid MSCs have been shown to alleviate colitis by elevating IL-10 level and decreasing TNF-α level after intraperitoneal injection.[100] Building on the these findings, MSC-EVs—acellular, membrane-bound particles carrying proteins, mRNAs, and miRNAs—exhibit excellent biocompatibility and mediate both local and systemic intercellular communication.[101] Upon endocytosis by recipient cells, RNA within MSC-EVs modulates protein expression. Moreover, MSC-EVs alleviate various IBD symptoms, including blood in stool, shortened colon length, mucosal damage, weight loss, and microscopic injury. Their efficacy is dose-dependent; in mouse models, effective doses typically range from 10 to 100 μg of protein. Injections of 100 μg significantly reduce colonic inflammation, and repeated dosing enhances immunomodulatory effects.[31,40]

The mode of administration significantly influences the efficacy of MSC-EVs. Intravenous injection results in predominant distribution to the lungs and liver, while intraperitoneal injection achieves broader distribution across the liver, pancreas, and gastrointestinal tract. Subcutaneous injection leads to significantly lower accumulation in all measured organs. Although intraperitoneal administration is considered effective for IBD with MSC-EVs,[102] oral administration is preferred for its non-invasiveness and lower risk of infection.[103] EV clearance from circulation is partially regulated by the innate immune system and facilitated by complement proteins, while their protein and lipid composition has minimal impact on clearance rate or biodistribution. Most injected EVs are rapidly taken up by macrophages in the reticuloendothelial system, regardless of administration route or cellular origin.[63] Despite their instability in circulation, EV cargo remains stable and exerts immunomodulatory effects. As a result, multiple injections are often required to mimic the continuous paracrine release observed in MSC-based therapies. However, the therapeutic effects of MSC-EVs—such as inflammation suppression and intestinal mucosal barrier repair—are typically short-term. It remains uncertain whether long-term use can prevent intestinal fibrosis and colitis-associated cancers.

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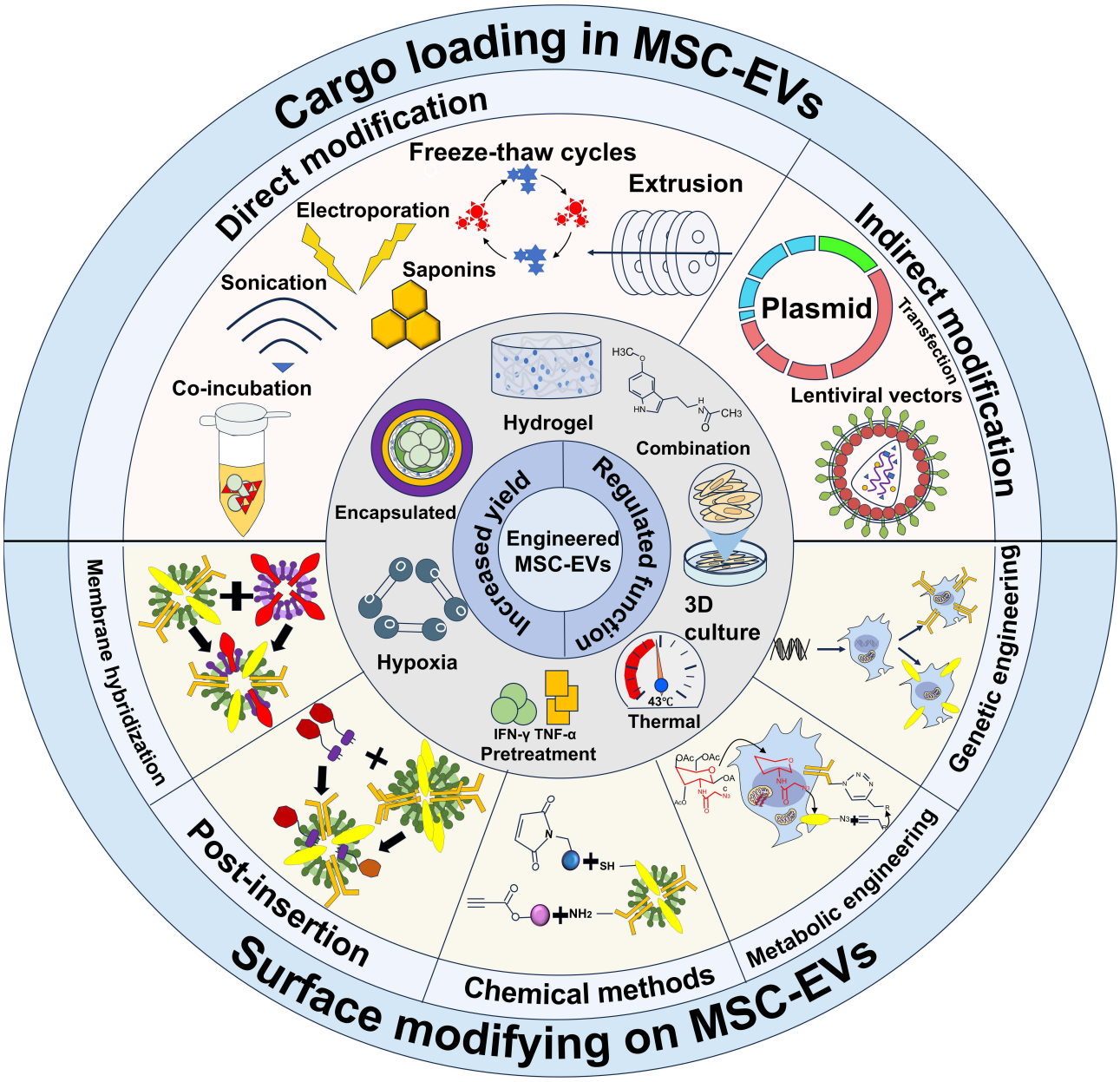

Figure 3 Potential application of MSC-EVs for the treatment of IBD. MSC-EVs, in addition to serving as therapeutic agents, function as carriers for drugs and can enhance receptor targeting through bioengineering techniques. Current cargo-loading methods include direct approaches such as co-incubation, electroporation, sonication, membrane permeabilization using saponins, and freeze-thaw cycles, as well as indirect modification strategies. Surface modification techniques that enhance the targeting capability of MSC-EVs include membrane hybridization, post-insertion methods, chemical modification, genetic engineering, and metabolic engineering. Additionally, the yield and therapeutic efficacy of MSC-EVs for treating IBD can be improved through approaches such as hypoxia preconditioning, pretreatment, encapsulation, hydrogel incorporation, thermal engineering, 3D culture, and combination strategies.
Abbreviations: MSC-EVs, mesenchymal stem cell-derived extracellular vesicles; IBD, inflammatory bowel disease.

Engineered MSC-EVs

Engineered MSC-EVs for Nano Drug Carriers

Compared to conventional drug carriers such as liposomes and inorganic mesoporous materials, EVs offer superior biocompatibility, low toxicity, and the potential for bioengineering to enhance drug-loading capacity.[104] MSC-EVs engineered through different strategies for IBD treatment are summarized in Table 4.

Current strategies for loading therapeutic molecules into EVs are generally classified into two categories. The first entails direct modification of EV contents using techniques such as co-incubation, electroporation, sonication, membrane permeabilization with saponins, and freeze-thaw cycles. Co-incubation, a passive loading method, enables drugs entry

