

Mechanism, current status, and prospects of MSC and its extracellular vesicles in the treatment of IBD

Zhao-Chun Chi^{1*}

¹Department of Gastroenterology, Qingdao Municipal Hospital, Qingdao 266011, China

*Correspondence to: Zhao-Chun Chi, Department of Gastroenterology, Qingdao Municipal Hospital, No. 1 Jiaozhou Road, Qingdao 266011, Shandong Province, China. E-mail: czchow123@163.com.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Peer review information

Gastroenterology & Hepatology Research thanks all anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

IBD, inflammatory bowel disease; MSC, mesenchymal stem cell; MSC-EXOs, mesenchymal stem cell-derived exosomes; ISCs, intestinal stem cells.

Citation

Chi ZC. Mechanism, current status, and prospects of MSC and its extracellular vesicles in the treatment of IBD. *Gastroenterol Hepatol Res.* 2023;5(4):19. doi: 10.53388/ghr2023-03-083.

Executive editor: Na Liu.

Received: 10 November 2023; Accepted: 30 December 2023;

Available online: 30 December 2023 2023.

© 2023 By Author(s). Published by TMR Publishing Group Limited. This is an open access article under the CC-BY license. (<https://creativecommons.org/licenses/by/4.0/>)

Abstract

The etiology of inflammatory bowel disease (IBD) is multifaceted, involving genetic susceptibility, immune dysregulation, alterations in the gut microbiota, and environmental factors. Both intrinsic and extrinsic factors can disrupt the intestinal mucosal barrier, leading to chronic nonspecific inflammation, local structural changes, and gastrointestinal dysfunction. Historically, due to a lack of effective treatments, recurrent inflammation and microcirculatory disturbances could result in complications such as intestinal fistulas, strictures, obstructions, perforations, gastrointestinal bleeding, sepsis, etc., thereby increasing the risk of intestinal cell carcinoma and mortality. While the overall incidence of IBD remains at 0.5% in North America and Europe, its annual incidence is increasing in Asia, Africa, and South America, resulting in a growing number of patients and warning significant attention. Recent research has highlighted mesenchymal stem cell (MSC) therapy as an innovative treatment option for IBD due to its capacity to modulate inflammatory immune responses and promote tissue regeneration. A current preclinical study has shown a promising result, with systemic administration of MSCs in patients with reduced intestinal inflammation and no intestinal inflammation. In addition, in a new study, the use of mesenchymal stem cell-derived exosomes (MSC-EXOs) was successful, a type derived from mesenchymal stem cells, was successful, especially in patients with refractory anal fistula. Consequently, MSC therapy has become a preferred approach in IBD treatment, showcasing the potential application prospects for stem cell-based therapy in IBD. However, clinical research in this field still needs to refine strategies and further explore to lay a solid foundation.

Keywords: therapy, Inflammatory bowel disease, MSC-derived exosomes, mesenchymal stem cells, therapeutic mechanisms, mesenchymal stem cell-derived extracellular vesicles, prospects

Background

Mesenchymal stem cells (MSCs) are a heterogeneous group of cells that originate from the mesoderm during early embryonic development, including stem cells and their differentiated descendants from both early and subsequent stages of embryonic development. MSCs can be isolated from various tissues such as dental pulp, dermis, umbilical cord, bone marrow, adipose tissue, tendon and muscle. Due to their therapeutic potential, both MSCs and MSC-EVs have been extensively studied for their utility. MSCs are stem cells or somatic precursors. Mesenchymal stem cells (MSCs) are also one of the most extensively studied pluripotent stem cells [1]. Furthermore, their ability to differentiate into multiple lineages and modulate inflammation is a key defining feature of mesenchymal stem cells. Mesenchymal stem cells are believed to promote a balanced inflammatory and regenerative microenvironment in tissues under conditions of severe inflammation. However, the differentiation and implantation of MSCs at the site of injury are very brief. MSCs exert their effects not through cellular replacement but by influencing tissue repair through paracrine factors, among which Mesenchymal Stem Cell-Exosomes (MSC-Exos) have garnered attention due to their ability to carry complex proteins, nucleic acids, lipids, rich miRNA, and deliver these contents to the recipient cells [2–4]. MSC-Exos can reprogram the behavior of target cells. It regulates physiological and pathological processes by affecting the proliferation, survival, gene expression, and migration of recipient cells. MSC-Exos are easier to transport and store than MSCs. [5].

MSCs have strong anti-inflammatory properties and can treat inflammation in many diseases. Currently, the potential of MSCs in the treatment of various inflammatory diseases such as lung injury, inflammatory bowel disease, renal injury, rheumatoid arthritis (RA), and osteoarthritis (OA) is under investigation.. MSCs can create a regenerative microenvironment and balance the inflammatory state when tissues are damaged. MSCs can stimulate the homing, differentiation, immune regulation and other functions of the body when the body is in a very severe inflammatory state. In addition, increasing evidence suggests that MSC derived extracellular vesicles (MSC-EXOs) are important for the effects in the human body mainly by transporting their components to recipient cells [6].

Research indicates that bone marrow-derived MSCs were initially discovered in bone marrow mononuclear cells, and due to differences in protein expression profiles, MSCs from different sources exhibit slight variations in characteristics. They generally possess self-renewal ability, multilineage differentiation potential, and immunomodulatory capabilities, all meeting the minimum identification criteria set by the International Society for Cellular Therapy. The basic definition of mesenchymal stem cells includes the following features: (1) maintain plasticity in standard culture media; (2) express surface markers CD90, CD105 and CD73, but do not express surface markers CD14, CD45, CD34, CD79a, CD19, CD11b, or HLA-DR (CD refers to Cluster of Differentiation antigens, including various specific antigens located on the surface of T cells). (3) differentiation into adipocytes, osteoblasts, and chondrocytes under standard in vitro conditions [7–10].

MSCs can modulate multiple effector functions due to their strong immunosuppressive effects. It also interacts with cells of the adaptive immune system and innate immunity. In addition, understanding experimental studies in animal models can better grasp the molecular, cellular and immune mechanisms related to IBD in cell therapy. Therefore, some animal model studies have shown improvements in colitis after MSC infusion, including reduced disease activity, decreased inflammatory markers, and increased survival rates [7, 8].

Extracellular vesicles

Extracellular vesicles (EVs) derived from host cells, including membrane vesicles (MVs) and extracellular vesicles (EVs), also take part in communication within the intestinal microenvironment [11, 12]. Accumulating evidence suggests that vesicles are essential for

maintaining intestinal homeostasis. At the same time, vesicles are an important way of cell-biota and cell-to-cell communication [13].

Origin, composition, and function of extracellular vesicles

EVs are mainly fallen into three major types: microvesicles, exosomes, apoptotic bodies, each with characteristic tags. Exosomes are small extracellular vesicles (sEVs) ranging from 40 to 200 nm (or 30 to 150 nm) in size, secreted by most cells. Exosomes are uniform vesicle populations formed by budding of the multivesicular body membrane into the lumen. Microvesicles formed by budding of the cell membrane are EVs ranging from 200 to 1000 nm in size. Apoptotic bodies (500 to 2000 nm) are products of apoptotic cell disintegration [14]. Both exosomes and microvesicles serve as appropriate carriers for passing molecules which contains genetic information on recipient cells.

