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Therapeutic Potential of M2 Macrophage-Derived Exosomes in Regenerative Medicine

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ABSTRACT

M2 macrophages and their exosome-derived products have therapeutic potential in regenerative medicine. M2 macrophages, characterized by their anti-inflammatory and tissue-repair functions, play pivotal roles in immune modulation, wound healing, and disease resolution. M2 macrophage-derived exosomes can modulate inflammatory responses, promote angiogenesis, and stimulate stem cell activity.

The review systematically examines their roles in diverse preclinical models, including diabetic fractures, periodontitis, neurodegenerative diseases, myocardial infarction, and chronic wounds. It addresses progress in bioengineering, such as combining M2-derived exosomes with biomaterials and scaffolds to improve targeted delivery and regenerative results. Although they show great potential, obstacles like exosome diversity, restricted scalability, and the need for standardized isolation techniques are recognized as hindrances to clinical application.

This review distinguishes M2 macrophage-derived exosomes as a promising acellular tool for personalized therapeutic applications and tissue repair by synthesizing existing literature and identifying promising directions for future research. It emphasizes the need for ongoing research to overcome technical and regulatory barriers to their successful translation to the clinical setting.

Keywords:

M2 Macrophages, M2 Macrophage-derived Exosomes, Regenerative Medicine, immunomodulation, tissue repair.

1. Introduction

Macrophages are versatile immune cells that play critical roles in host defense, tissue homeostasis, and repair. They can adopt different functional states, a process known as macrophage polarization, which allows them to respond dynamically to environmental cues. The two main polarized phenotypes are M1 (classically activated) and M2 (alternatively activated) macrophages, each with distinct roles and molecular characteristics (1). M1 Macrophages (Pro-inflammatory) are induced by microbial products such as lipopolysaccharide (LPS) and pro-inflammatory cytokines (2). M2 Macrophages (Anti-inflammatory) are induced primarily by cytokines IL-4 and IL-13 secreted by Th2 cells. M2 macrophages contribute to the resolution of inflammation, tissue remodeling, wound healing, and immune regulation (3).

M1 macrophages are critical in early defense against infections and tumor cells but can contribute to tissue damage and chronic inflammation if not regulated. M2 macrophages help resolve inflammation and promote tissue regeneration but can also be involved in pathological fibrosis and tumor progression. The balance between M1 and M2 polarization is crucial for immune homeostasis; dysregulation is linked to various diseases such as inflammatory bowel disease, autoimmune disorders, obesity, and cancer (4).

The therapeutic potential of M2

macrophages and their exosome-mediated signaling garner increasing attention due to their crucial roles in tissue repair and immune modulation. M2 macrophages, recognized for their anti-inflammatory and reparative functions, secrete exosomes—small extracellular vesicles that carry bioactive molecules, including miRNAs, cytokines, growth factors, and non-coding RNAs (5). These exosomes serve as pivotal mediators of intercellular communication, influencing the behavior of recipient cells and playing essential roles in tissue regeneration and immune regulation (6). Evidence suggests that M2 macrophage-derived exosomes (M2-Exos) accelerate healing processes in various models, such as diabetic fracture repair and wound healing. They enhance tissue repair by promoting the transition of pro-inflammatory M1 macrophages to the anti-inflammatory M2 phenotype, thereby reducing inflammation and fostering tissue regeneration through pathways like PI3K/AKT. Furthermore, M2-Exos facilitate angiogenesis, re-epithelialization, and the reduction of oxidative stress, all of which are critical for effective tissue repair (7).

Due to their biocompatibility, low immunogenicity, and ability to deliver therapeutic molecules, M2-derived exosomes are being explored as natural nanocarriers for drugs, miRNAs, and other therapeutic agents. Their lipid bilayer membrane ensures sustained release and efficient cellular uptake, providing advantages over conventional drug delivery systems. Current research is focused on optimizing exosome yield, targeting, and therapeutic efficacy to enhance their clinical application (8).

The growing research on M2 macrophages and their exosomes underscores their dual role as therapeutic agents and targets, facilitating tissue repair, immune regulation, and disease modulation. This has spurred the development of M2 macrophage-derived exosome-based therapies and cell-free therapy strategies for treating chronic wounds, ischemic injuries, and other inflammatory conditions (9). Ongoing investigations aim to optimize exosome production, targeting, and functional modification to fully realize their clinical potential.

This review will discuss the functions and mechanisms of M2 macrophages and their exosomes in regenerative medicine and cell-free therapy. Specifically, it addresses how they impact the resolution of inflammation, tissue repair, and immune modulation, with special attention to the molecular mechanisms and their preclinical therapeutic applications. Through synthesizing current evidence and identifying key challenges, this article seeks to highlight the translational potential of M2-Exos and recognize their importance as a novel platform in personalized and regenerative medicine.

2. Methodology

Studies were identified through a comprehensive search of relevant databases, including PubMed, Science Direct, SCOPUS, and Web of Science, using keywords such as M2 Macrophages and M2 Macrophage-derived Exosomes. Only peer-reviewed articles published in English between 2015-2024 were included to ensure relevance and quality. Inclusion criteria were studies that focused on studies that provided original data and had transparent methodologies. Exclusion criteria included case reports, reviews, editorials, and studies with incomplete data or unclear outcomes.

3. Overview of Macrophages

The origin and development of macrophages are complex and involve multiple sources and stages. Macrophages can originate from several sources, including yolk sac progenitors, fetal liver, and bone marrow. In primitive hematopoiesis, Yolk Sac unipotent myeloid progenitors give rise directly to macrophages without a monocyte intermediate and produce primitive macrophages, contributing to microglia in the brain and other early tissue-resident macrophages. Then, early in embryonic development, macrophages are produced by erythromyeloid progenitors (EMPs) in the yolk sac. These cells populate various tissues and can persist into adulthood. EMPs can differentiate into multiple myeloid cells, including macrophages. These macrophages migrate to the fetal liver, expanding and differentiating into tissue-resident macrophages that colonize different tissues (10,11).

As development progresses, the definitive hematopoiesis begins in the fetal liver and bone marrow. Hematopoietic stem cells (HSCs) give rise to all blood cell lineages, including monocytes. Monocytes produced in the bone marrow migrate to tissues and differentiate into macrophages, contributing to the adult macrophage population (12). Depending on local environmental cues, circulating monocytes can differentiate into tissue-specific macrophages upon entering tissues. Meanwhile, some tissue-resident macrophages, like microglia and Kupffer cells, can self-renew locally without relying on circulating monocytes. Overall, the origin and development of macrophages reflect a complex interplay between embryonic and adult hematopoiesis, with significant implications for immune function and tissue health (13).