Table 4 Engineered MSC-EVs for the Treatment of IBD

Engineering Strategies	Engineering Effects	Extracellular Vesicle Sources	Extracellular Vesicles Isolation Methods	In Vitro	In Vitro Effects	In Vivo	Animal Models of IBD	Infusion Methods	Dose of Injections	In Vivo Effects	Underline Mechanisms	References
Exo-Fect™ Exosome Transfection Kit	MSC-EVs loaded with IL1R2-siRNA	Mouse adipose	Ultracentrifugation	√	Reduced lipopoly saccharide-induced apoptosis and inflammation in IEC-6 cells	√	DSS-induced mouse model	/	/	Alleviated epithelial damage and immune infiltration, decreased apoptosis, and downregulated IL1R2/CCR2	Dual inhibition of IL1R2 and CCR2 pathways	[105]
Direct co-incubation	MSC-EVs loaded with Berberine	Human placenta	Exosome extraction reagent kit	√	Decreased the expression of inflammatory factors, inhibited macrophage apoptosis induced by ROS, hindered M1 phenotype, and facilitated M2 phenotype	√	DSS-induced mouse model	Tail vein injection	5 mg/ kg in 200 μL	Provided protection against oxidative damage to colonic tissues and reduce cellular apoptosis	Deactivation of the MAPK signaling pathway	[106]
Transduced with the lentiviral vectors	HIF and telomerase over-expression	Human dental pulp	Ultracentrifugation	√	Ameliorated fibrosis and suppressed inflammation and repolarize M1 to M2	√	TNBS-induced mouse model	Intraperitoneal injection	50 μg	Suppressed the inflammatory response by regulating the expression of cytokines and altering the ratio of M1/ M2 infiltration	/	[79]
Transduced with the lentiviral vectors	EphB2 over-expression	Rats bone marrow	Ultracentrifugation	/	/	√	DSS-induced rat model	Tail vein injection	100 μg	Maintained colonic immune homeostasis and attenuated inflammation and oxidative stress and protected the intestinal epithelial barrier	Restrain the activation of JAK-STAT3 signaling pathways	[47]
Transduced with the lentiviral vectors	PD-L1 over-expression	Rats bone marrow	Ultracentrifugation	/	/	√	DSS-induced rat model	Tail vein and Intraperitoneal injection	/	Mitigated colonal inflammation, apoptosis and oxidative stress	Block the activation of PTEN/PI3K/ AKT/mTOR axis and regulate the balance of Th17/Treg cells	[48]
Transduced with the lentiviral vectors	MiR-146a over-expression	Rats bone marrow	Ultracentrifugation	/	/	√	TNBS-induced rat model	Tail vein injection	100 μg	Down-regulated phosphorylation levels of NF-κB p65 and IκBα	Target TRAF6 and IRAK1 and suppress activation of the NF-κB pathway in inflamed colons	[107]
Thermal engineering	Enhancing EVs production and high levels of PD-L1	Human periodontal ligament	Ultracentrifugation	/	/	√	DSS-induced mice model	Tail vein injection	200 μg	Regulated the Th17/Treg cell balance	PD-1/PD-L1 signaling pathway	[108]
Transduced with the lentiviral vectors	EphB2 over-expression	Rats bone marrow	Ultracentrifugation	√	Restored intestinal barrier function and regulated the immune balance	/	/	/	/	/	Inhibit RhoA/ ROCK pathway	[109]
Transduced with the lentiviral vectors	Circ-CCND1 over-expression	Induced pluripotent stem cell	Ultrafiltration	√	Reduced pyroptosis in UC model cells	/	/	/	/	/	EV-delivered circ-CCND1 entered cells and bound KDM6B, limiting ELF3 activation, upregulating miR-342-3p, and suppressing KDM6B in a feedback loop that mitigated pyroptosis.	[110]
Transfected with siRNA (Lipofectamine 8000)	TSG-6–depleted exosomes	Mice bone marrow	Ultracentrifugation	√	Abolished the suppression of pyroptosis in Caco-2 intestinal epithelial cells.	√	DSS-induced mice model	Intraperitoneal injection	1 mg/ mouse	BMSC-EV suppressed NLRP3 inflammasome–mediated pyroptosis, but TSG-6 knockdown abolished these protective effects.	TSG-6–dependent inhibition of NLRP3/caspase-1/GSDMD pyroptosis in intestinal epithelial cells	[49]

Note: MSC-EVs engineered by different strategies for the treatment of IBD were summarized in Table 4, including Engineering Strategies, Engineering Effects, MSCs Sources, Extracellular Vesicles Isolation Methods, In Vitro, In Vitro Effects, In Vivo, Animal Models of IBD, Infusion Methods, Dose of Injections, In vivo Effects, Underline Mechanisms, and References. The tick symbol (√) indicates that the corresponding experiment/assessment was performed or data were reported; "/" indicates not reported/not applicable for that item.

into EVs via a concentration gradient.[111] For instance, Gao et al employed AMSC-EVs encapsulating IL1R2-siRNA to achieve dual inhibition of IL1R2 and CCR2 in DSS-induced colitis mice. AMSC-EVs/siIL1R2 significantly improved body weight, reduced epithelial apoptosis, and suppressed pro-inflammatory cytokines, likely through attenuation of CCR2-mediated macrophage recruitment and IL-1/IL1R2-driven NF-κB signaling.[105] Deng et al utilized hP-MSC-EVs to deliver the natural compound berberine, enhancing its solubility, bioavailability, and targeting of colon tissues.[106] MSC-EVs loaded with berberine promote epithelial repair, reduce apoptosis, and exhibit anti-inflammatory and anti-oxidant effects, likely through the inhibition of the MAPK signaling pathway (Figure 4). As shown in Figure 4, EVs-Ber reduced inflammatory mediators such as IL-6, TNF-α, PGE2, iNOS, and COX-2 in vitro, while also ameliorating colon

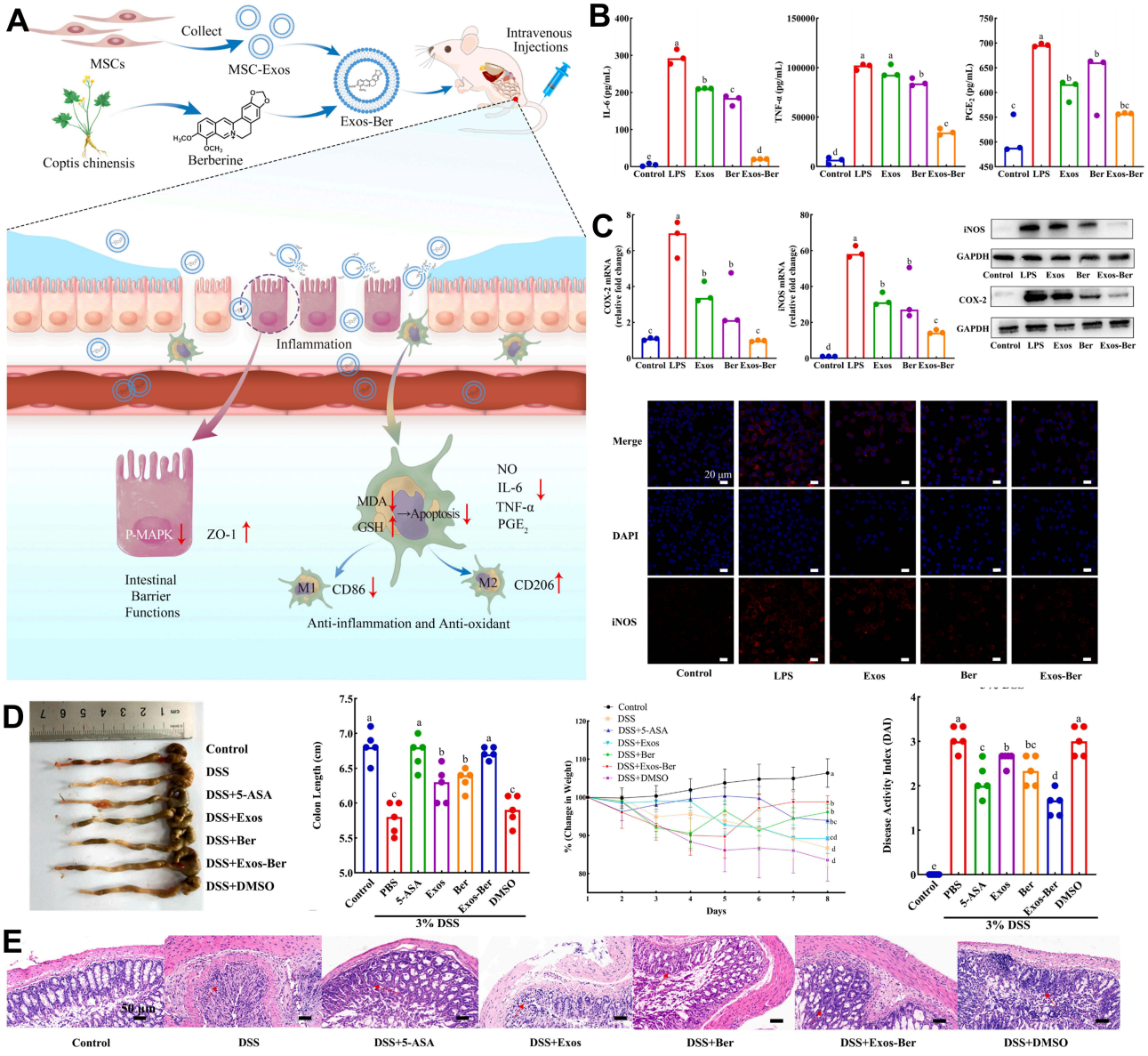

Figure 4 MSC-EVs as a drug delivery system for the treatment of IBD. (**A**) Preparation and mechanism of EVs-Ber for the treatment of UC. (**B**) In vitro anti-inflammatory properties of EVs-Ber, including reduction of IL-6, TNF-α and PGE2 levels. (**C**) EVs-Ber reduces the expression of inflammatory markers iNOS, COX-2 in vitro. (**D**) EVs-Ber effectively inhibited colon shortening and weight loss, and reduced DAI score. (**E**) H&E staining showed that EVs-Ber significantly improved colitis. Symbols and annotations: red arrows indicate increased/decreased expression or biological outcomes as depicted in the schematic; different colors represent different experimental groups, as labeled in each panel. Lowercase letters (a–e) above bars or data points indicate multiple-group statistical comparisons within each panel: groups with different letters differ significantly ($P < 0.05$), whereas groups sharing the same letter are not significantly different ($P > 0.05$). The letters do not denote fixed groups across the entire figure. Reproduced with permission from reference.[106] Copyright 2024, Elsevier.