The interior of EVs has a special cell-derived component, including proteins, lipids, nucleic acids, and glycoconjugates. Proteins are a crucial component of EVs, diverse in type and structurally complex, primarily categorized into two classes: structural proteins (such as Alix, CD9, TSG101, CD63, Hsp90) and specific proteins (such as MHC-I, MHC-II, CD80, TGF- β , CD86, FasL) [15]. Additionally, EVs carry nucleic acids, approximately 200 bp in size, which play a significant role in EV function, including genomic DNA, RNA and mitochondrial DNA. EVs can transport extracellular nucleic acids to other cells and tissues. EVs contain a variety of RNA types, mostly including mRNA and small regulatory RNAs, with exosomes being particularly enriched in small non-coding RNAs. In comparison to RNA, there is increasing evidence suggesting the presence of various DNA types in EVs, including single-stranded and double-stranded DNA, mitochondrial DNA, genomic DNA, and even reverse-transcribed complementary DNA (cDNA). These DNA components are believed to be involved in the adjustment of inflammation. These DNA components are also considered biomarkers for conditions like cancer, viral infections, and chemotherapy resistance [11].

In the gastrointestinal tract, EVs are primarily secreted by immune cells and intestinal epithelial cells (IECs). Extensive research has indicated that EVs are significant contributors to communication between IECs, the microbiota, endothelial cells, and immune cells [16]. EVs play a crucial role in the regulation of vascular and epithelial barrier functions in the context of inflammation and wound healing in the intestines. They also serve as important modulators for immune cell recruitment. The molecular cargo within extracellular vesicles in the digestive tract depends on the parent cell and determines their functions. Derived from immune cells EVs contribute to immune system evasion, while those derived from IECs have been shown to be obvious regulators of IEC-induced immune tolerance. These functions are essential for enterovirus mediated immune response, intestinal barrier regulation, and intestinal microbiota formation in the context of IBD [17].

Biogenesis, composition, and therapeutic roles of exosomes

Exosomes are a subset of EVs, typically ranging in size from 40 to 200 nm (or 30 to 150 nm), and play a crucial role in cell-to-cell communication between organs and cells. Almost all types of cells secrete exosomes, which contain a variety of biomolecules, including RNA, DNA, and proteins [18, 19]. Exosomes are endosomal vesicles that limit early membrane budding production, which gradually mature into intracavitary vesicles (ILVs) and prevent their degradation in cytoplasmic lysosomes by passing to multivesicles (MVBs) through endobudding. The endosomal sorting complex required for transport (ESCRT), a complex comprising approximately 30 proteins organized into four complexes (ESCRT-0, -I, -II, and -III), controls the development of ILVs. Additionally, two important proteins, TSG101 (a commonly used marker protein for exosomes) and ALIX (an exosome-associated protein), are involved in exosome biogenesis [20].

Exosome secretion can also occur through ESCRT-independent mechanisms. Exosome release is influenced by various physiological

factors and cellular conditions, such as lipopolysaccharides, tumor necrosis factor- α , interferons, and hypoxia. Other factors secreted by injured organs, containing healing RNA and induce stem cells, protein-rich exosomes, promoting tissue homeostasis maintenance [21].

Circulating exosomes are taken up by recipient cells through three different mechanisms, including fusion, ligand-receptor uptake, and endocytosis. Exosomes are precisely targeted and internalized by ligand receptor-mediated uptake to transport bioactive substances to the cell. Exosomes are internalized into target cells by specific receptors, such as integrins, CD9 (a leukocyte differentiation antigen), lysosome associated membrane protein-3 (CD63), and CD81 (a member of the quadransmembrane protein superfamily). Cell membrane of exosomes with the cell membrane releases the substances produced by exosomes into the cytoplasm of the target cell [22].

Four-transmembrane proteins, antigen-presenting proteins, adhesion proteins, membrane transport proteins, fusion proteins are four classes of proteins that constitute exosomes and serve as biomarkers characteristic of exosomes. Adhesion proteins play a role in exosome maturation and binding to target cells. Major histocompatibility complex (MHC) class I and II, along with other antigen-presenting proteins, are involved in immune regulation, immunomodulation, and activation. Examples of membrane transport and fusion proteins involved in exosome synthesis, secretion, and downstream cell fusion include synaptosome-associated protein (SNAP), annexins, and Ran-binding protein 5b (Ran5b) [23].

The elements of MSC-EXOs signify workable pursuits for the diagnosis and cure of IBD. For example, an *in vivo* find out about the usage of a mouse IBD mannequin confirmed that therapy with human umbilical cord-derived mesenchymal stem cell exosomes (hUC-MSC-EXOs) decreased macrophage infiltration in colon tissue, suppressed the expression of IL-7-carrying miR-146a in MSC-EXOs, efficaciously downregulated the expression of IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated issue 6 (TRAF6) [24].

Mechanisms of MSC therapy in IBD

The therapeutic outcomes of MSCs principally show up via paracrine signaling, soluble elements (growth elements and cytokines), and extracellular vesicles (EVs). Therefore, the MSC secretome can be considered a novel therapeutic product [25]. It offers several advantages, including (1) easier handling in clinical practice; (2) easier assessment of safety, dosage, and efficacy; (3) storage without toxic cryopreservatives; and (4) cost-effectiveness due to scalability, storage, and suitability for ready-to-use applications [6].

Anti-Inflammatory effects

Mesenchymal stem cells regulate immune adaptive cells from a plethora of T-cell effectors to a regulatory microenvironment rich in regulatory T cells (Tregs) through the secretion of paracrine factors, including hepatocyte growth factor (HGF), transforming growth factor- β (TGF- β), nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2). IL-10 is another immune-regulatory cytokine generated by MSCs, contributing to intestinal homeostasis, and its deficiency exacerbates DSS-induced colitis, while supplementation of IL-10 may serve as an alternative therapy for IBD [6]. Through these soluble factors, mesenchymal stem cells exert anti-inflammatory outcomes on a variety of sorts of immune cells. In this regard, it has been suggested that MSCs downregulate Th1 and Th17 responses whilst upregulating.

Treg-mediated responses and Th2, contributing to the amelioration of colonic infection [26]. Additionally, MSCs specific NOD2 and bind the ligand muramyl dipeptide (MDP), bettering the secretion of anti-inflammatory elements such as PGE2 and IL-10, stimulating the proliferation of Tregs. Therefore, the anti-inflammatory motion of umbilical cord-derived mesenchymal stem cells (UC-MSCs) is stronger via the activation of the cyclooxygenase-2 (COX-2) signaling by way of NOD2, subsequently decreasing the severity of sickness in a murine

colitis model. Furthermore, co-administration of MIS416 (a particle that prompts NOD2 and TLR9 signaling) with UC-MSCs can beautify the therapeutic outcomes of MSCs [27]. Additionally, different particular mechanisms are related with the anti-inflammatory outcomes of MSC-derived EVs.

Regenerative impact

Mesenchymal stem cells secrete a variety of biomolecules, including cytokines, growth factors, and lipids, that influence tissue renewal, migration, immunomodulation, and epithelial regeneration [28]. Proper tissue regeneration requires neovascularization to provide oxygen, nutrients, and growth factors. This suggests that inducing angiogenesis is another major mechanism of MSC action in tissue regeneration. MSCs promote endothelial cell proliferation and migration by secreting molecules such as vascular endothelial growth factor (VEGF), angiopoietin-1 and -2 (ANG-1 and -2), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor- β 1 (TGF- β 1), TGF- α , monocyte chemotactic protein-1 (MCP-1), chemokine ligand 5 (CXCL5), matrix metalloproteinases (MMPs), and more [29]. Studies suggest that MSCs promote the survival and limit colonic tissue damage and regeneration of colonic epithelial cells, such as atrophy, inflammation, and developmental deficiencies, through growth factors, cytokines, and EVs [30].