Tissue-resident macrophages (TRMs) are a diverse and specialized population of macrophages that permanently inhabit tissues throughout the body, performing critical roles beyond classical immune defense. They originate primarily from embryonic progenitors during early development—such as yolk sac and fetal liver precursors—before HSCs emerge, and they persist into adulthood through self-renewal, distinct from monocyte-derived macrophages circulating in the blood (14).

Differentiating TRMs from circulating macrophages requires a nuanced understanding of their developmental origins, functional specializations, and interactions with the local microenvironment. TRMs typically originate from embryonic or fetal precursors, such as yolk sac progenitors or fetal liver monocytes (15). These cells possess the capacity for local self-renewal and are generally maintained independently of circulating monocytes under homeostatic conditions. In contrast, circulating macrophages are derived from HSCs in the adult bone marrow. They enter the bloodstream as monocytes and differentiate into macrophages only upon migrating into tissues, particularly in response to injury or infection (16). TRMs functionally are adapted to fulfill tissue-specific roles, including maintaining homeostasis, maintaining immune surveillance, and participating in tissue development and repair. They are long-lived, exhibit low turnover rates, and can sustain their populations through in situ proliferation (17). Circulating macrophages, by comparison, are primarily involved in acute inflammatory responses and the rapid clearance of pathogens. They are generally short-lived and require continual replenishment from the monocyte pool, especially in high-turnover environments such as the intestinal mucosa (18).

Phenotypically, TRMs exhibit a high degree of specialization, characterized by distinct gene expression profiles shaped by their tissue environment. This allows them to perform finely tuned functions aligned with the physiological demands of their specific niche. Conversely, circulating macrophages display a more pro-inflammatory and less specialized phenotype, with gene expression patterns reflecting their role in immediate immune responses (19). Regarding environmental interaction, TRMs are deeply integrated into their tissue milieu, responding to local cues and maintaining close communication with non-immune cells. Circulating macrophages, however, are more reactive to systemic signals and inflammatory stimuli, often coordinating with other immune cells during acute responses. From a pathological standpoint, TRMs are frequently implicated in chronic inflammatory processes and tissue remodeling, contributing to conditions such as neurodegeneration and fibrosis. Circulating macrophages, on the other hand, are key players in the initial phases of inflammation and are central to host defense during infections. This distinction underscores context-specific macrophage biology's importance in health and disease (21).

Macrophages are adaptable immune cells with essential functions in host defense, maintaining tissue balance, and healing. They can assume various functional states, a process called macrophage polarization, enabling them to react adaptively to environmental signals. The primary polarized phenotypes are M1 (classically activated) and M2 (alternatively activated) macrophages, which have unique functions and molecular traits (21).

Macrophages are among the first cells to encounter pathogens, acting as sentinels that recognize and respond to microbial infections. They use pattern recognition receptors (PRRs) like toll-like receptors (TLRs) and scavenger receptors to identify pathogen-associated molecular patterns (PAMPs). Upon recognizing pathogens, macrophages initiate an immune response by secreting pro-inflammatory cytokines, which recruit other immune cells to the site of infection. Through phagocytosis, macrophages engulf and digest pathogens, cellular debris, and foreign particles. During phagocytosis, macrophages undergo a respiratory burst, producing reactive oxygen species (ROS) to kill ingested microbes (22,23). Additionally, macrophages are involved in the initial inflammatory response by secreting pro-inflammatory cytokines, which recruit other immune cells to the injury site.

On the other hand, they contribute to resolving inflammation by producing anti-inflammatory cytokines and promoting the clearance of apoptotic cells and debris. Macrophages phagocytose dead cells, cellular debris, and pathogens, essential for creating a clean environment conducive to healing (24). Macrophages secrete growth factors that stimulate the proliferation and differentiation of cells necessary for tissue regeneration, such as fibroblasts, endothelial cells, and myogenic cells. Macrophages promote angiogenesis by interacting with endothelial cells and supporting the formation of new blood vessels, which are crucial for delivering oxygen and nutrients to healing tissues (25). Macrophages transform inflammation into tissue repair by modulating their functional phenotypes. Pro-inflammatory macrophages are involved in the initial response, while anti-inflammatory macrophages facilitate tissue repair and regeneration. Overall, macrophages are essential for coordinating the complex processes involved in tissue injury repair, ensuring efficient healing, and minimizing the risk of fibrosis or chronic inflammation (26).

4. M1 vs. M2 Macrophages

Macrophage polarization is a dynamic process in which macrophages adopt different functional phenotypes in response to environmental cues. They are primarily classified into M1 and M2 types. This process is crucial for immune responses, tissue repair, and disease progression. Macrophage polarization involves the differentiation of monocytes into distinct macrophage phenotypes based on signals from their microenvironment (27). M1 macrophages are pro-inflammatory, involved in pathogen clearance and inflammation, while M2 macrophages are anti-inflammatory, promoting tissue repair and immune tolerance.

M1 macrophages are characterized by several key features that enable them to play a crucial role in the immune response, particularly in inflammation and pathogen clearance. M1 macrophages are known for secreting high levels of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, IL-12, and IL-23. These cytokines are essential for initiating and maintaining an inflammatory response and recruiting other immune cells to the site of infection or injury (28). M1 macrophages promote Th1 cell differentiation and recruitment, further enhancing the inflammatory response. M1 macrophages are highly efficient in producing ROS and NO through activating enzymes like Nox2 and inducible nitric oxide synthase (iNOS). These reactive species are crucial for killing pathogens and contributing to the bactericidal activity of M1 macrophages. M1 macrophages express high levels of surface markers such as MHC II, CD68, CD80, and CD86, facilitating antigen presentation to T cells (1).

M2 macrophages produce anti-inflammatory cytokines such as IL-10, TGF-β, and IL-22. These cytokines help suppress inflammation and promote a healing environment. M2 macrophages inhibit the production of pro-inflammatory cytokines like IL-12, facilitating a shift from Th1 to Th2 immune responses, which are more conducive to tissue repair (29). M2 macrophages are crucial for wound healing because they secrete factors that promote the formation of the extracellular matrix (ECM), such as fibronectin and collagen. They also facilitate the differentiation of fibroblasts into myofibroblasts, which aids in wound contraction and closure. M2 macrophages support angiogenesis (forming new blood vessels) and tissue remodeling by producing angiogenic factors essential for delivering nutrients and oxygen to healing tissues (26). M2 macrophages efficiently phagocytose apoptotic cells, preventing secondary necrosis and inflammation, which is crucial for maintaining tissue integrity during repair processes. Unlike M1 macrophages, which rely on glycolysis, M2 macrophages primarily use oxidative phosphorylation for energy production, supported by β-fatty acid oxidation and glutamine metabolism (28).