Abbreviation: EVs-Ber, MSC-EVs loaded with Berberine.

shortening, weight loss, disease activity, and histopathological damage in vivo, supporting the therapeutic advantage of EV-based berberine delivery. Beyond therapeutic efficacy, the effects of engineering strategies on EV properties also merit attention. Engineering strategies may affect EV membrane integrity, loading efficiency, and stability. Conventional methods such as sonication and extrusion may compromise membrane properties or particle retention, whereas tonicity control improves loading yield while preserving EV morphology and surface markers.[112] Protective encapsulation, such as PEGylated metal–phenolic network shells, can further enhance EV stability during storage and transport.[113] Overall, preserving EV integrity and manufacturability is critical for clinical translation.[114]

The second strategy involves indirect modification, in which the genome of EV donor cells is engineered to promote the expression and encapsulation of therapeutic drugs within secreted EVs. A common approach is the transfection of MSCs with recombinant lentiviruses encoding the target gene, enabling stable overexpression of target RNAs in EVs for therapeutic delivery.[115] For instance, MSC-EVs overexpressing PD-L1 via lentivirus transfection alleviate IBD by blocking the PI3K/Akt/mTOR pathway, restoring Th17/Treg cell homeostasis,[48] inhibiting inflammatory immune cells, and promoting tissue repair via the PD-1/PD-L1 pathway.[116] Similarly, BMSC-EVs overexpressing EphB2 specifically target damaged colonic tissue, strengthen intestinal barrier function, and maintain colonic immune homeostasis by inhibiting the RhoA/ROCK pathway and STAT3 activation.[47,109] Additionally, EVs released by HIF-1α- and telomerase-overexpressing DPSCs reverse the pro-inflammatory environment, eliminate inflammation, and promote tissue repair.[79] MSC-EVs overexpressing miR-146a alleviate IBD by targeting and inhibiting TRAF6, IRAK1, and NF-κB signaling in the inflamed colon.[107] In vitro studies demonstrated that engineering iPSC-derived MSC-EVs to overexpress circ-CCND1 enabled their delivery of circ-CCND1 into colonic epithelial cells, where it bound to KDM6B and suppressed its activation of the ELF3 gene. The resulting downregulation of ELF3 elevated miR-342-3p expression, which subsequently inhibited KDM6B, thereby establishing a feedback loop that mitigated pyroptosis.[110] Meanwhile, Tang et al found that thermally engineered MSC-EVs, generated by exposing cells to 43°C for 1 hour, overexpress PD-L1 to regulate Th17/Treg cells through the PD-1/PD-L1 pathway, thereby suppressing inflammation in colitis.[108]

Oral administration is preferred for MSC-EVs in IBD treatment due to improved patient comfort and compliance. However, the harsh gastrointestinal environment and low bioavailability hinder efficient delivery to the lesion site.[117] Hydrogels, biocompatible semisolid polymers composed of natural or synthetic polymer networks infused with water, have gained attention in tissue engineering and drug delivery.[118] Hydrogel microparticles encapsulating MSC-EVs can be formulated into suspensions or aggregates (pro-cellular infiltration microscaffolds) for oral or minimally invasive injection. Alternatively, they can be embedded in bulk hydrogels to form composites with multiscale behaviors.[119] Notably, hydrogels can also be used to deliver MSC-CM to relieve colitis.[120] Li et al synthesized an injectable, biodegradable nanofiber-hydrogel composite matrix by mixing maleimide-modified polycaprolactone with a precursor solution of acrylated hyaluronic acid and polyethylene glycol dithiol. This matrix, loaded with MSC-EVs, effectively alleviates inflammation at fistula site and promotes tissue regeneration through macrophage polarization and neovascularization.[121] Nie et al engineered a dopamine methacrylamide (DMA)-modified hydrogel by cross-linking gelatin methacrylate with DMA and incorporated IL-27–overexpressing MSC-EVs using microfluidic technology. This microcarrier adheres firmly to the colonic surface after transrectal administration, enabling sustained therapeutic release, inflammation reduction, and barrier repair (Figure 5).[122] Figure 5 further shows that this adhesive hydrogel system improved colon length and histological injury, reduced pro-inflammatory cytokines such as IL-6 and TNF-α, and restored epithelial junction proteins including claudin, occludin, and ZO-1, thereby demonstrating enhanced mucosal protection and barrier repair. Similarly, Gan et al designed a multi-layered microcapsule consisting of MSC-EVs encapsulated in sodium alginate (SA) hydrogel microspheres, coated with gelatin and an outer enteric Eudragit FS30D layer (Figure 6). Figure 6 outlines that the triple-layered system enhanced colonic accumulation after oral administration and improved therapeutic outcomes in colitis. This structure preserves MSC-EVs stability and bioactivity, enabling targeted delivery to the damaged colon and effectively treating UC.[123]

In addition to hydrogels, biomaterials such as non-toxic polysaccharides like chitosan and glucan—selectively degradable by colonic microbiota-derived enzymes—offer promising options for encapsulating MSC-EVs in IBD treatment. These materials serve dual functions of payload protection and site-specific colonic release. A layer-by-layer self-assembly (LbL) strategy has been developed by Deng et al to encapsulate MSC-EVs, effectively shielding