MSCs therapy for IBD-associated intestinal fibrosis

Currently, there is very confined lookup on the feature and mechanisms of MSCs in intestinal fibrosis. However, the existing few studies largely demonstrate promising prospects for MSCs in the treatment of intestinal fibrosis, offering potential opportunities for prevention and treatment. MSCs adjust intestinal fibrosis by secreting EVs, boom factors, or using producing cytokines. MSCs inhibit the activation and proliferation of pro-fibrotic immune cells, such as mast cells, Th17 cells, M1 macrophages, and Th2 cells, and promote the technology of anti-fibrotic Tregs and M2 macrophages. Additionally, MSCs inhibit epithelial-mesenchymal transition (EMT) and the technology of myofibroblasts.

Both allogeneic and autologous mesenchymal stem mobile transplants are secure and consequently therapeutic choices for fibrotic diseases, fistulizing colitis (e.g., Crohn's disease), and refractory connective tissue diseases, as they are non-immunogenic [31]. Furthermore, systemic administration of MSCs for treating refractory radiation-induced colitis is effective and secure, relieving pain, diarrhea, inflammation, bleeding, and fistula formation whilst regulating an extend in lymphocyte subsets closer to Tregs and lowering activated effector T cells. One study showed that intravenous infusion and intravenous injection of MSCs significantly reduced fibrosis and scar tissue compared to the phosphate-buffered saline (PBS) treatment group after rat anal sphincter muscle injury [32].

EVs derived from MSCs impact the improvement of intestinal fibrosis via their proteins and RNA. One study by Yang and his colleagues indicated that exosomes containing miR-200b alleviated colon inflammation-related fibrosis by preventing epithelial-mesenchymal transition (EMT) [33]. An other recent experimental investigation on CD examined the effects of MSCs overexpressing telomerase and hypoxia-inducible factor 1- α (HIF-1 α) on pro-inflammatory stimulation-induced activated endothelial fibrosis and inflammation. The researchers discovered that EV-MSC-T-HIFC not only inhibited inflammation but also stopped TGF- β -treated fibroblasts from differentiating into myofibroblasts [34].

Fibrosis and the development of perianal fistulas are intimately associated. Mesenchymal stem cells appear to be an alternate treatment option for fistulas, according to studies. Patients suffering from fistulizing Crohn's disease (CD) may experience fewer fistulas when treated with allogeneic adipose-derived mesenchymal stem cells. Furthermore, it is safe to inject autologous MSCs produced from adipose tissue, which can heal 57% of patients with fistulas and lessen

secretion in the remaining patients [35]. Moreover, long-term clinical trials have demonstrated the safety and efficacy of both autologous and allogeneic adipose-derived mesenchymal stem cells in the treatment of fistulas, with encouraging outcomes [36, 37].

Reduction of Apoptosis

There has been an increase in interest in studies examining MSCs' anti-apoptotic pathways in different organ damage models [38]. Adipose tissue-derived mesenchymal stem cells (AT-MSCs) cause the anti-apoptotic markers B-cell lymphoma-2 (BCL2) and survivin, as well as the cell proliferation marker nuclear protein Ki67, to be upregulated, while apoptosis indicators like TUNEL staining, annexin V (a reagent for detecting cell apoptosis), caspase-3, and caspase-9 are downregulated [39]. Extracellular vesicles (EVs) containing miRNA-146a-5p are also present in bone marrow-derived mesenchymal stem cells (BM-MSCs). These EVs decrease inflammation and neuronal death by downregulating nuclear factor of activated T-cells 5 (NFAT5) and IL-1 receptor-associated kinase 1 (IRAK1). Mesenchymal stem cells that produce IL-37b have been suggested as a treatment for IBD because they can stop cell death and inflammation. Moreover, EVs generated by BM-MSCs prevent cell death in animal models by lowering the cleavage of caspase-9, caspase-8, and caspase-3 [40].

Antioxidant effects

One prevalent and significant pathogenic mechanism in illnesses is oxidative stress [41]. An imbalance between pro-oxidants, or free radicals, and antioxidants causes oxidative stress, which alters the structure of lipids, proteins, and DNA and causes pathological damage or injury to cells or tissues. The most researched free radicals are reactive oxygen species (ROS), which include superoxide anion (O_2^-), hydroxyl radicals ($\cdot OH$), and hydrogen peroxide (H_2O_2). Tissue homeostasis depends on the dynamic balance between ROS production and metabolism; when this balance is upset, oxidative stress and tissue damage result [42].

The constitutive expression of antioxidant enzymes like superoxide dismutase 1 (SOD1), SOD2, catalase (CAT), glutathione peroxidase (GPx), high levels of the antioxidant glutathione (GSH), and other proteins like heat shock protein 70 (HSP70) and sirtuins (SIRT) may be the reason for the resistance of MSCs to oxidative damage, as reported in several studies [43]. Mesenchymal stem cells have been shown to have anti-oxidative properties in both vitro and in vivo settings across a range of disease models, such as ischemia damage, aging, and gastrointestinal inflammation. Numerous methods have been found, such as the recovery of free radicals, the enhancement of the body's natural antioxidant defense, immunological modulation via the suppression of reactive oxygen species (ROS), and the repair of damaged cells' mitochondrial activity [39, 41]. Furthermore, MSCs can lower ROS and macrophage and monocyte myeloperoxidase (MPO) activity, which lowers the pro-inflammatory phenotype of these cells [44]. EVs generated by BM-MSCs lowered malondialdehyde (MDA) and MPO activity, enhanced antioxidant enzymes (SOD and GSH), and decreased oxidative disturbances in a rat colitis model [40].

These findings imply that mesenchymal stem cells induce immune regulation to prevent oxidative damage based on their antioxidant properties.

Immune system modulation

In the gut, MSCs control how each immune cell behaves. Mesenchymal stem cells encourage M1 macrophages to polarize toward the M2 phenotype by suppressing inflammation in them. MSCs downregulate CD80, CD86, and IL-12 in DCs, which prevents DCs from maturing and activating. Additionally, PGE2, IL-10, TGF- β , indoleamine 2,3-dioxygenase (IDO), and IL-6 can be secreted by MSCs to restrict the growth of T cells and NK cells, lower the production of inflammatory cytokines in Th1 and Th17 cells, and so reduce immunological responses and inflammation [45].

Tumor necrosis factor-alpha-stimulated gene 6 (TSG-6) and Prostaglandin E2 (PGE2) can be secreted by MSCs under inflammatory

activation [46]. It has been discovered that TSG-6 inhibits the migration of neutrophils, the signal transduction of immune cells residing in tissue, and the polarization of macrophages, approaching the M2 subtype. Additionally, PGE2 has the ability to attach to macrophage receptors, converting them from the M1 subtype to the M2 subtype and reducing inflammation [47].

Research indicates that MSCs have the ability to suppress DC maturation and activation by downregulating the expression of CD80, CD86, and IL-12 in DCs [48]. Moreover, MSCs have the ability to inhibit mature DC migration and reduce their antigen-presenting capacity. Furthermore, MSCs can cause DCs to differentiate into tolerogenic dendritic cells (TDCs), which effectively reduces inflammation by suppressing effector T cells and encouraging the activation of Treg cells.