M2 macrophages are characterized by specific surface markers and the production of various cytokines and factors that support their anti-inflammatory and tissue repair functions. CD206, CD163, and CD209 are commonly expressed by M2 macrophages, facilitating their roles in tissue repair and anti-inflammatory responses (30). CD163 is a scavenger receptor involved in the clearance of hemoglobin-haptoglobin complexes, associated with anti-inflammatory responses (31). CD206 (the mannose receptor) facilitates endocytosis and tissue repair. M2 macrophages produce factors supporting angiogenesis, such as VEGF, essential for forming new blood vessels during tissue repair. M2 macrophages contribute to wound healing by secreting ECM components like fibronectin and collagen. They produce Arginase-1, which converts L-arginine to L-ornithine, which is involved in collagen synthesis and tissue repair. These characteristics highlight the role of M2 macrophages in resolving inflammation, promoting tissue repair, and supporting immune homeostasis (32).

M2 macrophages are further divided into subtypes, M2a, M2b, M2c, and M2d, each with distinct functions and characteristics. M2a Macrophages are Primarily involved in tissue repair and remodeling, activated by IL-4 and IL-13. M2a Macrophages are Primarily activated by IL-4 and IL-13, which induce the expression of the mannose receptor CD206. They are involved in tissue repair and remodeling. They produce IL-10, TGF-β, CCL17, CCL18, and CCL22, promoting cell growth and endocytosis. M2a macrophages can contribute to tumor growth by producing angiogenic factors like VEGF and PDGF, facilitating tumor invasion and metastasis. They produce L-ornithine, a precursor for collagen synthesis, and promote fibrosis (29). M2b Macrophages are Known for their role in immune regulation and tumor progression. They are activated by immune complexes, like TLR ligands and IL-1β. They regulate immune responses and inflammation by secreting pro-inflammatory (e.g., TNF-α, IL-1β, IL-6) and anti-inflammatory cytokines (e.g., IL-10). They produce anti-inflammatory cytokines like IL-10 and facilitate immune escape mechanisms. They are Involved in the regulation of humoral immunity and Th2 differentiation (33).

M2c Macrophages are Characterized by their anti-inflammatory activity, producing IL-10 and efficiently phagocytosing apoptotic cells. Glucocorticoids, IL-10, and TGF-β activate M2c Macrophages. They also express MerTK, facilitating the phagocytosis of apoptotic cells, and are engaged in controlling neutrophil chemotaxis and promoting tissue repair. M2d Macrophages are Induced by TLR antagonists, IL-6, and adenosines. They Produce high levels of IL-10 and VEGF, promoting angiogenesis and tumor progression. They also secrete CCL5, CXCL10, and CXCL16 (34). M2d macrophages are commonly found in the tumor microenvironment, supporting tumor growth and angiogenesis. Each subtype of M2 macrophages plays a distinct role in tissue repair, immune regulation, and disease progression, highlighting the complexity and versatility of macrophage functions in different contexts (35).

These subtypes differ in their activation stimuli, secretory profiles, and biological effects, including their exosome secretion and cargo, which influence therapeutic outcomes, especially in tissue repair and regeneration. While it is established that M2 macrophages secrete exosomes that carry proteins, miRNAs, long non-coding RNAs (lncRNAs), and other bioactive molecules, the specific differences in exosome cargo among M2 subtypes are not fully characterized in current studies. However, the cargo composition critically influences the biological effects of these exosomes on recipient cells (36). The differences in exosome cargo among M2 subtypes likely translate into varied regenerative outcomes. M2a-derived exosomes may predominantly support tissue repair and anti-inflammatory processes due to their growth factor and anti-inflammatory cytokine cargo. On the other hand, M2b-derived exosomes might have a dual role, balancing pro- and anti-inflammatory signals, which could modulate immune responses during regeneration (37). Although direct comparative studies of exosome cargo across M2 subtypes are limited, the evidence suggests that the functional heterogeneity of M2 macrophages extends to their exosomal secretions, which critically regulate recipient cell behavior and therapeutic outcomes in inflammation resolution, tissue repair, fibrosis, and cancer progression.

5. Exosomes: Characteristics, Functions, and Therapeutic Applications

According to MISEV2023, extracellular vesicles (EVs) are particles released from cells, delimited by a lipid bilayer, and incapable of replicating independently (i.e., they do not contain a functional nucleus). While specific subtypes like exosomes and microvesicles are traditionally used, MISEV2023 encourages operational terms based on physical characteristics, biological constituents, or cellular origins rather than relying on particular biogenesis pathways. Two classifications include Small EVs and medium/large EVs. Based on Biogenesis, though not strictly defined, terms like exosomes (endosome-derived) and microvesicles (plasma membrane-derived) are used (38).

Exosomes are small, membrane-bound extracellular vesicles, typically 50-150 nm in size, produced in the endosomal compartment of eukaryotic cells. They are formed through the inward budding of late endosomes, also known as multivesicular bodies (MVBs). Exosomes contain proteins, lipids, and nucleic acids, reflecting the metabolic state of their parent cells. They play a crucial role in intercellular communication by transferring signals between cells. Exosomes are found in various bodily fluids and are involved in physiological and pathological processes. Exosomes have potential applications as biomarkers and therapeutic agents due to their role in disease processes (39).

Exosomes are complex extracellular vesicles containing diverse bioactive molecules, including proteins, lipids, and RNA cargo. These components play crucial roles in intercellular communication and are involved in physiological and pathological processes. Proteins like Tetraspanins (CD9, CD63, and CD81) are commonly used as exosomal markers and are involved in cell adhesion and signaling. Heat Shock Proteins, including HSP70 and HSP90, are associated with stress response and antigen presentation. ESCRT Proteins like ALIX and TSG101 are essential for exosome biogenesis and secretion. Additionally, Integrins and Glycoproteins facilitate interactions with recipient cells (40).