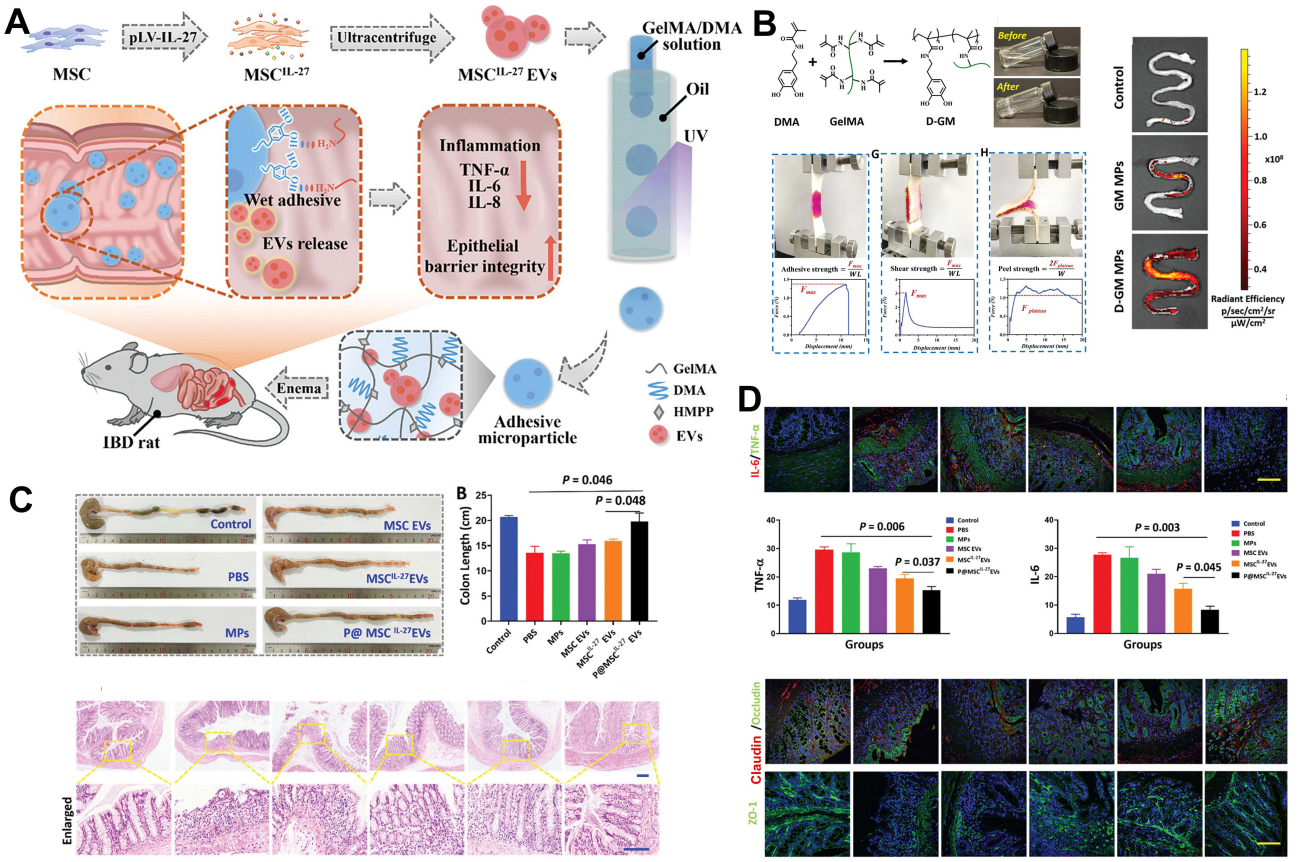

Figure 5 DMA-modified GelMA hydrogels loaded with MSC-EVs overexpressing IL-27 were used to treat IBD. (**A**) Schematic illustration of the fabrication and application of DMA/GelMA hydrogels loaded with MSC-EVs overexpressing IL-27 for the treatment of IBD. (**B**) DMA/GelMA hydrogel preparation, optical images before and after ultraviolet radiation, adhesive strength, shear strength, peeling strength and fluorescence images of colon receiving GelMA and DMA/GelMA. (**C**) Colon length images of healthy mice (control), IBD model with PBS, pure particles (MPs), MSC-EVs, MSC-EVs overexpressing IL-27 (MSCIL-27-EVs) and DMA/GelMA hydrogel MPs loaded with MSCIL-27-EVs (P@MSCIL-27 EVs) groups, colon length statistical analysis and H&E staining. For the H&E images (from left to right), the groups are: Control, PBS, MPs, MSC EVs, MSC^IL-27-EVs, and P@MSC^IL-27-EVs. Scale bar = 50 μm. (**D**) Images of IL-6 and TNF-α immunostaining, gray value of different groups, calaudin, occludin and ZO-1 immunostaining in colon sections of different groups. For the immunofluorescence images (IL-6/TNF-α, from left to right), the groups are: Control, PBS, MPs, MSC EVs, MSC^IL-27-EVs, and P@MSC^IL-27-EVs. For the immunofluorescence images (claudin/occludin/ZO-1, from left to right), the groups are: Control, PBS, MPs, MSC EVs, MSC^IL-27-EVs, and P@MSC^IL-27-EVs. Scale bar = 50 μm. Symbols and annotations: group labels (Control, PBS, MPs, MSC EVs, MSC^IL-27-EVs, and P@MSC^IL-27-EVs) correspond to the treatment conditions shown in each panel; red arrows indicate increased/decreased biological outcomes as depicted in the schematic (eg, ↓ inflammatory cytokines and ↑ epithelial barrier integrity); the color bars in the quantitative graphs indicate the respective experimental groups; scale bars are as indicated in the original images Reproduced with permission from reference.[122] Copyright 2023, Wiley.

Abbreviations: DMA, dopamine methacrylamide; GelMA, gelatin methacrylate. MPs:microparticles; MSC^IL-27-EVs, MSC-EVs overexpressing IL-27; P@MSC^IL-27-EVs, DMA/GelMA MPs loaded with MSC^IL-27-EVs; ZO-1, zonula occludens-1.

them from enzymatic degradation while enabling targeted delivery to inflamed colonic tissues. Following transoral administration, this system alleviates UC by suppressing the MAPK/NF-κB signaling pathway (Figure 7).[124] Figure 7 further demonstrates that LbL-encapsulated MSC-EVs improved colonic accumulation, ameliorated histological injury, restored ZO-1 expression, and partially normalized gut microbiota composition in DSS-induced colitis. The engineered vesicles utilized N-(2-hydroxy)propyl-3-trimethyl ammonium chitosan chloride and oxidized konjac glucomannan, both biodegradable polysaccharide derivatives. Notably, the oxidized konjac glucomannan was exclusively cleaved by β-mannanase, a colon-targeted enzyme, ensuring localized therapeutic action. Meanwhile, Liu et al employed a modified double emulsion technique to fabricate poly (lactic-co-glycolic acid)-encapsulated hucMSC-EVs particles, which, upon oral administration, reversed IBD-induced epithelial–mesenchymal transition and alleviated inflammation in a mouse model of IBD.[125]

In conclusion, the development of biomaterials science has facilitated the implementation of diverse materials, including hydrogels, in MSC-EV-based therapeutic strategies for IBD treatment (Table 5). However, the dual role of MSC-EVs—both as therapeutic carriers and as encapsulated agents within protective matrices—remains incompletely

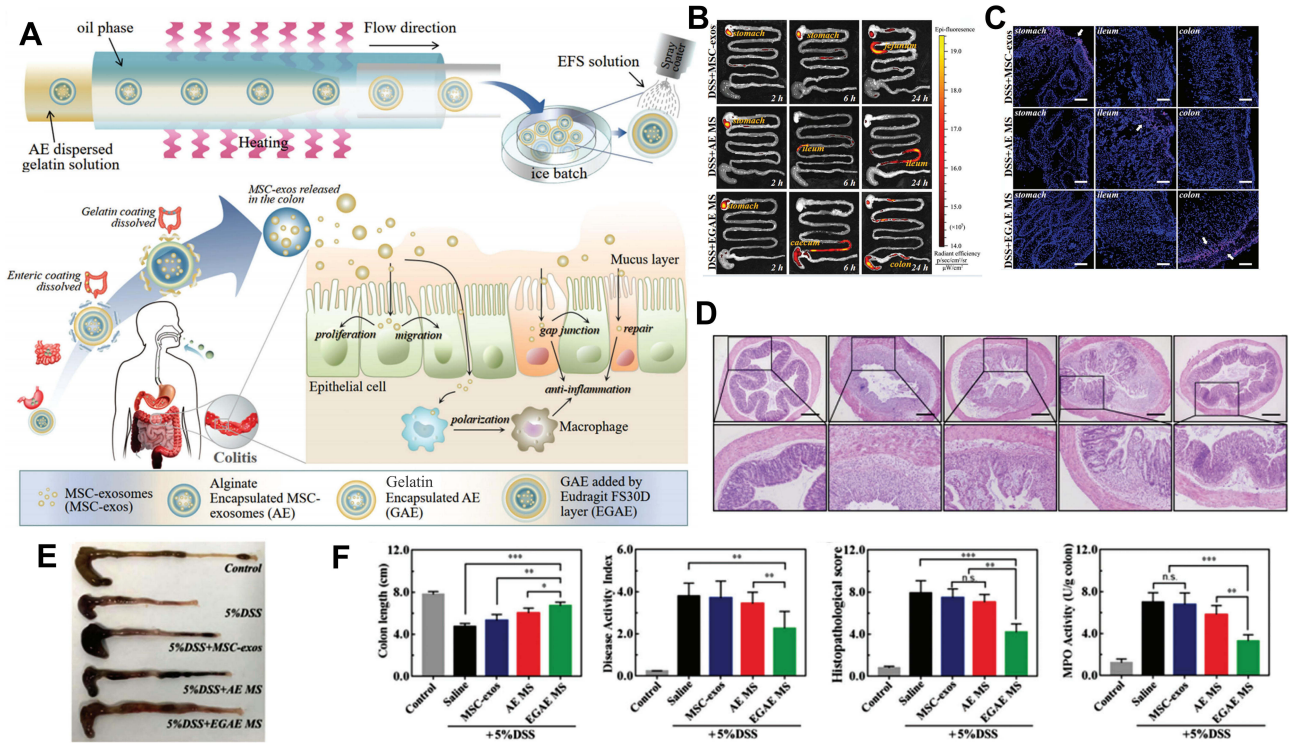

Figure 6 MSC-EVs was wrapped with sodium alginate (SA) hydrogel, gelatin, and enteric-coated-Eudragit FS30D (EFS) sequentially from the inside out to treat IBD. (**A**) Illustration of the fabrication process of the triple-wrapped MSC-EVs (EGAE) and the mechanism of treatment of IBD. (**B**) EV vivo biodistribution images of MSC-EVs over time (2.6.12h) after oral administration of MSC-EVs at a dose of 20 mg MSC-EVs/kg body weight in all three groups: the MSC-EVs treatment group, the MSC-EVs treatment group encapsulated in SA, and the EGAE microparticles treatment group. (**C**) Representative fluorescent images showing three digestive organs from each group at 24 hours. Scale bar =100 μm. (**D**) H&E images of the colon tissues. The groups are shown from left to right as: Control, DSS+Saline, DSS+MSC-exos, DSS+AE MS, and DSS +EGAE MS. Scale bar =100 μm. (**E**) Colon appearance. (**F**) Length of colon, DAI score, histopathological score of colon tissue and MPO activity in different treatment groups on day 8. *P < 0.05; **P < 0.01; ***P < 0.001. Reproduced with permission from reference.[123] Copyright 2022, Wiley.

Abbreviation: ns, no significance.

characterized, necessitating further mechanistic investigations. Critical priorities include optimization of therapeutic molecule integration and comprehensive evaluation of in vivo safety profiles for drug-loaded or encapsulated MSC-EV systems.