Mucosal barrier repair

One important step in the therapy of IBD is the repair of the epithelial barrier. MSCs regulate T cell immunity and epithelial cell proliferation to both protect and promote the repair of the epithelial barrier [49]. Studies reveal that the primary mechanism by which mesenchymal stem cells restore the intestinal mucosal barrier is through the release of vesicles or cytokines. Initially, MSCs secrete specific molecules that control the development microenvironment of intestinal stem cells (ISCs) and encourage their differentiation into intestinal epithelial cells (IECs). Second, mesenchymal stem cells produce substances that lessen intestinal wall permeability and encourage the healing of injured IECs. Furthermore, it has been demonstrated that MSC-secreted vesicles inhibit the death of IECs in an IBD animal model by lowering the cleavage of caspase-3, caspase-8, and caspase-9. By secreting pro-angiogenic substances, MSCs can also encourage lymphatic endothelial cells' (LECs) migration, proliferation, and development of lymphatic vessels.

Although MSCs that have migrated to the intestinal mucosa have the ability to differentiate into IECs and myofibroblasts, this is not the main mechanism of healing and is not very common. The homing rate of IBD lesions is greatly increased by local injection of MSC preparations, exhibiting positive local effects and establishing the groundwork for the therapeutic use of local injection treatment for perianal fistulas in Crohn's disease (CD) [50, 51].

Enhancing the bacterial microbiota

MSCs release a variety of antimicrobial peptides, including IL-10, PGE2, IDO, and IL-17, all of which have been proven in several studies to have antimicrobial action [44].

Lactobacilli promote intestinal stem cell regeneration by activating the STAT3 signaling pathway and secreting IL, protecting the integrity of the intestinal mucosa [52]. Lactobacilli also modulate oxidative stress and immune responses, alleviating DSS-induced IBD. Furthermore, lactobacilli regulate the Th17/Treg cell balance in DSS-induced mouse colitis, which is widely recognized as crucial for maintaining normal intestinal homeostasis [53]. In the IBD model, MSC therapy dramatically boosts Firmicutes' abundance, which could be one of its therapeutic benefits. While the specific processes by which MSCs regulate these probiotic abundances are yet unknown, the control of probiotics—like Firmicutes and Lactobacillus—is essential to the healing of inflammatory bowel disease. This study raises the possibility that MSC-gut microbiota interactions might be important in the management of IBD. In IBD models caused by DSS, immune cells, TGF- β , gut microbiota, and metabolites are significant factors. The regeneration of gut microbiota, miRNA expression, and metabolite synthesis are all facilitated by MSC treatment. Thus, the makeup of the gut microbiota altered by MSC therapy may impact a patient's vulnerability to DSS-induced colitis [54].

Please note that this translation is quite lengthy, and if you have any specific questions or need further clarification on any section, feel free to ask.

Experimental treatment and clinical application of MSCs in IBD

Intestinal stem cells (ISCs)

Innovative methods have demonstrated the viability of Intestinal stem cells (ISCs), which are seen as a viable approach for stem cell treatment. Preclinical research has also shown preliminary validation for the effectiveness of ISCs in treating IBD. Advances in 3D cultivation techniques and the development of an in vitro ISC growth system have opened up new possibilities for individuals suffering from refractory gastrointestinal illnesses like IBD [55].

Recently, Watanabe and colleagues cultured "intestinal organoids" from human colon fragments and murine colon fragments in a 3D in vitro culture system. These organoids were then injected into the intestines of DSS-induced IBD model mice and sutured to the mice's anus. Fluorescence imaging confirmed that the transplanted intestinal organoids adhered to the damaged areas of the mouse intestines, retaining the intestinal epithelial structure. Subsequently, it was discovered that the attached intestinal organoids could expand within the mouse colonic mucosa and reconstruct new intestinal epithelial tissues. Finally, examination of mouse colonic specimens and tissue showed that the transplantation of intestinal organoids significantly reduced colonic inflammation and repaired intestinal damage [56].

iPSCs (Pluripotent stem cells induced)

Induced pluripotent stem cells, or iPSCs, have shown encouraging outcomes in preclinical research. In a mouse model of inflammatory bowel disease (IBD), it was discovered that induced mesenchymal stem cells (iMSCs) could stimulate intestinal angiogenesis and the proliferation of Lgr5+ ISCs and intestinal epithelial cells (IECs) just as well as adipose-derived mesenchymal stem cells (ASCs). In a different investigation, animals with DSS-induced colitis were given injections of iPSCs that were generated from mouse skin fibroblasts. Within a day of receiving iPSCs, mice's clinical symptom ratings significantly improved. Following a 72-hour period, the mice exhibited notably longer and heavier colon specimens, as well as lower levels of transmural inflammation, mucosal ulcers, and inflammatory cell infiltration in the colonic mucosa.

Spence and colleagues successfully generated definitive endoderm from human pluripotent stem cells by inducing them with Activin A, a TGF- β -related molecule. They then transferred the cells to a culture environment containing Wnt3a protein (Wnt3a) and fibroblast growth factor 4 (FGF4) to generate "intestinal organoids" with crypt-like structures, containing various types of IECs and enteroendocrine cells [45, 57].

Preclinical Research on mesenchymal stem cell therapy in bone marrow

Because of their immunomodulatory and anti-inflammatory qualities, bone marrow mesenchymal stem cells (BM-MSCs) are showing potential as an IBD therapeutic strategy. Furthermore, by increasing the level of circulating insulin-like growth factor 1 (IGF-1), BM-MSCs promote the proliferation of IECs and hence support the integrity and repair of the colonic epithelium [58].

Recent research suggests that intraperitoneal injection of MSCs (2×10^6) can reduce the development of colitis and decrease serum levels of IL12-, IL-6, and IL-1 β in a colitis model [59]. This anti-inflammatory effect may be attributed to Gal-3 secreted by MSCs, which inhibits the inflammatory phenotype of DCs in colonic tissue. Systemic injection of adipose tissue-derived MSCs (AT-MSCs) (1×10^6) inhibits DSS-induced colitis by improving Tregs frequency and promoting M2 polarization [60].

Transplanting human umbilical cord-derived MSCs (UC-MSCs) reduced experimental colitis in a mouse model of TNBS-induced colitis by stimulating CD5+ B cells and interleukin-10 (IL-10)-secreting CD5+ regulatory B cells (Bregs) [79]. In the colonic tissue of treated mice, human UC-MSC therapy also increased Tregs and decreased Th1/Th17 cell numbers [61]. Treatment reduced symptoms, enhanced macroscopic and histological scores, and raised the overall survival rate [62].

Subsequent investigation revealed that mesenchymal stem cells may release thrombospondin-1 (THBS1), which may stimulate IL-10 +

regulatory B cells (Bregs), hence regulating the course and incidence of colitis.

Presently, systemic infusion of UC-MSCs resulted in the activation of endoplasmic reticulum (ER) stress-related proteins within 6 hours in a mouse model of colitis [63]. Sustaining intestinal homeostasis and facilitating protein folding, modification, and secretion brought on by MSC treatment depend on adequate ER activity.