CD9, CD63, and CD81 are highly enriched in exosome membranes and are commonly used to identify exosomes in research and clinical studies. Nonetheless, these tetraspanins are also present on the plasma membrane. They can be found in other EV types, such as microvesicles (MVs) or ectosomes, which bud directly from the cell surface. The presence of tetraspanins varies quantitatively and qualitatively depending on the cell type and even within EV populations from the same cell, indicating heterogeneity in exosome composition (41).

Exosome isolation methods vary in principle, efficiency, purity, and suitability depending on the sample type and downstream application. Differential ultracentrifugation is the traditional gold standard method for separating exosomes based on size and density through multiple high-speed spins. It provides moderate to high purity and yield but is time-consuming, requiring several hours and expensive ultracentrifuge equipment (42). It may also co-isolate protein contaminants if not combined with additional purification steps. Density gradient centrifugation improves purity by separating exosomes according to their buoyant density using sucrose or iodixanol gradients. This method achieves very high purity but at the cost of lower yield and longer processing times. It is labor-intensive and best suited for applications demanding highly pure exosomes (43). Size-exclusion chromatography (SEC) isolates exosomes by size exclusion through porous beads, offering high purity with minimal protein contamination. It is relatively fast (30 to 60 minutes) and cost-effective but typically yields moderate exosomes. SEC is widely used for clinical samples and can be combined with other methods for enhanced purity (44). Ultrafiltration concentrates exosomes by filtering samples through membranes with defined pore sizes. It is faster than ultracentrifugation and can yield moderate to high amounts of exosomes. However, this method may deform vesicles or cause some loss, and it is often paired with SEC to improve purity (45). Precipitation-based kits use polymers like polyethylene glycol to precipitate exosomes quickly and easily without requiring specialized equipment. These kits provide high yield but generally lower purity due to co-precipitation of non-exosomal proteins and aggregates, which can affect downstream applications (46). Methods like density gradient centrifugation, immunoaffinity capture, metal oxide affinity, and SEC provide the highest purity, while precipitation kits and metal oxide affinity yield the most exosomes. Ultracentrifugation methods require more time and specialized equipment but remain widely used. Combining techniques, such as ultrafiltration followed by SEC, often achieves the best balance of purity and yield. The method choice depends on the experiment's specific needs, including sample type, desired purity, yield, time constraints, and available resources.

Exosomes play a crucial role in intercellular communication as messengers between cells. They transport bioactive molecules such as proteins, mRNAs, and microRNAs (miRNAs) from donor to recipient cells, influencing various physiological and pathological processes. This intermediate role allows exosomes to mediate cell interactions over long distances, which is crucial in intercellular communication (47). Mechanisms of Intercellular Communication via Exosomes include binding and uptake, signal transmission, and cross-tissue communication. Exosomes in the binding and uptake mechanism interact with recipient cells through adhesion molecules, receptor-mediated endocytosis, or phagocytosis, facilitating the transfer of their cargo. In the signal transmission pathway, exosomes can modulate signaling pathways in recipient cells by delivering signaling molecules like proteins and miRNAs, which interact with receptors or influence gene expression. Furthermore, Exosomes can cross biological barriers, such as the blood-brain barrier, to communicate between distant tissues and organs (48).

Exosomes resemble their parent cells and mediate their functions. Exosomes contain diverse molecules, including proteins, lipids, and nucleic acids, that reflect their parent cells' metabolic state and characteristics. This means that the composition of exosomes can provide insights into the condition and function of the cells from which they are derived. Exosomes' resemblance to their origin cells makes them potential disease diagnosis biomarkers. For example, exosomes from cancer cells can carry tumor-specific antigens or miRNAs, aiding in cancer detection (40,49).

Exosomes have emerged as promising tools in therapeutic applications due to their unique properties and natural ability to transport bioactive molecules. They offer a natural, versatile, and relatively safe platform for therapeutic delivery. They provide unique advantages such as biocompatibility, cargo protection, and crossing biological barriers. However, challenges related to targeting specificity, cargo loading, stability, and manufacturing standardization must be overcome to realize their clinical potential fully. Continued research and technological advances are essential to address these limitations and enable widespread therapeutic use of exosomes (50,51).

Exosomes have many advantages in therapeutic applications, including biocompatibility and safety features. They are also low in immunogenicity and toxicity, making them well-tolerated in vivo. Unlike whole-cell therapies, exosomes are non-tumorigenic and less likely to provoke adverse immune reactions. Due to their surface proteins and lipids, exosomes naturally target specific cells or tissues. Engineering their surface molecules can further enhance this targeting, improving delivery precision. Exosomes protect their therapeutic cargo—such as proteins, RNAs, and drugs—from enzymatic degradation in the extracellular environment (50,52). They can also cross biological barriers, including the BBB, to deliver hard-to-reach tissues. Exosomes are relatively stable in biological fluids and more straightforward to preserve than living cells. They can be produced from various cell sources, allowing scalable manufacturing. Compared to cell therapies, exosomes are more straightforward to isolate, purify, and store, facilitating large-scale production and quality control (53,54).

It is essential to note the limitations of exosomes for therapeutic application. The immune system can rapidly clear exosomes, which may shorten their therapeutic window and effectiveness. While exosomes have some intrinsic targeting ability, they are generally inadequate for targeted delivery and must be subjected to advanced engineering to enhance specificity. Loading therapeutic molecules into exosomes remains challenging, and current methods may result in low or variable cargo incorporation (55,56). Although more stable than cells, exosomes often require ultra-low temperature storage (e.g., −80 °C), making logistics difficult and costly. Exosome composition variation based on cell source and isolation method creates reproducibility and regulatory challenges (57). Despite promising preclinical data, exosome-based therapies face clinical development challenges, including limited long-term safety data and a lack of definitive regulatory guidance.

6. M2 Macrophage-Derived Exosomes: Therapeutic Applications

M2 macrophage-derived exosomes exert their therapeutic effects through three main mechanisms: inflammation inhibition, angiogenesis promotion, and stem cell activity stimulation. M2 macrophage-derived exosomes carry anti-inflammatory cytokines (such as IL-4, IL-10, and TGF-β) and immunomodulatory molecules that promote tissue repair and resolve inflammation. These properties make them ideal candidates for personalized cell-free therapies targeting chronic inflammatory and autoimmune diseases like rheumatoid arthritis and inflammatory bowel disease.

M2 macrophage exosomes can also be engineered to deliver drugs specifically to inflamed tissues, enhancing therapeutic efficacy while minimizing systemic side effects. Moreover, they can induce polarization of pro-inflammatory M1 macrophages toward the anti-inflammatory M2 phenotype, amplifying their regenerative and immunomodulatory effects (37).