Targeted Modification of MSC-EVs

EVs derived from specific cells inherently target particular cell types, leading to differential distribution throughout the body. Target cells internalize MSC-EVs mainly via endocytosis or phagocytosis, while receptor–ligand interactions and direct membrane fusion also contribute to uptake.[126] Despite their natural advantages over conventional nanomedicines in terms of target cell uptake, the limited targeting capability of MSC-EVs remains a significant barrier to clinical application, requiring further modifications.[127] Five principal methods for modifying MSC-EV biofilms include membrane hybridization, post-insertion techniques, chemical methods, genetic engineering, and metabolic engineering.[128] Among these, genetic engineering is the most established, utilizing plasmid vectors or lentiviruses to fuse ligands with specific functions to transmembrane proteins, such as CD9, CD86, and GPI, on the EV surface.[129]

Streptavidin had been genetically engineered onto BMSC-EVs surfaces by Meng et al, enabling high-affinity binding to biotinylated molecules. This approach demonstrated that pH-sensitive fusion peptides and anti-IL12/IL23 surface-modified BMSC-EVs achieve targeted therapeutic delivery and inflammation relief in hypoxic tumor microenvironment and arthritic joints.[130] Furthermore, Golgi glycoprotein 1-functionalized EVs loaded with Wnt agonist 1 exhibit bone-targeted efficacy, accelerating fracture repair in colitis models (Figure 8).[43] Figure 8 highlights that this targeted strategy not only attenuated IBD-associated bone loss, but also redirected BMSC differentiation toward osteoblastogenesis rather than adipogenesis within the bone marrow niche. While modified MSC-EV-based targeted therapies are extensively investigated in oncology, their application in IBD remains understudied. Critical barriers to clinical translation persist,

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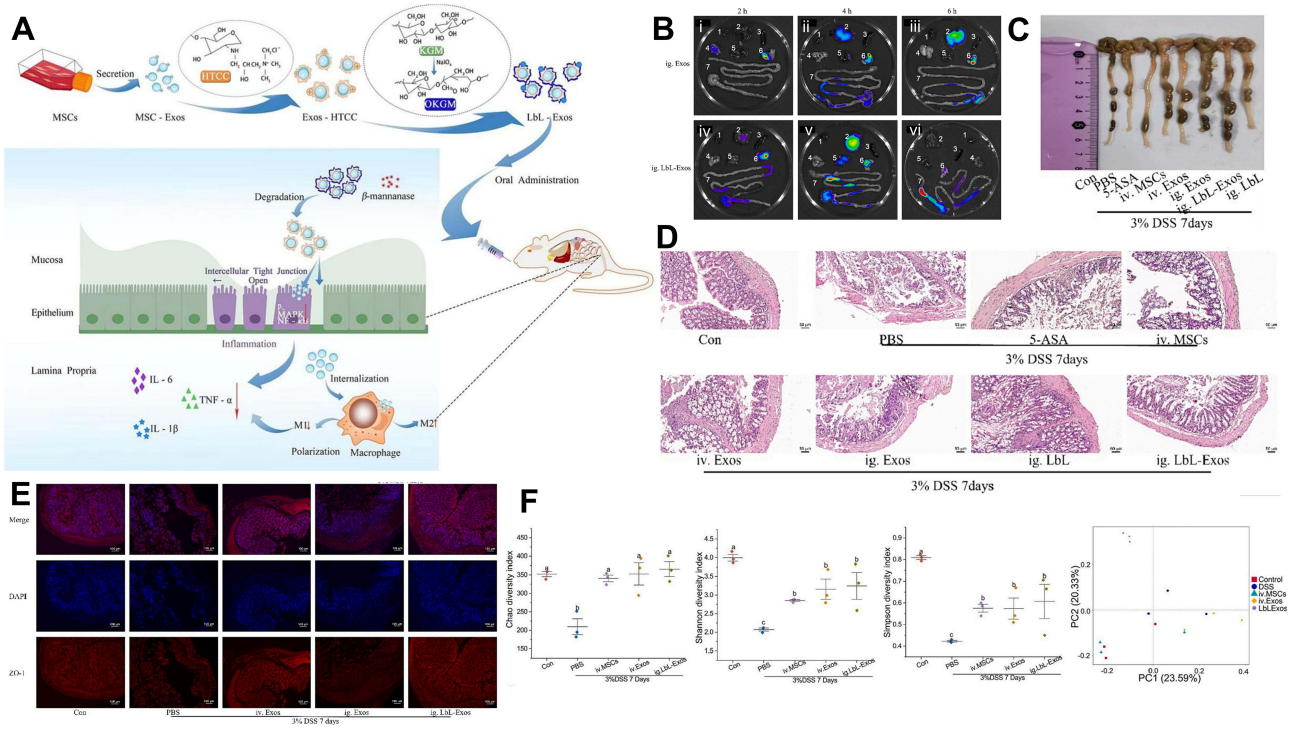


Figure 7 LbL(layer-by-layer)-encapsulated MSC-EVs for the treatment of IBD. (**A**) Schematic diagram demonstrating the preparation of LbL-MSC-EVs and the mechanism of treatment of IBD. Red downward arrows indicate decreased levels/biological effects. (**B**) Live animal imaging of DiR-labeled MSC-EVs in DSS-treated mice. Representative IVIS image of tissues from mice upon intragastric administration of (i–iii) Exos after 2 h (i), 4 h (ii), and 6 h (iii) or (iv–vi) LbL-Exos after 2 h (iv), 4 h (v), and 6 h (vi). 1: heart; 2: liver; 3: spleen; 4: lungs; 5: kidneys; 6: stomach; 7: intestine. IVIS: live animal imaging system; ig: intragastric (**C**) Representative colonic image from day 11. (**D**) H&E staining of the colon in different treatment groups. Scale bar: 50μm. (**E**) The expression of tight junction protein ZO-1 in colon tissues. ZO-1: red, DAPI: blue, scale bar: 100 μm. (**F**) LbL-EVs regulates the gut microbiota of mice with DSS-induced colitis. Alpha diversity was assessed by Chao, Shannon and Simpson indices. Structural changes in the gut microbiome as measured by PCoA-based beta diversity. Alphabetical indicators (a, b, c) denote statistical significance groups in the corresponding plots; groups sharing the same letter are not significantly different, whereas groups with different letters are significantly different ($P < 0.05$). Reproduced with permission from reference.[124] Copyright 2023, Elsevier.

including the identification of optimal MSC-EV sources for modification and the development of engineering strategies with maximal efficacy in IBD alleviation.

Diagnostic Applications

EVs present in saliva, plasma, and other body fluids serve as promising non-invasive indicators for the early diagnosis of IBD, offering convenience and minimal side effects. The saliva-derived exosomal proteasome alpha subunit type 7, associated with immune and inflammatory responses, is significantly overexpressed in IBD patients and may serve as a valuable diagnostic marker for disease progression.[131] During the active phase of IBD, dysregulation of RNA and protein expression is observed, including the upregulation of serum exosomal pregnancy zone protein,[132] miR-144-3p,[133] and lncRNA H19.[134] These molecules show potential as diagnostic and monitoring biomarkers. Meanwhile, miRNAs such as miR-21, miR-92, miR-223, and miR-375 have been identified as promising biomarkers for UC diagnosis, detection, and prognosis.[135] Cheung et al reported that MSC-secreted factors, particularly PGE2 and CCL2, closely correlate with clinical responses in CD patients. Peripheral blood mononuclear cells from these patients induce MSC apoptosis, enhancing PGE2 secretion, which can serve as a predictive biomarker for clinical responses. This finding supports the use of MSC-secreted products for patient stratification and efficacy assessment in MSC-based therapies.[50] Although trends in miRNA and protein expression have been observed, quantitative analysis remains limited. Standardization of assay protocols is essential to accurately define expression thresholds. EVs secreted by MSCs, particularly their miRNA cargo, hold strong potential as biomarkers for IBD diagnosis, monitoring, and prognosis. Collectively, patient-derived EVs/exosomal cargo from saliva and blood are being explored as promising non-invasive biomarkers for IBD diagnosis, monitoring, and prognosis. In contrast, MSC-derived products (including MSC-secreted

Table 5 Encapsulated MSC-EVs for the Treatment of IBD

Encapsulated Materials	Encapsulated Effects	Encapsulated Contents	Extracellular Vesicles Isolation Methods	Animal Models of IBD	Infusion Methods	Dose of Injections	Extracellular Vesicles Reactive Molecules	In Vivo Effects	Underline Mechanisms	References
Dopamine methacrylamide-modified hydrogel	Sustained release effect and effective wet adhesion property	IL-27 high expressive HucMSC-EVs	Ultracentrifugation	DSS-induced rat colitis model	Rectal administration	500 μL	IL-27	Reduced the inflammatory response, repaired the damaged barrier and regulated the differentiation of T cells	/	[122]
SA hydrogel, gelatin layer and Eudragit FS30D	Maintained the stability and bioactivity of MSC-EVs	HucMSC-EVs	Ultracentrifugation	DSS-induced mice colitis model	Oral administration	20 mg/kg	/	Reduced the proinflammatory cytokines levels and impaired colonic epithelial cells	/	[123]
HTCC and OKGM polysaccharides	Provided colon targeting and protected EVs from degradation	HP-MSC-EVs	Isolation kit	DSS-induced mice colitis model	Intragastric administration	200 μg	/	Anti-inflammatory and tissue repair effects	MAPK/NF-κB signaling pathway inhibition	[124]
Nanofiber-hydrogel composite microgel matrix	Extended local retention and sustained release of EVs	Rat AMSC-EVs	Ultracentrifugation	TNBS-induced rat CD model	Rectal administration	3×10^{11}	/	Promoted tissue regeneration and fistula healing	/	[121]
Poly (lactic-co-glycolic acid) polymer	Maintained EV stability, enabled oral delivery, and provided sustained release	HucMSC-EVs	Ultracentrifugation	TNBS-induced rat CD model	Oral administration	150 μL/20 g volume	/	Alleviated inflammation by reversing IBD-induced epithelial-mesenchymal transition	/	[125]

Note: MSC-EVs encapsulated by different materials for the treatment of IBD were summarized in Table 5, including Encapsulated Materials, Encapsulated Effects, Encapsulated Contents, Extracellular Vesicles Isolation Methods, Animal Models of IBD, Infusion Methods, Dose of Injections, Extracellular Vesicles Reactive Molecules, In Vivo Effects, Underline Mechanisms, and References. Abbreviations: HTCC:N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride; OKGM: oxidized konjac glucomannan; SA: sodium alginate. "/" indicates not reported/not applicable for that item.