Clinical treatment studies with MSCs

Autologous. In a recent experiment, Lightner and colleagues showed that colonic infusion of autologous adipose tissue-derived MSCs (35×10^6 cells per patient) caused no serious adverse effects in 5 patients with refractory Crohn's fistulas. In a 6-week follow-up, three of the patients completely stopped drainage [64]. Another phase I trial involving 12 CD patients (NCT03803917) showed that autologous AT-MSCs administered intracolonicly were safe, with 57% of patients achieving complete fistula closure. The most common side effect was persistent stomach discomfort for many days following surgery. In addition, one patient suffered urine retention, one had mild bleeding during liposuction, and two patients had tiny abscesses [35].

Similarly, intracolonic administration of autologous adipose tissue-derived MSCs ($1 - 2 \times 10^7$ cells per patient) was demonstrated to be safe and viable in a phase I study including CD patients [65]. In a different trial, 10 patients with refractory Crohn's fistulas received an intravenous injection of autologous BM-MSCs ($1 - 2 \times 10^6$ cells/kg) without experiencing any serious side effects. Within six weeks of starting treatment, three patients had their condition Activity Index (DAI) lowered by intervention; nevertheless, three more patients needed surgery as a result of their condition progressing [66]. Additionally, Dhere et al. documented the viability and safety of administering autologous MSCs generated from bone marrow via systemic injection (1×10^7 cells/kg) to 12 patients with CD [67].

Allogeneic. In a study by Zhang and colleagues, systemic injection of 1×10^6 UC-MSCs/kg decreased corticosteroid dosages, disease activity indices (DAI), and Harvey-Bradshaw indices (HBI) in 82 patients (41 in the intervention group and 41 in the control group) without causing serious adverse effects [68]. Perianal fistula repair was enhanced by local allogeneic 3×10^7 MSC injection per patient. Furthermore, allogeneic BM-MSCs ($3 - 9 \times 10^7$) injected colonically decreased the size of fistula tracts in CD patients four years later without causing any long-term negative consequences [69]. In a phase I/IIa clinical trial, local administration of allogeneic adipose tissue-derived MSCs (20×10^6 cells per patient) resulted in complete closure of all existing fistula tracts in 30% of patients, reduced fistula drainage counts in 69.2% of patients, and achieved complete fistula healing in 56.3% of patients. The patients included 24 CD patients. The outcomes show that using allogeneic AT-MSCs to treat CD patients is safe, feasible, and successful [70].

Prospects and future directions

MSCs have been investigated as a new treatment for inflammatory bowel disease (IBD) because of their immunomodulatory and anti-inflammatory qualities. Mesenchymal stem/stromal cells (MSCs) have been linked to improved tissue healing through potent anti-inflammatory and immunomodulatory activities, according to a growing body of research [71, 72]. In numerous animal studies of IBD and some clinical trials involving IBD patients, MSCs have demonstrated satisfactory therapeutic effects [73]. The therapeutic properties of MSCs may be replicated by removing MSC-derived exosomes (MSC-Exos) from MSC growing media, which increases the viability and safety of clinical MSC applications [74].

There are still a number of obstacles in the way of MSC-Exos' clinical use in IBD. First of all, MSC-Exo preparations vary widely [75]. The biological properties of MSC-Exos can be influenced by the MSCs' source and the culture environment, leading to variations in their composition and biological roles [76]. Technically speaking, the extraction of MSC-Exos lacks very sensitive and specific molecular

markers; instead, it depends mostly on size and density for separation and purification. As various subtypes of small extracellular vesicles (sEVs) or biologically similar-sized macromolecules are mixed together, it is hard to acquire comparatively pure MSC-Exo the preparations [77]. The variety of extraction approaches leads to differences in the purity of MSC-Exo preparations, affecting the rigor of research and the similarities and reliability of studies. The recognition methods for MSC-Exo preparations are varied and not yet mature. Therefore, errors yet exist in deciding the purity and structure of MSC-Exo preparations [78]. Both the physical properties and production techniques of MSC-Exos need to be standardized, compared, optimized, improved, and unified for better application in clinical practice.

Second, it might be difficult to gauge MSC-Exos' efficacy. Evaluating MSC-Exos' activity and potency is more difficult than determining their purity and composition [77]. Predicting the therapeutic benefits of MSC-Exos on IBD and quantifying the activity and composition of effector molecules inside them are challenging problems. Further investigation is still needed to fully understand the chemical makeup of MSC-Exos in IBD, their effector molecules, and their precise mechanisms of action. As of right now, the most pertinent expected biological activities of MSC-Exos can only be used to evaluate the therapeutic efficacy of these preparations; this may not accurately reflect the molecular processes behind MSC-Exo preparations' actions in IBD [78]. Moreover, opinions about the best way and amount to administer MSC-Exo to patients with IBD are divided [79].

Achieving safe, effective, and standardized stem cell therapy for IBD still requires significant progress. First and foremost, it's critical to achieve homogeneity in stem cell preparations. Significant variation arises from the effect of culturing conditions and tissue sources on stem cells. It is difficult to establish uniformity when screening IBD patients since there are currently no consistent criteria and controlled comparisons for the tissue sources and growth conditions of stem cells. Because of this variability, clinical research on IBD is not as consistent as it might be. Individual variances in study outcomes can be substantial, which makes it difficult to share and use the data and ultimately restricts clinical translation.

Second, one must think about the possible risks associated with stem cell treatment. Stem cells are much more sophisticated than chemical medications or biologics, and little is known about their structure and function at this time. The complete scope of stem cell activity in vivo is still largely understood, and observations of stem cell function in vivo are now restricted to certain illnesses. Clinical translation is restricted by these possible therapeutic hazards.

Thirdly, stem cell treatment has to be standardized. There are currently no established guidelines for the administration of stem cells, including doses, timing, and methods; also, there are no established indications or contraindications.

At this time, the majority of stem cell research in IBD is conducted in the preclinical phase, with little clinical use in the treatment of refractory IBD and isolated fistulas. On the other hand, stem cells are anticipated to develop into a standard "weapon" in clinical treatment as methods for using them advance, providing encouraging opportunities for the management of IBD patients.