M2 macrophage-derived exosomes possess unique properties characterized by their specific cargo, which includes anti-inflammatory cytokines, growth factors, and microRNAs such as miR-21 and miR-146a. These exosomes play a significant role in modulating inflammation and promoting tissue repair. Key miRNAs like miR-21, miR-146a, and miR-93-5p are enriched in M2 exosomes. These miRNAs regulate immune responses, inhibit inflammatory signaling pathways, and promote angiogenesis and tissue regeneration (58).

M2 macrophage-derived exosomes (M2-EXOS) have demonstrated diverse therapeutic applications in preclinical studies across multiple disease models, with emerging potential for clinical translation. Their key roles involve immune modulation, promotion of tissue regeneration, and anti-inflammatory effects. M2-exos exhibit broad therapeutic potential in preclinical neurological injury, inflammation, tissue regeneration, and cardiovascular disease models (59). While clinical applications remain in the early stages, advances in exosome engineering and delivery pave the way for future clinical use.

M2 macrophage-derived exosomes play a pivotal role in bone regeneration by modulating cellular differentiation, immune responses, and signaling pathways. In a study, M2-Exos were found to effectively treat osteonecrosis of the femoral head (ONFH) by modulating the communication between neutrophils and endothelial cells, specifically by reducing the formation of neutrophil extracellular traps (NETs) and promoting endothelial phenotype transition. The therapeutic effect of M2-Exos was attributed to its high content of miR-93-5p, a microRNA known for its anti-inflammatory and pro-angiogenic properties. These findings suggest that M2-Exos, through the delivery of miR-93-5p, offers a promising therapeutic strategy for ONFH by modulating immune responses and promoting tissue repair (60). In a diabetic mouse model, administration of M2-Exos significantly accelerated diabetic fracture healing compared to untreated controls. Specific PI3K/AKT pathway inhibitors attenuated the beneficial effects of M2-Exos, confirming the pathway's role in macrophage polarization and bone healing. This study showed the potential of M2-Exos as a cell-free therapeutic strategy for modulating immune responses and promoting tissue regeneration in diabetic fractures (7). Another study suggested that M2-exos inhibit osteoclastogenesis by downregulating Colony-stimulating factor 2 (CSF2) expression, which in turn inactivates TNF-α signaling pathways. These findings provide insights into the potential therapeutic application of M2-exos in treating bone diseases characterized by excessive osteoclast activity (61).

Accumulating evidence highlights the therapeutic potential of M2-Exos in treating periodontitis. Periodontitis is characterized by inflammation and destruction of periodontal tissues, leading to tooth loss. A study on periodontitis concludes that M2-Exos promotes osteogenesis and suppresses inflammation in Human periodontal ligament stem cells (hPDLSCs) through the upregulation of CXCL12. These findings propose M2-exos as a promising therapeutic strategy for periodontitis by enhancing tissue regeneration and modulating inflammatory responses.

Simultaneously, M2-exos reduced inflammatory cytokines IL-1β, IL-6, and TNF-α in hPDLSCs, indicating anti-inflammatory effects (62). Another study investigated the therapeutic potential of M2-Exos in preventing alveolar bone loss associated with periodontitis. In vitro experiments demonstrated that M2-Exos promoted osteogenic differentiation in bone marrow stromal cells (BMSCs) and inhibited osteoclastogenesis in bone marrow-derived macrophages (BMDMs). The therapeutic effects of M2-Exos were attributed to the delivery of interleukin-10 (IL-10) mRNA, leading to increased IL-10 cytokine expression in recipient cells (63). Another study showed how M2-Exos influence the osteogenic differentiation of human periodontal ligament stem cells (hPDLSCs). M2-exos significantly enhanced mineralized nodule formation and upregulated osteogenic markers such as alkaline phosphatase (ALP) and osteocalcin (OCN) in hPDLSCs. The study underscores the importance of macrophage polarization states in influencing stem cell differentiation via exosomal miRNA cargo (64). Furthermore, Researchers engineered M2-like macrophages by silencing the gene encoding casein kinase 2 interacting protein-1 (Ckip-1), resulting in stable M2 polarization with enhanced regenerative potential. In vitro experiments demonstrated that these exosomes effectively rescued *P. gingivalis*-suppressed cementoblast mineralization and promoted cementogenesis. The study's findings suggest that exosomal delivery of specific microRNAs can modulate gene expression in target cells, promoting tissue regeneration even under pathogenic conditions (65). Another study using an M2 macrophage-conditioned medium (CM2) and transwell coculture with M2 macrophages (Trans-M2) showed enhanced cementoblast mineralization compared to controls. These findings suggest that M2 macrophages could play a significant role in cementum regeneration, offering potential therapeutic strategies for periodontal tissue repair (66).

Macrophages are pivotal in wound healing, with M1 macrophages initiating inflammation and M2 macrophages facilitating tissue repair. Exosomes derived from M2 macrophages (M2-EXO) have emerged as promising cell-free therapeutic agents for enhancing wound healing. A study showed enhanced angiogenesis and accelerated wound closure Compared to controls when the M2-Exo-loaded scaffold was implanted into murine wound models, suggesting the therapeutic efficacy of the M2-Exo-loaded scaffold. This approach holds promise for developing advanced therapeutic strategies in regenerative medicine, particularly for chronic wounds and tissue defects requiring enhanced angiogenesis and remodeling (67). Another study investigated the therapeutic potential of M2-Exos in promoting wound healing by reprogramming pro-inflammatory M1 macrophages into anti-inflammatory M2 phenotypes. In vitro experiments demonstrated that M2-Exo could reprogram M1 macrophages into M2 phenotypes, as evidenced by increased Arginase-1 and decreased iNOS expression. In vivo studies involved subcutaneous injection of M2-Exo into mice's wound sites, which accelerated wound closure compared to controls. In conclusion, The study suggests that M2-Exos contain cytokines and growth factors that facilitate macrophage reprogramming and tissue regeneration (68). A survey of enhancing wound healing through angiogenesis demonstrated that M2-Exos promoted the proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVECs), indicating enhanced angiogenic activity. The angiogenic effects were associated with increased expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1-alpha (HIF-1α) in HUVECs. In vivo experiments using a mouse skin wound model showed that M2-Exos treatment accelerated wound closure and improved tissue vascularization (69). The findings suggest that M2-Exos could be developed into a novel treatment modality for chronic wounds and other conditions requiring enhanced tissue regeneration. Furthermore, another research investigated whether M2-exos could enhance skin flap survival by promoting angiogenesis through the HIF1AN/HIF-1α/VEGFA signaling pathway. M2-exosome treatment increased survival area and microvascular density in the skin flaps compared to controls. These findings suggest that M2-exosomes could serve as a potential therapeutic strategy for improving the outcomes of skin flap surgeries and other ischemic tissue conditions (70).