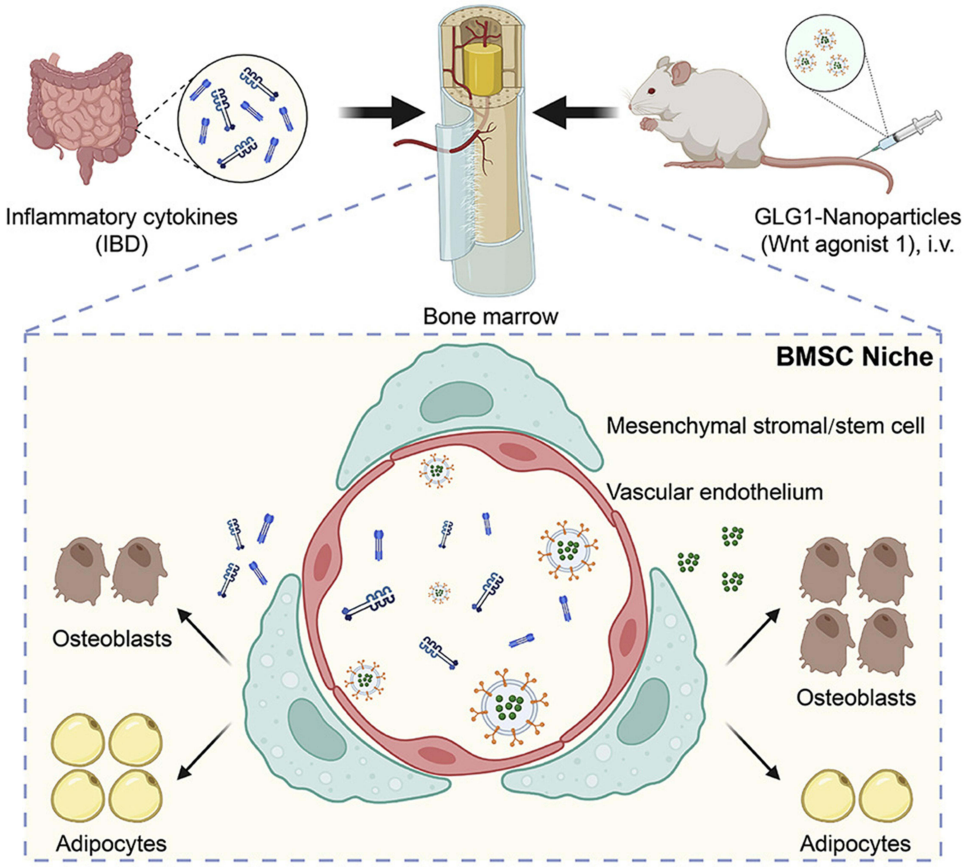


Figure 8 Extracellular vesicle-based targeted drug delivery attenuates bone loss and promotes bone formation in IBD by relocalizing BMSC differentiation to osteoblasts. Golgi glycoprotein 1 (GLG1)–functionalized EVs loaded with Wnt agonist 1 show bone-targeted efficacy in colitis models, promoting osteoblastogenesis over adipogenesis within the bone marrow BMSC niche and thereby accelerating fracture repair. Symbols and annotations: arrows indicate the direction of the proposed changes in cell fate/differentiation; dashed outlines indicate the magnified (zoomed-in) region of the bone marrow niche; bold labels identify the anatomical site and target microenvironment (eg., "Bone marrow" and "BMSC niche"). Reproduced with permission from reference.[43] Copyright 2023, Elsevier.

factors and MSC-EVs) may be more appropriately considered as therapy-associated or surrogate biomarkers to support patient stratification and response assessment in MSC/MSC-EV–based interventions, rather than primary diagnostic markers for IBD.

Clinical and Translational Potential of MSC-EVs for IBD

Current Clinical Evidence

Since the first successful use of MSCs to treat refractory rectovaginal fistulae in 2003,[136] multiple Phase I, II, and III clinical trials have confirmed the safety and efficacy of MSCs in CD. Notably, an AMSC-derived product (Alofisel/darvadstrocel, Takeda) has been approved in the EU and Japan for treating perianal fistula in CD patients.[137] These advances also provide an important clinical basis for the development of MSC-EV-based therapies.

At present, the clinical application of MSC-EVs in IBD remains limited, and the available evidence is mainly focused on refractory perianal fistulizing CD. Pak et al reported that local injection of MSC-EVs resulted in clinical improvement in 10 of 11 patients, with 5 achieving complete healing after 6 months of treatment.[138] Hadizadeh et al defined refractory perianal fistulas as those unresponsive to at least one course of anti-TNF-α therapy and enrolled 20 patients accordingly.[139] Each patient received 5 mL of hucMSC-EVs (0.5×10^{10} particles/mL, equivalent to 50 μg/mL) injected directly into the fistulas, followed by three treatment sessions at two-month intervals. Complete closure was achieved in 12 patients (60%), partial closure in 4, and no response in 4. Among the 43 total fistulas treated in this Phase II trial, 30 (69.7%) achieving full closure. Histopathological analysis revealed marked reductions in local inflammation and

enhanced tissue regeneration. In another study, Nazari et al classified refractory perianal fistulas as those unresponsive to anti-TNF-α therapy within six months. Five patients with refractory perianal CD fistulas received a single 5 mL dose of 50 μg/mL hucMSC-EVs. After 6 months, four patients showed clinical improvement, with three (60%) achieving complete healing, whereas one patient showed no response and continued to experience fistula discharge. Importantly, no local or systemic adverse events were reported. (Figure 9).[140] Figure 9 presents the overall workflow of MSC-EV preparation and administration in this Phase I trial, together with representative MRI images showing fistula resolution after treatment.

Taken together, these preliminary studies suggest that MSC-EVs may be feasible and well tolerated in the treatment of refractory perianal fistulizing CD, with encouraging signals of efficacy. However, the currently available evidence is still limited by small sample sizes, non-randomized or open-label designs, and relatively short follow-up periods. In addition, clinical evidence for luminal ulcerative colitis or luminal Crohn's disease remains absent. Current clinical studies and their key characteristics are summarized in Table 6.

Translational Opportunities and Remaining Barriers

MSC-EVs have considerable translational potential for IBD because they retain the immunomodulatory and reparative properties of MSCs while offering the advantages of a cell-free therapeutic platform. Compared with live MSCs, they are easier to store, transport, and engineer, making them attractive for repeated administration and for incorporation into targeted delivery systems.

The current focus of clinical studies on refractory perianal fistulizing Crohn's disease may also reflect a favorable translational setting. Perianal fistulas are localized lesions that permit direct injection of MSC-EVs into or around the tract, which may improve local retention and facilitate therapeutic assessment through fistula closure and tissue repair. This makes fistulizing disease an important early clinical entry point for MSC-EV-based therapies.