References

- Serafini MA, Sirena DH, da Silveira ABT, et al. Mesenchymal stromal cell-derived membrane particles: A novel cell-free therapy for inflammatory bowel diseases. *Int Immunopharmacol* 2023;118:110076. Available at: <http://doi.org/10.1016/j.intimp.2023.110076>
- Liu H, Li R, Liu T, Yang L, Yin G, Xie Q. Immunomodulatory Effects of Mesenchymal Stem Cells and Mesenchymal Stem Cell-Derived Extracellular Vesicles in Rheumatoid Arthritis. *Front Immunol* 2020;11. Available at: <http://doi.org/10.3389/fimmu.2020.01912>
- de Vos JD, Hillberg O, Perch M, Jensen JU, Wilcke JT, Alpha-1-antitrypsin deficiency. *Ugeskr Laeger*. 2021;183:V02210150.
- Cai X, Zhang Z, Yuan J, et al. hucMSC-derived exosomes attenuate colitis by regulating macrophage pyroptosis via the miR-378a-5p/NLRP3 axis. *Stem Cell Res Ther* 2021;12(1). Available at: <http://doi.org/10.1186/s13287-021-02492-6>
- Wang S, Lei B, Zhang E, et al. Targeted Therapy for Inflammatory Diseases with Mesenchymal Stem Cells and Their Derived Exosomes: From Basic to Clinics. *Int J Nanomedicine* 2022;17:1757–81. Available at: <http://doi.org/10.2147/IJN.S355366>
- Eiro N, Fraile M, González-Jubete A, González LO, Vizoso FJ. Mesenchymal (Stem) Stromal Cells Based as New Therapeutic Alternative in Inflammatory Bowel Disease: Basic Mechanisms, Experimental and Clinical Evidence, and Challenges. *Int J Mol Sci* 2022;23(16):8905. Available at: <http://doi.org/10.3390/ijms23168905>
- Shi Y, Wang Y, Li Q, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. *Nat Rev Nephrol* 2018;14(8):493–507. Available at: <http://doi.org/10.1038/s41581-018-0023-5>
- Zhang W, Wang Y, Kong Y. Exosomes Derived From Mesenchymal Stem Cells Modulate miR-126 to Ameliorate Hyperglycemia-Induced Retinal Inflammation Via Targeting HMGB1. *Invest Ophthalmol Vis Sci* 2019;60(1):294. Available at: <http://doi.org/10.1167/iovs.18-25617>
- Wu P, Zhang B, Shi H, Qian H, Xu W. MSC-exosome: A novel cell-free therapy for cutaneous regeneration. *Cytotherapy* 2018;20(3):291–301. Available at: <http://doi.org/10.1016/j.jcyt.2017.11.002>
- Brown C, McKee C, Bakshi S, et al. Mesenchymal stem cells: Cell therapy and regeneration potential. *J Tissue Eng Regen Med* 2019;13(9):1738–55. Available at: <http://doi.org/10.1002/term.2914>
- Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem* 2019;88(1):487–514. Available at: <http://doi.org/10.1146/annurev-biochem-013118-111902>
- Toyofuku M, Nomura N, Eberl L. Types and origins of bacterial membrane vesicles. *Nat Rev Microbiol* 2018;17(1):13–24. Available at: <http://doi.org/10.1038/s41579-018-0112-2>
- Chu H, Khosravi A, Kusumawardhani IP, et al. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Science* 2016;352(6289):1116–20. Available at: <http://doi.org/10.1126/science.aad9948>
- Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. Review of the Isolation, Characterization, Biological Function, and Multifarious Therapeutic Approaches of Exosomes. *Cells* 2019;8(4):307. Available at: <http://doi.org/10.3390/cells8040307>
- He C, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. *Theranostics* 2018;8(1):237–255. Available at: <http://doi.org/10.7150/thno.21945>
- Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol* 2014;14(3):195–208. Available at: <http://doi.org/10.1038/nri3622>
- Shen Q, Huang Z, Yao J, Jin Y. Extracellular vesicles-mediated interaction within intestinal microenvironment in inflammatory bowel disease. *J Adv Res* 2022;37:221–33. Available at: <http://doi.org/10.1016/j.jare.2021.07.002>
- Taheri B, Soleimani M, Fekri Aval S, Esmaili E, Bazi Z, Zarghami N. Induced pluripotent stem cell-derived extracellular vesicles: A novel approach for cell-free regenerative medicine. *J Cell Physiol* 2018;234(6):8455–64. Available at: <http://doi.org/10.1002/jcp.27775>
- Gao X, Ran N, Dong X, et al. Anchor peptide captures, targets,