M2 macrophages have shown significant therapeutic potential in treating neurodegeneration by modulating neuroinflammation, promoting neuroprotection, and enhancing neural regeneration. A study investigated the neuroprotective effects of M2 microglia-derived exosomes in a model of neuronal injury induced by oxygen-glucose deprivation and reoxygenation (OGD/R), which leads to neuronal injury characterized by increased reactive oxygen species (ROS) accumulation. Treatment with M2-exos significantly improved cell proliferation and reduced ROS accumulation, Fe^{2+} levels, and lipid peroxidation in OGD/R-conditioned HT22 cells. These findings suggest that M2-exosomes deliver miR-124-3p to HT22 cells, leading to the downregulation of nuclear receptor coactivator 4 (NCOA4) and subsequent inhibition of ferroptosis. The study highlights the therapeutic potential of M2 microglia-derived exosomes in treating neurodegenerative conditions associated with ferroptosis, providing a basis for future clinical applications (71). A study investigated the roles of M1 and M2 macrophages in spinal cord injury (SCI) and their impact on neuronal survival and axon growth. Following SCI, macrophages infiltrate the injury site and can adopt either a pro-inflammatory M1 phenotype or an anti-inflammatory M2 phenotype. The high M1/M2 macrophage ratio at the injury site correlates with poor outcomes in SCI repair. These findings suggest modulating macrophage polarization could be a therapeutic strategy to enhance recovery after SCI (72). Another study aimed to evaluate whether M2-Exos modified with viral macrophage inflammatory protein II (vMIP-II) and lysosomal-associated membrane protein 2b (Lamp2b) could enhance targeting to the spinal cord injury model and modulate immune responses. Treatment with vMIP-II-Lamp2b-M2 exosomes decreased the levels of proinflammatory cytokines such as IL-1β, IL-6, IL-17, IL-18, TNF-α, and iNOS while promoting the production of anti-inflammatory cytokines like IL-4 and Arg1. Behavioral assessments indicated significant improvements in motor function, suggesting effective neuroprotection and repair. The study concludes that vMIP-II-Lamp2b-M2 exosomes offer a promising therapeutic strategy for SCI by enhancing targeted delivery to the injury site, modulating immune responses, and promoting tissue repair (73). Furthermore, Macrophage-derived exosomes have been shown to cross the Blood Brain Barrier without chemical modification or targeting peptides. In vivo experiments demonstrated that intravenous administration of naive Macrophage-derived exosomes allowed the delivery of brain-derived neurotrophic factor (BDNF) to the brain. These findings highlight the potential of utilizing Macrophage-derived exosomes as natural nanocarriers for targeted protein delivery to the brain. Such an approach could offer a novel, minimally invasive strategy for treating various CNS disorders characterized by inflammation (74).

A study on the atherosclerosis model showed that M2-Exos, through the regulation of miR-221-3p expression, promotes human umbilical vein endothelial cell (HUVEC) proliferation and inhibits inflammation and apoptosis, offering a potential therapeutic strategy for endothelial dysfunction in atherosclerosis. M2-Exos treatment reduced the apoptotic rate and decreased pro-apoptotic protein expression while increasing anti-apoptotic protein expression in HUVECs. The expression levels of inflammatory cytokines were lower in the M2-Exos group compared to the control group (75). Another study demonstrated that M2-Exos significantly inhibited platelet-derived growth factor-BB (PDGF-BB)-induced vascular smooth muscle cells (VSMCs) proliferation and migration. These findings highlight the potential of M2-Exos as a therapeutic agent in atherosclerosis by targeting VSMC behavior (76).

In addition to these studies, M2-Exos have been investigated in many different waysn vivo studies using diabetic mouse models showed that administration of M2-exo led to enhanced vascular remodeling and reduced vascular leakage in the retina. The therapeutic effects of M2-exos were further amplified by their ability to promote M2 polarization of retinal microglia, creating a positive feedback loop. These findings propose M2-exo as a promising cell-free therapeutic strategy for treating diabetic retinopathy by promoting vascular remodeling and reducing inflammation (77). A study investigated the therapeutic potential of M2-Exos in treating acute myocardial infarction (AMI). In vivo studies using a mouse model of AMI showed that treatment with M2-Exos improved cardiac function and reduced infarct size. These findings suggest that M2-Exos, through delivery of miR-1271-5p, alleviate cardiac injury in AMI by down-regulating SOX6 (78). Another study investigated the role of M2-Exos in the progression of infantile hemangiomas (IHs). Their findings suggested that M2-exosome-derived MIR4435-2HG promotes IH progression by modulating HNRNPA1 and NF-κB signaling, highlighting potential therapeutic targets for IHs (79). A study on knee osteoarthritis (KOA) in rats demonstrated that treatment with M2-Exos significantly reduced inflammatory responses and pathological damage in the articular cartilage of KOA rats. Key cartilage-related proteins such as Aggrecan, Collagen-10, SOX6, and Runx2 were upregulated, while the cartilage-degrading enzyme MMP-13 was suppressed following M2-Exo treatment. The therapeutic effects were primarily mediated by inhibiting the PI3K/AKT/mTOR signaling pathway, which is often overactivated in KOA. By downregulating this pathway, M2-Exos helped restore the balance between cartilage degradation and regeneration (80). Another study investigated the therapeutic potential of M2b macrophage-derived exosomes (M2b-Exos) in treating dextran sulfate sodium (DSS)-induced colitis in mice. Histological examination revealed reduced inflammatory cell infiltration, crypt loss, submucosal edema, and goblet cell loss in the colons of M2b-Exos-treated mice. Compared to exosomes derived from other macrophage phenotypes, M2b-Exos demonstrated superior efficacy in alleviating DSS-induced colitis (81).