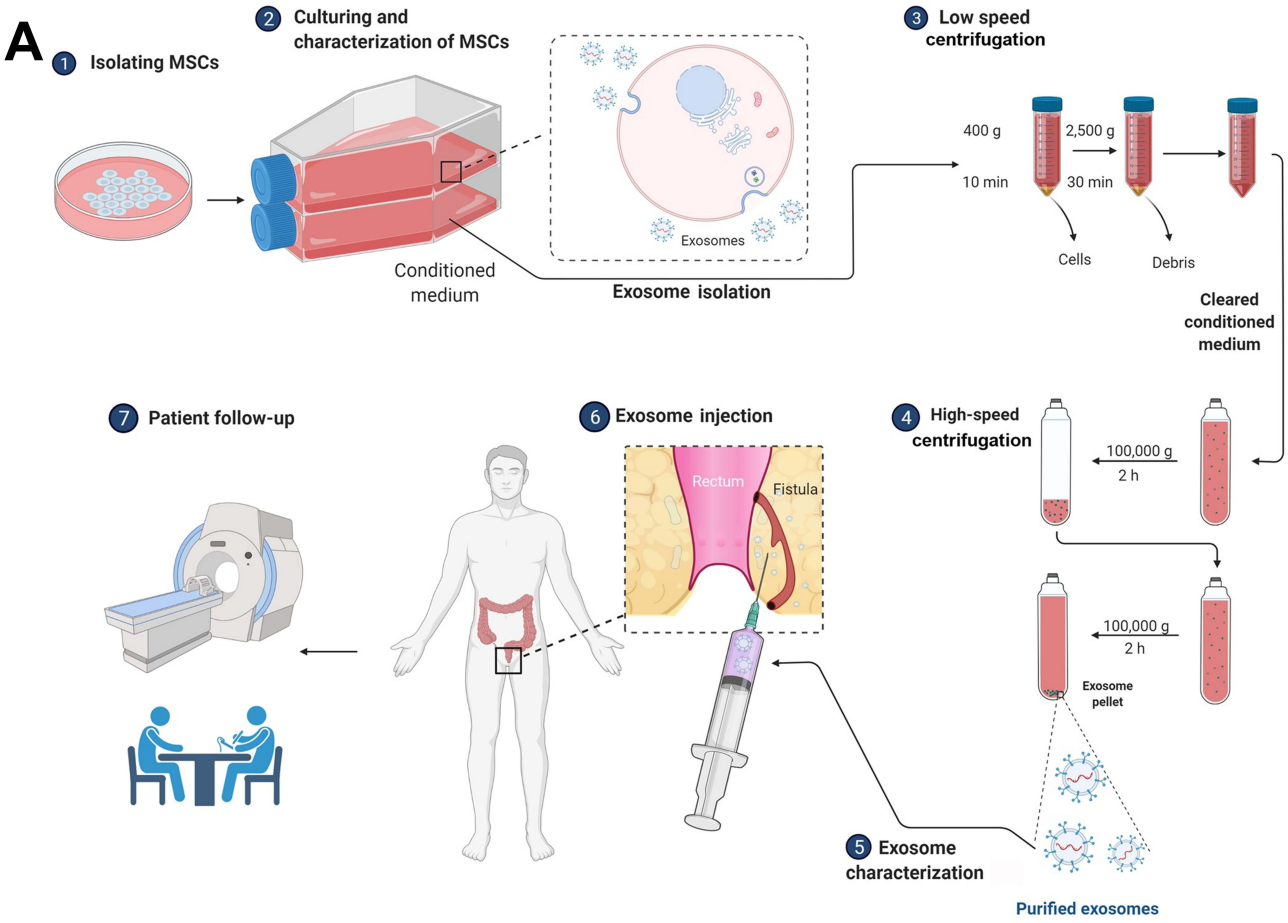
Another major translational opportunity lies in the adaptability of MSC-EVs as an engineering platform. Their therapeutic cargo can be enhanced through preconditioning or genetic modification of parent MSCs, and their delivery may be improved by biomaterial-assisted systems such as hydrogels, microcapsules, and colon-targeted carriers. These advances may expand the application of MSC-EVs from local fistula therapy to luminal ulcerative colitis and Crohn's disease in the future.

However, current clinical evidence remains preliminary and is still confined to a limited disease setting. Further studies are needed to determine whether the encouraging results observed in fistulizing disease can be extended to broader IBD phenotypes. Overall, MSC-EVs represent a promising translational strategy whose clinical value may be further strengthened by continued advances in engineering and targeted delivery.

Challenges in Developing MSC-EV-Based Therapies for IBD

Scalable and Standardized MSC-EV Production

Therapeutic efficacy typically requires approximately 10^{13} EVs per dose, while current methods yield only 10^{9}–10^{11} EVs per liter of CM.[141] Allogeneic manufacturing processes are therefore necessary to enable large-scale production and the development of ready-to-use products.[101] Production rates vary significantly depending on the MSC source; for instance, DPSCs proliferate faster than BMSCs and AMSCs, influencing overall yield and cost.[142] Thus, identifying an optimal cell source is critical for improving production efficiency. Enhancing MSC-EV yield involves both upstream and downstream processing. Upstream approaches include replacing traditional 2D culture with 3D systems,[143] employing hollow-fiber bioreactors or stirred tanks,[144] and using chemical stimuli[45,46] or mechanical forces to boost EV secretion.[145] Downstream efforts focus on improving EV isolation and purification from CM.The standardized collection of high-quality, uniform MSC-EVs preserves their intrinsic properties, minimizes contaminant-induced side effects, and facilitates clinical applications. However, current isolation and purification techniques lack consistency in ensuring EV content and quality, as variations in cell sources and culture conditions significantly affect their properties. Given the distinct advantages and limitations of each method, careful selection based on intended use is required. Therefore, the development of standardized protocols for MSC-EV production, purification, and isolation remains a critical priority.



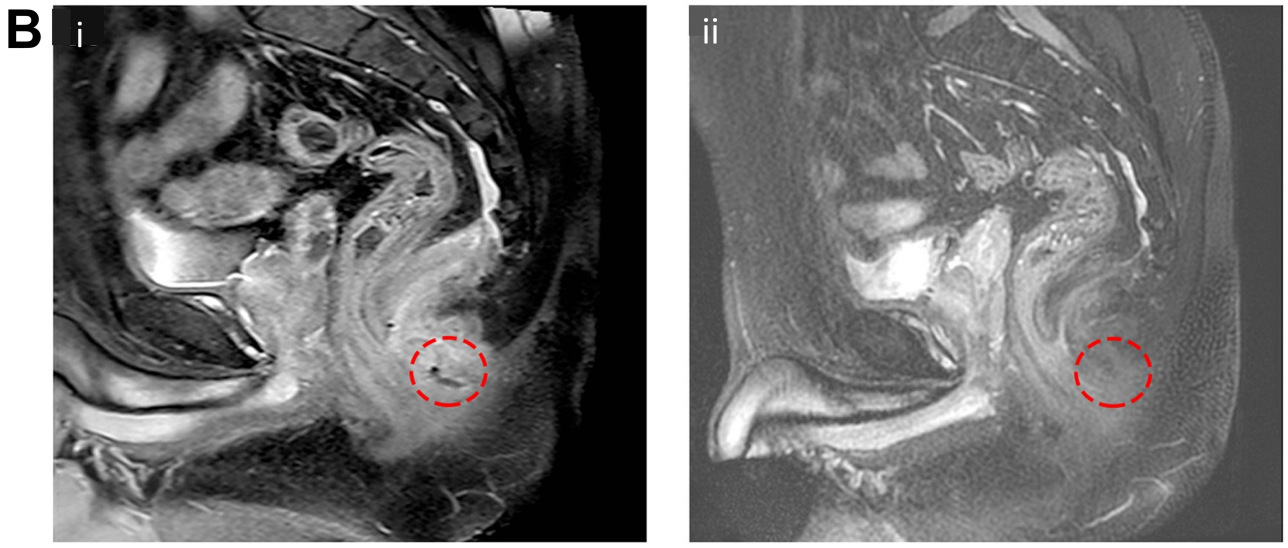


Figure 9 A clinical phase I trial of MSC-EVs for the treatment of patients with IBD. (**A**) Procedure of MSC-EVs extraction and clinical aspects of this study. Bold text within the schematic highlights the main procedural steps/stages of the trial workflow (eg, exosome isolation, characterization, injection, and patient follow-up) as labeled in the figure (**B**) MRI images of a patient with a fistula before (i) and after (ii) 6 months of MSC-EVs injection showing resolution of the fistula. The red dotted circle indicates the fistula/lesion region of interest on the MRI images. Reproduced with permission from reference.[140] Copyright 2022,Oxford University Press.

Table 6 Clinical Trials of MSC-EVs for the Treatment of Perianal Fistula

First Author, Year	Country	EV Source	Indication/ patient population	Administration Routes	Number of EVs Infused	Male/ female	Age	Follow-up Period	Study Design	Main Outcomes	Adverse Effects and Adverse Events	Translational Implications	References
Pak, 2023	Iran	Human placenta MSC-EVs	Complex perianal fistula (n = 11)	Injected along the fistula tract near the internal orifice	5 mL EVs containing 50μg/mL,total of three times	8/3	Average age was 43.27 years (35–56 years)	6 months	Phase I open-label clinical trial	Clinical improvement in 10/11 patients; complete healing in 5/11 after 6 months	No	Provides early proof-of-concept for local MSC-EV therapy in perianal fistula	[138]
Hadizadeh, 2024	Iran	Human umbilical cord MSC-EVs	Refractory perianal fistulizing CD (n = 20)	Injected into the adjacent tissue of the tract	5mL EVs 50 μg/mL total of three times	9/11	Mean age was 34.52 ± 11.02 years	6 months	Nonrandomized, nonblinded single-center phase II clinical trial	Complete closure in 12/20 patients; partial closure in 4/20; 30/43 fistulas achieved full closure; histological improvement observed	No	Supports repeated local hucMSC-EV administration as a feasible strategy for refractory fistulizing CD, although controlled validation is still needed	[139]
Nazari, 2022	Iran	Human umbilical cord MSC-EVs	Refractory perianal CD fistula (n = 5)	Injected surrounding the tract	5 mL and 50-μg/mL	3/2	Median age of 35 years (range 31–47 years)	6 months	Phase I open-label clinical trial	Clinical improvement in 4/5 patients; complete healing in 3/5 after 6 months	No	Suggests favorable short-term safety and efficacy of single-dose local hucMSC-EV therapy, but evidence remains preliminary due to the small sample size	[140]

Note: Clinical trials of MSC-EVs for the treatment of perianal fistula were summarized in Table 6, including First Author, Year, Country, EV Source, Indication / patient population, Administration Routes, Number of EVs Infused, Male/ female, Age, Follow-up Period, Study Design, Main Outcomes, Adverse Effects and Adverse Events, Translational implications and References.