- and loads exosomes of diverse origins for diagnostics and therapy. *Sci Transl Med* 2018;10(444). Available at: <http://doi.org/10.1126/scitranslmed.aat0195>
20. Yuan YG, Wang JL, Zhang YX, Li L, Reza AMMT, Gurunathan S. Biogenesis, Composition and Potential Therapeutic Applications of Mesenchymal Stem Cells Derived Exosomes in Various Diseases. *Int J Nanomedicine* 2023;Volume18:3177–3210. Available at: <http://doi.org/10.2147/IJN.S407029>
 21. van der Pol E, Böing AN, Harrison P, Sturk A, Nieuwland R. Classification, Functions, and Clinical Relevance of Extracellular Vesicles. Mattson MP, editor. *Pharmacol Rev* 2012;64 (3):676-705. Available at: <http://doi.org/10.1124/pr.112.005983>
 22. Mulcahy LA, Pink RC, Carter DRF. Routes and mechanisms of extracellular vesicle uptake. *J Extracell Vesicles* 2014;3(1). Available at: <http://doi.org/10.3402/jev.v3.24641>
 23. Jeske R, Bejoy J, Marzano M, Li Y. Human Pluripotent Stem Cell-Derived Extracellular Vesicles: Characteristics and Applications. *Tissue Eng Part B Rev* 2020;26(2):129–44. Available at: <http://doi.org/10.1089/ten.teb.2019.0252>
 24. Wu H, Fan H, Shou Z, et al. Extracellular vesicles containing miR-146a attenuate experimental colitis by targeting TRAF6 and IRAK1. *Int Immunopharmacol* 2019;68:204–12. Available at: <http://doi.org/10.1016/j.intimp.2018.12.043>
 25. Teixeira FG, Salgado AJ. Mesenchymal stem cells secretome: current trends and future challenges. *Neural Regen Res* 2020;15(1):75. Available at: <http://doi.org/10.4103/1673-5374.264455>
 26. Alves VBF, de Sousa BC, Fonseca MTC, et al. A single administration of human adipose tissue-derived mesenchymal stromal cells (MSC) induces durable and sustained long-term regulation of inflammatory response in experimental colitis. *Clin Exp Immunol* 2019;196(2):139–54. Available at: <http://doi.org/10.1111/cei.13262>
 27. Lee BC, Shin N, Lee JY, et al. MIS416 Enhances Therapeutic Functions of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells Against Experimental Colitis by Modulating Systemic Immune Milieu. *Front Immunol* 2018;9. Available at: <http://doi.org/10.3389/fimmu.2018.01078>
 28. Fernández-Francos S, Eiro N, Costa LA, Escudero-Cernuda S, Fernández-Sánchez ML, Vizoso FJ. Mesenchymal Stem Cells as a Cornerstone in a Galaxy of Intercellular Signals: Basis for a New Era of Medicine. *Int J Mol Sci* 2021;22(7):3576. Available at: <http://doi.org/10.3390/ijms22073576>
 29. Ahangar P, Mills SJ, Cowin AJ. Mesenchymal Stem Cell Secretome as an Emerging Cell-Free Alternative for Improving Wound Repair. *Int J Mol Sci* 2020;21(19):7038. Available at: <http://doi.org/10.3390/ijms21197038>
 30. Sendon-Lago J, Rio LG, Eiro N, et al. Tailored Hydrogels as Delivery Platforms for Conditioned Medium from Mesenchymal Stem Cells in a Model of Acute Colitis in Mice. *Pharmaceutics* 2021;13(8):1127. Available at: <http://doi.org/10.3390/pharmaceutics13081127>
 31. Hertegard S, Nagubothu SR, Malmstrom E, LeBlanc K. Treatment of Vocal Fold Scarring With Autologous Bone Marrow-Derived Human Mesenchymal Stromal Cells-First Phase I/II Human. *Stem Cell Res Ther* 2020;11(1):128. <http://doi.org/10.1186/s13287-020-01632-8>.
 32. Salcedo L, Penn M, Damaser M, Balog B, Zutshi M. Functional Outcome After Anal Sphincter Injury and Treatment With Mesenchymal Stem Cells. *Stem Cells Transl Med* 2014;3(6):760–67. Available at: <http://doi.org/10.5966/sctm.2013-0157><http://doi.org/10.1186/s13287-020-01762-z>
 33. Chen B, Han Z, Zhou Q, Liu H, Xu P, Zhi F. Efficacy of infliximab in treatment-naïve patients with stricturing small bowel Crohn's disease. *Scand J Gastroenterol* 2021;56(7):812–19. Available at: Li Z, Dong X, Tian M, et al. Stem cell-based therapies for ischemic stroke: a systematic review and meta-analysis of clinical trials. *Stem Cell Res Ther* 2020;11(1). Available at: <http://doi.org/10.1080/00365521.2021.1922748>
 34. Gómez-Ferrer M, Amaro-Prellezo E, Dorronsoro A, et al. HIF-Overexpression and Pro-Inflammatory Priming in Human Mesenchymal Stromal Cells Improves the Healing Properties of Extracellular Vesicles in Experimental Crohn's Disease. *Int J Mol Sci* 2021;22(20):11269. Available at: <http://doi.org/10.3390/ijms222011269>
 35. Dige A, Hougaard HT, Agnholt J, et al. Efficacy of Injection of Freshly Collected Autologous Adipose Tissue Into Perianal Fistulas in Patients With Crohn's Disease. *Gastroenterology* 2019;156 (8):2208-2216.e1. Available at: <http://doi.org/10.1053/j.gastro.2019.02.005>
 36. Serrero M, Grimaud F, Philandrianos C, Visée C, Sabatier F, Grimaud JC. Long-term Safety and Efficacy of Local Microinjection Combining Autologous Microfat and Adipose-Derived Stromal Vascular Fraction for the Treatment of Refractory Perianal Fistula in Crohn's Disease. *Gastroenterology* 2019;156 (8):2335-2337.e2. Available at: <http://doi.org/10.1053/j.gastro.2019.01.032>
 37. Barnhoorn MC, Wasser MNJM, Roelofs H, et al. Long-term Evaluation of Allogeneic Bone Marrow-derived Mesenchymal Stromal Cell Therapy for Crohn's Disease Perianal Fistulas. *J Crohns Colitis* 2019;14(1):64–70. Available at: <http://doi.org/10.1093/ecco-jcc/jjz116>
 38. Duan S, Wang F, Cao J, Wang C. & Exosomes Derived from MicroRNA-146a-5p-Enriched Bone Marrow Mesenchymal Stem Cells Alleviate Intracerebral Hemorrhage by Inhibiting Neuronal Apoptosis and Microglial M1 Polarization. *Drug Des Dev Ther* 2020;14:3143-58. Available at: <http://doi.org/10.2147/DDDT.S255828>
 39. Qian C, Meng Q, Lu J, Zhang L, Li H, Huang B. Human amnion mesenchymal stem cells restore spermatogenesis in mice with busulfan-induced testis toxicity by inhibiting apoptosis and oxidative stress. *Stem Cell Res Ther* 2020;11(1). Available at: <http://doi.org/10.1186/s13287-020-01803-7>
 40. Yang J, Liu XX, Fan H, et al. Extracellular Vesicles Derived from Bone Marrow Mesenchymal Stem Cells Protect against Experimental Colitis via Attenuating Colon Inflammation, Oxidative Stress and Apoptosis. Camussi G, editor. *PLoS ONE* 2015;10(10):e0140551. Available at: <http://doi.org/10.1371/journal.pone.0140551>
 41. Stavely R, Nurgali K. The emerging antioxidant paradigm of mesenchymal stem cell therapy. *Stem Cells Transl Med*. 2020;9:985-1006. <http://doi.org/10.1002/sctm.19-0446>
 42. Tian T, Wang Z, Zhang J. Pathomechanisms of Oxidative Stress in Inflammatory Bowel Disease and Potential Antioxidant Therapies. *Oxid Med Cell Longev* 2017;2017:1–18. Available at: <http://doi.org/10.1155/2017/4535194>
 43. Gorbunov NV, Garrison BR, McDaniel DP, et al. Adaptive Redox Response of Mesenchymal Stromal Cells to Stimulation with Lipopolysaccharide Inflammagen: Mechanisms of Remodeling of Tissue Barriers in Sepsis. *Oxid Med Cell Longev* 2013;2013:186795. Available at: <http://doi.org/10.1155/2013/186795>
 44. Guillén MI, Platas J, Pérez del Caz MD, Mirabet V, Alcaraz MJ. Paracrine Anti-inflammatory Effects of Adipose Tissue-Derived Mesenchymal Stem Cells in Human Monocytes. *Front Physiol* 2018;9:661. Available at: <http://doi.org/10.3389/fphys.2018.00661>
 45. Tian CM, Zhang Y, Yang MF, et al. Stem Cell Therapy in Inflammatory Bowel Disease: A Review of Achievements and