M2 macrophages, known for their immunosuppressive properties, secrete exosomes that influence the tumor microenvironment. A study explored how M2-Exos contributes to tumor resistance against immunotherapy. The study highlights that tumor cells receiving exosomes from M2 macrophages exhibit reduced expression of tumor antigens. This reduction in antigen presentation impairs the activation of CD8+ T cells, which are crucial for targeting and eliminating tumor cells. Consequently, the effectiveness of immune checkpoint blockade (ICB) therapies is diminished, as these therapies rely on the activation of T cells to combat cancer cells. The findings suggest that targeting the communication between M2 macrophages and tumor cells could enhance the efficacy of immunotherapies. Overall, the study underscores the importance of understanding the role of M2-Exos in tumor resistance to improve cancer treatment strategies (82).

7. Future Perspectives and Challenges

Exosomes have developed as a helpful tool in translational medicine, with vast potential in personalized therapies and incorporation with cutting-edge biomedical technologies. Integration with Biomaterials and Tissue Scaffolds is one of the potential applications of exosomes. Sustained and Controlled Release can be obtained by Incorporating exosomes into biomaterials like hydrogels, β-tricalcium phosphate, or bioactive glass scaffolds. This allows for controlled, localized release of bioactive cargo to promote tissue regeneration and repair (83). Strategies like exosome-anchoring peptides targeting tetraspanin CD63 have functionalized 3D-printed scaffolds to enhance exosome retention and therapeutic outcomes in bone tissue engineering (84). Incorporating exosomes in stimuli-responsive hydrogels (e.g., MMP-9-sensitive) provides on-demand release by reacting to the wound microenvironment, as shown in diabetic wound healing models (85). Exosomes incorporated in biomaterials and tissue scaffolds offer improved therapeutic potential by delivering controlled, localized, and sustained release of bioactive cargo. For instance, exosome-loaded hydrogels, β-tricalcium phosphate, and bioactive glass scaffolds were employed for bone tissue engineering, angiogenesis, and inflammatory regulation. Stimulus-responsive, smart hydrogels also release exosomes in response to environmental stimulation (e.g., enzymatic activity, temperature), allowing precise spatiotemporal control of the therapy, as shown in diabetic wound models (86).

Clinical translation issues of exosome therapies are primarily founded on scalability, manufacturing, standardization, and safety. Low efficiency and yield are still significant bottlenecks to producing high quantities of uniform-quality exosomes, hindering large-scale production and clinical use. There is no standardization of isolation and purification protocols for exosomes, which generates heterogeneity in exosome preparations, making reproducibility and regulation more difficult. Therapeutic agents are difficult to load into exosomes efficiently, and inadequate drug loading and the lack of control over cargo packaging decrease therapeutic effects. Exosomes are heterogeneous in size, content, and function, making quality control and batch standardization more challenging. Exosomes are cleared quickly from the plasma because of fast uptake by macrophages, and targeted delivery to tissues is still difficult, with potential off-target effects and diminished therapeutic effects.

Overcoming these obstacles involves pushing forward scalable and standardizable manufacturing processes, improved cargo loading and target strategies, and stringent safety assessments, including choosing suitable cell sources and modulation of exosome content. Researchers, clinicians, and industrial partners must work together to surmount these hurdles and facilitate successful clinical translation of exosome-based treatments.

The regulatory landscape for exosome-based therapies, including those derived from macrophages, is complex and evolving, with significant gaps and challenges. In the United States, the FDA regulates exosome products primarily as drugs and biological products under the Public Health Service (PHS) Act and the Federal Food, Drug, and Cosmetic (FD&C) Act. These products require rigorous premarket review and approval to ensure safety and efficacy. As of October 2023, no exosome products have FDA approval, and the FDA has issued multiple warning letters to clinics marketing unapproved exosome therapies with unsubstantiated claims (87). In Europe, the EMA classifies exosome products based on their content and function. Exosomes containing functionally translated RNA with therapeutic effects are considered Advanced Therapy Medicinal Products (ATMPs) and are regulated accordingly by the Committee for Advanced Therapies (CAT). Products without such active components are classified differently, with biological specifications applied. Other regions such as Japan, South Korea, and Taiwan have their own regulatory units and guidelines focusing on how exosomes are obtained and their quality control, emphasizing good manufacturing practices (GMP) from raw material to final product (87). Despite these frameworks, regulatory gaps exist due to the novelty and complexity of exosome therapies. Challenges include defining exosome characterization standards, ensuring consistent manufacturing quality, and establishing clear clinical trial pathways. The heterogeneous nature of exosomes, their diverse cargo, and variable biological effects complicate regulatory classification and safety evaluation. Moreover, the rapid commercialization and marketing of exosome products without sufficient clinical evidence pose safety risks and regulatory enforcement challenges, as highlighted by FDA warning. M2 macrophage-derived exosomes have been implicated in promoting tumor progression and therapy resistance. They can transfer molecules such as miRNAs and proteins that suppress tumor suppressor pathways, inhibit apoptosis, and stimulate tumor cell proliferation and migration. For example, M2 exosomes enriched with arginase-1 promote glioblastoma cell migration and proliferation (58). In various cancers, M2 exosomes modulate signaling pathways (e.g., IL-6R/STAT3, NF-kB) to induce chemoresistance and tumor expansion. They can also transfer drug efflux pumps like P-glycoprotein, facilitating chemotherapy resistance by exporting drugs out of tumor cells (88). These pro-tumorigenic effects raise significant safety concerns for therapeutic applications of M2 macrophage-derived exosomes, especially in oncology. There is a risk that exosome therapies could inadvertently enhance tumor growth or resistance if not carefully controlled or if derived from M2 macrophages in a tumor-promoting state (89).

8. Conclusion

M2 macrophages and their exosomes are a new frontier of regenerative medicine and cell-free therapeutic approaches. These alternatively activated macrophages are key orchestrators of the resolution of inflammation, tissue repair, and immune regulation, and their activities are crucial in both the physiology of healing and therapy. Their exosomes, rich in anti-inflammatory cytokines, pro-regenerative growth factors, and regulatory microRNAs, can reprogram immune responses, induce angiogenesis, and activate stem cells.

Preclinical investigations in a range of conditions, from diabetic fracture and periodontitis to myocardial infarction and neurodegenerative disorders, consistently reveal the therapeutic potential of M2-derived exosomes. Their capacity to activate key signaling pathways such as PI3K/AKT and their capacity to transport targeted miRNA cargo such as miR-21, miR-146a, and miR-93-5p make them pertinent to precision medicine. Partnering with bioengineered matrices and delivery vehicles further enhances their clinical pertinence by facilitating sustained, localized, and environment-sensitive therapeutic interventions.