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Enhancing the Therapeutic Potential of MSC-EVs

The culture environment modulates MSC behavior, influencing their exosomal cargo composition and release dynamics, which may enhance immunomodulatory and regenerative properties. Various stimuli—including pro-inflammatory factors,[46,67] hypoxia,[45] and genetic modifications[48,79,109]—have been employed to enhance MSC-EV function, often through upstream processing with chemical inducers to boost yield. Pre-treatment with inflammatory factors such as LPS, TNF-α, and IFN-γ alters MSC-EV protein, miRNA, and cytokine profiles, thereby enhancing their immunosuppressive capacity in colitis models.[46,67] Hypoxia, a hallmark of immune niches and the MSC native microenvironment, further enhances MSC-EV therapeutic potential by upregulating HIF-1α expression.[45] In addition, combining MSC-EVs with bioscaffolds and nanoscaffold materials holds promise for improving therapeutic outcomes in IBD treatment. However, these pre-treatments may induce unintended changes in MSC-EVs, necessitating rigorous evaluation of their efficacy and safety before clinical application.

Deeper Understanding of Treatment Mechanisms

Current experimental models—including DSS, trinitrobenzene sulfonic acid, oxazolone, and IL-10 knockout—only partially replicate IBD mechanisms due to their inherent complexity. Models for CD remain especially scarce, highlighting the need for more comprehensive systems to elucidate underlying etiological differences and enable more definitive conclusions. MSC-EVs have been shown to modulate immune responses, such as promoting $CD4^+$ T cell differentiation into Treg cells by inducing monocyte polarization toward the M2 phenotype. Meanwhile, interactions between gut microbiota and macrophages increases intestinal permeability.[146] Therefore, further investigation is needed to clarify the regulatory effects of MSC-EVs on immune cells, intestinal epithelial cells, and gut microbiota, as well as their interrelationships. Although several studies have reported that MSC-EV treatment is associated with shifts in gut microbial composition and metabolite profiles,[71,147] the direct causal mechanisms remain largely unclear. In particular, it is still unknown whether MSC-EVs regulate microbial ecology directly or indirectly through reshaping host immune responses, epithelial barrier integrity, and intestinal metabolic homeostasis. At the metabolomics level, analyses beyond transcriptomic and proteomic data may provide deeper insights. It is also paramount to recognize that EVs secreted by MSC in vivo may differ significantly from those generated in vitro, complicating cross-study comparisons. Furthermore, the concentrations, pharmacokinetics, pharmacodynamics, biodistribution, and systemic effects of MSC-EVs require further evaluation.

Determining the Appropriate Cell Source Types

Different MSC sources may influence EV production and physicochemical characteristics. Comparative analyses show that EVs derived from bone marrow-, umbilical cord-, and adipose-derived MSCs differ in yield and size distribution. BM- and UC-MSCs produced comparable maximum EV yields (~1×10^{10}particles/mL), whereas adipose-derived MSCs generated lower yields (~5×10^{9}particles/mL). Median particle sizes also varied, with UC-MSC-EVs averaging approximately 173nm, BM-MSC-EVs 158nm, and adipose-MSC-EVs 143nm.[148] Another comparative study of placenta-, endometrium-, and dental pulp-derived MSC-EVs further revealed substantial differences in morphology, surface protein expression, and biochemical composition, highlighting the heterogeneity of MSC-EVs across tissue sources and the importance of selecting appropriate MSC sources for therapeutic applications.[149]

Among the various MSC sources, hucMSC-EV, BMSC-EVs, and AMSC-EVs are the most extensively explored for IBD treatment. Among them, hucMSC-EVs receive growing attention due to their non-invasive acquisition, low immunogenicity, and high compatibility, which minimize the risk of immune rejection. These EVs also demonstrate potent anti-inflammatory and regenerative properties, superior scalability, and fewer ethical concerns, as they originate from postnatal tissue. Meanwhile, studies have shown that hucMSC-EVs not only treat IBD independently but also, when co-administered with hucMSCs via intraperitoneal injection, produce stronger therapeutic effects on UC than EVs alone.[150] Based on these advantages, while Clua-Ferré et al suggested that AMSC-EVs may represent a more suitable source for IBD therapy,[21] we propose that hucMSC-EVs possess superior translational potential and represent

a particularly promising candidate for clinical application. In contrast, EVs derived from sources such as PDLSC, DPSC, OE-MSC, and HF-MSC require further investigation due to limited evidence.

Translational Challenges of Engineered MSC-EVs

The complexity of MSC-EVs contents, limited targeting capability, and unclear therapeutic mechanisms contribute to the instability of their therapeutic efficacy, necessitating resolution through engineering strategies. MSC-EV engineering primarily involves indirect methods, such as lentiviral or plasmid transfection of parent cells, and direct methods like co-incubation, sonication, or electroporation. Engineered EVs function as vaccines, therapeutic agents, drug delivery vehicles, or biomarkers. While EVs and EV-like nanoparticles exhibit favorable bio-distribution and biocompatibility as oral drug delivery vectors, effectively targeting EV-encapsulated drugs to damaged gastrointestinal tissues remains challenging. Moreover, rapid clearance by macrophages restricts their systemic use. Ligand modification improves organ-specific accumulation, although total delivery remains suboptimal. Incorporation into biomaterials such as hydrogels extends MSC-EV stability, modulates release kinetics, and enhances tissue targeting, thereby improving therapeutic outcomes.

However, despite these promising advances, the clinical translation of engineered MSC-EVs still faces important challenges related to safety, cost, and manufacturing scalability. For example, genetic modification of parental MSCs may enhance EV cargo loading or targeting ability, but it may also introduce potential safety concerns, including unintended gene expression changes, off-target effects, and possible immunogenicity. In addition, the large-scale production of engineered EVs remains technically demanding and may increase manufacturing complexity and cost compared with native EVs.

Future research should prioritize the engineering of MSCs and their EVs, alongside the development of application-specific biomaterials, to optimize clinical efficacy. At the same time, more attention should be paid to standardized, scalable, and clinically compliant manufacturing strategies to facilitate the safe translation of engineered MSC-EVs into clinical applications.

Limited Clinical Trial Exploration

MSC-EVs exhibit negligible immunogenicity and their repeated application poses no toxicity concerns. However, their limited ability to penetrate fibrotic tissue reduces efficacy in treating fistulas surrounded by fibrosis, particularly at the tract and internal openings. Most research findings remain at the preclinical or early clinical stage, with existing human data still limited. In addition, most clinical trials exclude pediatric patients and rarely address complex or refractory fistulas, such as those with multiple openings, rectovaginal or abdominal fistulas, or cases involving prior surgical interventions beyond drainage. Current clinical studies also differ in administration route, dosage, and treatment frequency. Most trials have used local injection, but the rationale for route selection and the impact of different dosing strategies remain unclear. In addition, dosage has been reported using different units, such as particle number and protein concentration, making direct comparison across studies difficult. Comprehensive investigations into administration route, dosage, and potential adverse effects are required before clinical application. Furthermore, large-scale, long-term clinical trials are necessary to validate their efficacy. The high cost of pharmaceutical-grade MSC-EVs limits their feasibility as an affordable treatment. Moreover, the production of MSC-derived therapeutics must include final sterilization, purification, and virus removal. Successful clinical translation relies on interdisciplinary collaboration across biology, pharmacology, toxicology, clinical medicine, and the pharmaceutical manufacturing.

Conclusion

MSC-EVs represent a promising cell-free therapeutic strategy for IBD by regulating immune responses, suppressing inflammation, restoring epithelial barrier integrity, and promoting tissue repair. As discussed in this review, MSC-EVs from different sources exhibit distinct therapeutic advantages and can alleviate colitis through multiple mechanisms, including modulation of macrophage polarization, Th17/Treg balance, inflammasome activation, ferroptosis, fibrosis, and gut microbial homeostasis. Moreover, recent advances in engineered MSC-EVs and biomaterial-based delivery systems, such as hydrogels and colon-targeted encapsulation platforms, have further expanded their therapeutic potential.

Nevertheless, several challenges continue to hinder clinical translation, including the lack of scalable and standardized production methods, heterogeneity in EV quality and cargo composition, insufficient targeting efficiency, and limited knowledge regarding pharmacokinetics, biodistribution, and long-term safety. In addition, current preclinical models do not fully capture the complexity of human IBD, especially fistulizing and fibrotic Crohn's disease.

Future studies should focus on standardizing MSC-EV manufacturing and quality control, identifying the most suitable MSC sources, clarifying the mechanisms underlying EV-mediated immunoregulation, and optimizing engineering strategies for targeted and sustained delivery. The integration of MSC-EVs with gene engineering, preconditioning approaches, and intelligent biomaterials may promote the development of more precise, effective, and non-invasive therapies. In particular, future research may further explore combination strategies, such as the co-administration of MSC-EVs with anti-inflammatory agents, biologics, or biomaterial-based platforms, to enhance therapeutic efficacy and durability. At the same time, targeted engineering approaches, including surface modification, cargo loading, and ligand-directed delivery, may improve tissue specificity and therapeutic precision in inflamed intestinal lesions. With continued advances in these areas and validation in large-scale clinical trials, MSC-EVs are expected to become an important therapeutic platform for IBD and other immune-mediated inflammatory disorders.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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