- Challenges. *J Inflamm Res* 2023;Volume16:2089–2119. Available at: <http://doi.org/10.2147/JIR.S400447>
46. Li Y, Ma K, Zhang L, Xu H, Zhang N. Human Umbilical Cord Blood Derived-Mesenchymal Stem Cells Alleviate Dextran Sulfate Sodium-Induced Colitis by Increasing Regulatory T Cells in Mice. *Front Cell Dev Biol* 2020;8. Available at: <http://doi.org/10.3389/fcell.2020.604021>
 47. Cao X, Duan L, Hou H, et al. IGF-1C hydrogel improves the therapeutic effects of MSCs on colitis in mice through PGE2-mediated M2 macrophage polarization. *Theranostics* 2020;10(17):7697–7709. Available at: <http://doi.org/10.7150/thno.45434>
 48. García JR, Quirós M, Han WM, et al. IFN- γ -tethered hydrogels enhance mesenchymal stem cell-based immunomodulation and promote tissue repair. *Biomaterials* 2019;220:119403. Available at: <http://doi.org/10.1016/j.biomaterials.2019.119403>
 49. Parikh K, Antanaviciute A, Fawcner-Corbett D, et al. Colonic epithelial cell diversity in health and inflammatory bowel disease. *Nature* 2019;567(7746):49–55. Available at: <http://doi.org/10.1038/s41586-019-0992-y>
 50. Zhang HM, Yuan S, Meng H, et al. Stem Cell-Based Therapies for Inflammatory Bowel Disease. *Int J Mol Sci* 2022;23(15):8494. Available at: <http://doi.org/10.3390/ijms23158494>
 51. Liang X, Zhang L, Wang S, Han Q, Zhao RC. Exosomes secreted by mesenchymal stem cells promote endothelial cell angiogenesis by transferring miR-125a. *J Cell Sci* 2016;129(11):2182–2189. Available at: <http://doi.org/10.1242/jcs.170373>
 52. Lavelle A, Sokol H. Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol* 2020;17(4):223–237. Available at: <http://doi.org/10.1038/s41575-019-0258-z>
 53. Hou Q, Ye L, Liu H, et al. Lactobacillus accelerates ISCs regeneration to protect the integrity of intestinal mucosa through activation of STAT3 signaling pathway induced by LPLs secretion of IL-22. *Cell Death Differ* 2018;25(9):1657–1670. Available at: <http://doi.org/10.1038/s41418-018-0070-2>
 54. Wang H, Sun Y, Xiao FJ, et al. Mesenchymal Stem Cells Ameliorate DSS-Induced Experimental Colitis by Modulating the Gut Microbiota and MUC-1 Pathway. *J Inflamm Res* 2023;Volume16:2023–39. Available at: <http://doi.org/10.2147/JIR.S402592>
 55. Watanabe M. Retraction: Adult tissue stem cell therapy for gastrointestinal diseases by Mamoru Watanabe. *J Gastro Hepatol* 2017;33 (1):329–329. Available at: <http://doi.org/10.1111/jgh.12555>
 56. Watanabe S, Kobayashi S, Ogasawara N, et al. Transplantation of intestinal organoids into a mouse model of colitis. *Nat Protoc* 2022;17(3):649–671. Available at: <http://doi.org/10.1038/s41596-021-00658-3>
 57. Spence JR, Mayhew CN, Rankin SA, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature* 2010;470(7332):105–109. Available at: <http://doi.org/10.1038/nature09691>
 58. Xu J, Wang X, Chen J, et al. Embryonic stem cell-derived mesenchymal stem cells promote colon epithelial integrity and regeneration by elevating circulating IGF-1 in colitis mice. *Theranostics* 2020;10(26):12204–12222. Available at: <http://doi.org/10.7150/thno.47683>
 59. Saadh MJ, Mikhailova MV, Rasolzadegan S, et al. Therapeutic potential of mesenchymal stem/stromal cells (MSCs)-based cell therapy for inflammatory bowel diseases (IBD) therapy. *Eur J Med Res* 2023;28(1):47. Available at: <http://doi.org/10.1186/s40001-023-01008-7>
 60. Kawata Y, Tsuchiya A, Seino S, et al. Early injection of human adipose tissue-derived mesenchymal stem cell after inflammation ameliorates dextran sulfate sodium-induced colitis in mice through the induction of M2 macrophages and regulatory T cells. *Cell Tissue Res* 2019;376(2):257–271. Available at: <http://doi.org/10.1007/s00441-018-02981-w>
 61. Chao K, Zhang S, Qiu Y, et al. Human umbilical cord-derived mesenchymal stem cells protect against experimental colitis via CD5+ B regulatory cells. *Stem Cell Res Ther* 2016;7(1):109. Available at: <http://doi.org/10.1186/s13287-016-0376-2>
 62. Liu J, Lai X, Bao Y, et al. Intraperitoneally Delivered Mesenchymal Stem Cells Alleviate Experimental Colitis Through THBS1-Mediated Induction of IL-10-Competent Regulatory B Cells. *Front Immunol* 2022;13:853894. Available at: <http://doi.org/10.3389/fimmu.2022.853894>
 63. Banerjee A, Bizzaro D, Burra P, et al. Umbilical cord mesenchymal stem cells modulate dextran sulfate sodium induced acute colitis in immunodeficient mice. *Stem Cell Res Ther* 2015;6(1):1–14. Available at: <http://doi.org/10.1186/s13287-015-0073-6>
 64. Lightner AL, Dozois EJ, Dietz AB, et al. Matrix-Delivered Autologous Mesenchymal Stem Cell Therapy for Refractory Rectovaginal Crohn's Fistulas. *Inflamm Bowel Dis* 2019;26(5):670–677. Available at: <http://doi.org/10.1093/ibd/izz215>
 65. Cho YB, Lee WY, Park KJ, Kim M, Yoo HW, Yu CS. Autologous Adipose Tissue-Derived Stem Cells for the Treatment of Crohn's Fistula: A Phase I Clinical Study. *Cell Transplant* 2013;22(2):279–285. Available at: <http://doi.org/10.3727/096368912X656045>
 66. Duijvestein M, Vos ACW, Roelofs H, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. *Gut* 2010;59(12):1662–1669. Available at: <http://doi.org/10.1136/gut.2010.215152>
 67. Dhere T, Copland I, Garcia M, et al. The safety of autologous and metabolically fit bone marrow mesenchymal stromal cells in medically refractory Crohn's disease – a phase 1 trial with three doses. *Aliment Pharmacol Ther* 2016;44(5):471–81. Available at: <http://doi.org/10.1111/apt.13717>
 68. Zhang J, Lv S, Liu X, Song B, Shi L. Umbilical Cord Mesenchymal Stem Cell Treatment for Crohn's Disease: A Randomized Controlled Clinical Trial. *Gut Liver* 2018;12(1):73–78. Available at: <http://doi.org/10.5009/gnl17035>
 69. Barnhoorn MC, Wasser MNJM, Roelofs H, et al. Long-term Evaluation of Allogeneic Bone Marrow-derived Mesenchymal Stromal Cell Therapy for Crohn's Disease Perianal Fistulas. *J Crohns Colitis* 2019;14(1):64–70. Available at: <http://doi.org/10.1093/ecco-jcc/jjz116>
 70. de la Portilla F, Alba F, García-Olmo D, Herrerías JM, González FX, Galindo A. Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. *Int J Colorectal Dis* 2012;28(3):313–323. Available at: <http://doi.org/10.1007/s00384-012-1581-9>
 71. Markov A, Thangavelu L, Aravindhan S, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. *Stem Cell Res Ther* 2021;12(1):1–30. Available at: <http://doi.org/10.1186/s13287-021-02265-1>
 72. Tavakoli S, Ghaderi Jafarbigloo HR, Shariati A, et al. Mesenchymal stromal cells; a new horizon in regenerative medicine. *J Cell Physiol* 2020;235(12):9185–9210. Available at: <http://doi.org/10.1002/jcp.29803>
 73. Soontarak S, Chow L, Johnson V, et al. Mesenchymal Stem

- Cells (MSC) Derived from Induced Pluripotent Stem Cells (iPSC) Equivalent to Adipose-Derived MSC in Promoting Intestinal Healing and Microbiome Normalization in Mouse Inflammatory Bowel Disease Model. *Stem Cells Transl Med* 2018;7(6):456–467. Available at: <http://doi.org/10.1002/sctm.17-0305>
74. Mendt M, Rezvani K, Shpall E. Mesenchymal stem cell-derived exosomes for clinical use. *Bone Marrow Transplant* 2019;54(S2):789–792. Available at: <http://doi.org/10.1038/s41409-019-0616-z>
 75. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018;7(1). Available at: <http://doi.org/10.1080/20013078.2018.1535750>
 76. Deng H, Sun C, Sun Y, et al. Lipid, Protein, and MicroRNA Composition Within Mesenchymal Stem Cell-Derived Exosomes. *Cell Reprogram* 2018;20(3):178–186. Available at: <http://doi.org/10.1089/cell.2017.0047>
 77. Witwer KW, Van Balkom BWM, Bruno S, et al. Defining mesenchymal stromal cell (MSC)-derived small extracellular vesicles for therapeutic applications. *J Extracellular Vesicle* 2019;8(1). Available at: <http://doi.org/10.1080/20013078.2019.1609206>
 78. Gimona M, Brizzi MF, Choo ABH, et al. Critical considerations for the development of potency tests for therapeutic applications of mesenchymal stromal cell-derived small extracellular vesicles. *Cytotherapy* 2021;23(5):373–380. Available at: <http://doi.org/10.1016/j.jcyt.2021.01.001>
 79. Baghaei K, Tokhanbigli S, Asadzadeh H, Nmaki S, Reza Zali M, Hashemi SM. Exosomes as a novel cell-free therapeutic approach in gastrointestinal diseases. *J Cell Physiol* 2018;234(7):9910–26. Available at: <http://doi.org/10.1002/jcp.27934>