Yet, notwithstanding promising results, there are many challenges ahead. Limitations to large-scale production, cargo load capacity, targeting specificity, and immune clearance must be overcome to enable the bench-to-bedside transition. Standardization of isolation protocols, in addition to regulatory guidelines, is also paramount to clinical translation.

M2 macrophage-derived exosomes represent an innovative strategy for regenerative and personalized therapies. As we continue to clarify their mechanisms and refine delivery methods, the upcoming research can unleash their full therapeutic capabilities and cement their position as pillars of the future of immunomodulatory and regenerative medicine.

Conflict of interests

The authors declare no conflict of interest.

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Appendix

Table 1. Preclinical applications on M2 macrophage-derived exosomes

Disease/Condition	Study Title / Reference	Source of Exosomes	Key Mechanisms	Experimental Model	Key Outcomes	Clinical Relevance/Potential
ONFH	M2 Macrophages-Derived Exosomes for ONFH	M2 macrophages	miR-93-5p	ONFH mouse model	Reduced NETs, improved vascularization	Bone regeneration via immune modulation
Periodontitis	M2-exos promote osteogenesis via CXCL12	IL-4-induced RAW264.7	CXCL12	LPS-hPDLSCs	Enhanced osteogenesis, reduced inflammation	Periodontal tissue regeneration
Periodontitis	IL-10 mRNA M2-Exos prevent bone loss	Reparative M2-like	IL-10 mRNA	Mouse periodontitis model	Reduced bone resorption, increased IL-10	Anti-inflammatory bone therapy
Periodontitis	miRNA-mediated osteogenesis in hPDLSCs	M2 macrophages	hsa-miR-6085, miR-4800-5p	hPDLSCs, exosome treatment	Upregulated ALP, OCN, mineralization	Stem cell-based periodontal regeneration
Cementum Regeneration	Genetically engineered sh-Ckip-1-EXOs	sh-Ckip-1 M2	Let-7f-5p	Pg-inhibited cementoblasts	Cementum formation, mitochondrial biogenesis	Novel cementum therapy for periodontitis
Atherosclerosis	M2-Exos regulate miR-221-3p in HUVECs	M2 macrophages	miR-221-3p	HUVEC + oxLDL/TNF-α	Reduced apoptosis, cytokines	Endothelial protection in CVD
Cementum	M2 macrophages enhance mineralization	M2 macrophages	p38 signaling	Pg-stimulated cementoblasts	Enhanced mineralization	Regenerative periodontal therapy
Diabetic Fractures	M2-Exos modulate osteoimmunity via PI3K/AKT	M2 macrophages	PI3K/AKT	Diabetic fracture mouse model	Better callus formation	Targeted immune modulation in diabetes
Osteoclast Regulation	M2-Exos regulate CSF2/TNF-α axis	M2 (Raw264.7)	CSF2, TNF-α signaling	BMDM osteoclast precursors	Reduced osteoclastogenesis	Anti-resorptive therapy potential
Diabetic Retinopathy	M2-microglia Exos remodel vasculature	M2 microglia	Unspecified miRNAs	DR mouse model, retinal cells	Angiogenesis, reduced leakage	Eye therapy for diabetic microangiopathy
Wound Healing	3D-bioprinted M2-Exo hydrogels	M2 via hydrogel scaffold	JAK/STAT, PPAR pathways	Murine wound model	Enhanced healing, angiogenesis	Bioengineering wound solutions
Wound Healing	M2-Exos reprogram M1 macrophages	M2 macrophages	iNOS down, Arg1 up	Mice wound injection	Re-epithelialization, collagen deposition	Chronic wound treatment strategy

Disease/Condition	Study Title / Reference	Source of Exosomes	Key Mechanisms	Experimental Model	Key Outcomes	Clinical Relevance/Potential
Flap Survival	M2-Exos act via HIF1AN/HIF-1α/VEGFA	M2 macrophages	HIF-1α, VEGFA	Skin flap model (mouse)	Flap survival, angiogenesis	Graft surgery adjunct therapy
Wound Healing	M2-Exos drive angiogenesis	M2 macrophages	VEGF, HIF-1α	HUVECs, mouse wound model	Capillary density, faster closure	Pro-angiogenic therapy
Atherosclerosis	Exo^M2 stabilize plaques via VSMC phenotype	M2 macrophages	Not specified	ApoE-/- mice + PDGF-BB VSMCs	↓ plaque size, ↑ contractile markers	Atherosclerosis therapeutic angle
Myocardial Infarction	M2-Exos deliver miR-1271-5p	M2 macrophages	miR-1271-5p → SOX6	MI mouse model, hypoxia-cardiomyocytes	Reduced infarct size, apoptosis	Post-infarction repair strategy
KOA	M2-Exos suppress PI3K/AKT/mTOR	M2 macrophages	PI3K/AKT/mTOR inhibition	KOA rat model	↓ MMP-13, ↑ cartilage proteins	Novel osteoarthritis therapy
Brain Inflammation	Mϕ-Exos deliver BDNF to brain	Naive macrophages	BDNF	Inflamed BBB, CNS tissue	Crossed BBB, enhanced delivery	CNS protein delivery vehicle
Neuronal Injury	miR-124-3p M2-exos inhibit ferroptosis	M2-type microglia	miR-124-3p → NCOA4	OGD/R HT22 cells	↓ ROS, ↓ lipid peroxidation	Anti-ferroptosis neurotherapy
SCI	vMIP-II-Lamp2b M2-Exos target injury site	Engineered M2	Targeted delivery, MAPK inhibition	SCI mouse model	↓ cytokines, ↑ motor recovery	Advanced spinal cord repair
SCI	M1 vs. M2 in neurotoxicity	Macrophage types	M1 = toxic, M2 = regenerative	In vivo SCI, in vitro neurons	M2 = axon growth	M2-targeted recovery post-SCI
Colitis	M2b-Exos reduce DSS colitis	M2b macrophages	CCL1/CCR8 axis	DSS-induced colitis model	↓ cytokines, ↑ Tregs	IBD immunotherapy strategy
Hemangiomas	MIR4435-2HG M2-Exos activate NF-κB	M2 macrophages	MIR4435-2HG → HNRNPA1	HemECs, IH tissues	↑ angiogenesis, invasion	Infantile hemangioma target
Cancer	M2-Exos confer immunotherapy resistance	M2 macrophages	miRNAs, proteins reducing antigens	Tumor cells + ICB therapies	↓ CD8+ T activation	Overcoming immune resistance