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REVIEW

Macrophages in dental pulp: dynamic regulators of microenvironmental homeostasis and therapeutic targets in pulpitis

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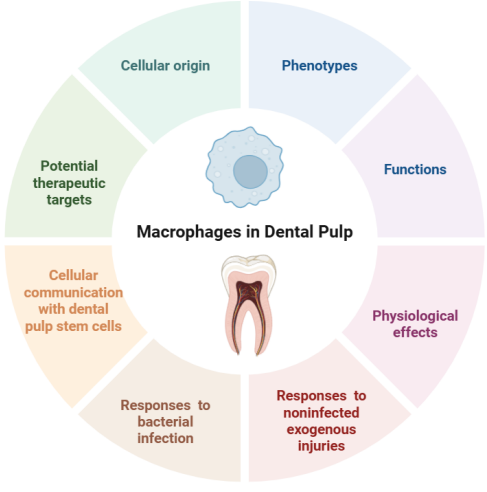
ABSTRACT: The dental pulp is a soft connective tissue enclosed by mineralized dentin, containing odontoblasts, dental pulp stem cells (DPSCs), fibroblasts, endothelial cells, nerves, and immune cells. Under physiological and pathological conditions, these cells collectively regulate the dentin-pulp microenvironment. Macrophages are key immune cells in this niche, participating not only in host defense and inflammatory amplification but also in apoptotic cell clearance, angiogenesis, extracellular matrix remodeling, and reparative dentinogenesis. Recent studies indicate that dental pulp macrophages should not be interpreted solely through the classical M1/M2 dichotomy; instead, their activation states are shaped by local microbial, neural, vascular, and stromal signals. In this review, we summarize current evidence on the origin, phenotype, and function of macrophages in the dental pulp, with particular emphasis on their interaction with DPSCs under inflammatory conditions. We further discuss macrophage-related therapeutic strategies for pulpitis and regenerative endodontics, including modulation of macrophage recruitment, inflammatory resolution, paracrine signaling, extracellular vesicles, and biomaterial-based immunomodulation. Finally, we highlight current limitations and future directions, particularly the need to integrate clinical pulp samples, single-cell/spatial omics, and functional validation to define macrophage states within the dentin-pulp-immune axis.



KEYWORDS: macrophages, polarization, dental pulp stem cells, pulpitis, regenerative endodontics

1 Introduction

The dentine-pulp complex is regarded as an integrated biological entity that shares the same ectomesenchymal origin from the dental papilla[1,2]. It senses external stimuli from the oral cavity, supplies nutrients and innervation to the tooth, and contributes to dentine formation and repair[3]. Under caries, trauma, or restorative procedures, changes in dentinal permeability and pulpal inflammation may disrupt this microenvironment; conversely, controlled inflammation can initiate repair and tertiary dentine formation[4,5]. For a long time, studies on dentine repair have focused primarily on odontoblasts, the direct producers of dentine matrix[6]. Extensive work has characterized odontoblast differentiation, signaling pathways, and matrix secretion[7-9]. However, accumulating evidence indicates that dentine-pulp repair is not driven by odontoblasts alone. Immune cells, vascular cells, nerves, fibroblasts, and dental pulp stem cells (DPSCs) jointly shape the local microenvironment and determine whether an injury resolves toward repair or progresses to irreversible pulpitis[10,11].

The American Association of Endodontists has emphasized the concept of the "dentine-pulp-immune axis," highlighting that immune regulation is intrinsically coupled with the regenerative potential of the pulp[12]. Within this axis, macrophages are particularly important, as they rapidly sense damage- and pathogen-associated signals, coordinate inflammatory responses, clear cellular debris, and provide repair-promoting cues[13,14]. Therefore, understanding dental pulp macrophages requires attention not only to classical macrophage biology but also to tissue-specific conditions of the dental pulp, including dentinal tubules, microbial invasion, hypoxia, neurovascular regulation, and DPSC-mediated repair.

Despite significant progress in understanding macrophage-mediated inflammation, microenvironmental remodeling, and tissue regeneration, several questions remain unresolved: (i) how dental pulp macrophages transition among inflammatory, resolving, and reparative states during disease progression; (ii) how resident macrophages and recruited monocyte-derived macrophages differentially contribute to pulp homeostasis and

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injury repair; (iii) how macrophages communicate with DPSCs, odontoblast-lineage cells, and other stromal cells; and (iv) how macrophage-related mechanisms can be translated into vital pulp therapy and regenerative endodontics[15-18]. This review summarizes current evidence on these issues and discusses future directions within the dentin-pulp-immune axis.

2 Macrophages and macrophages in dental pulp

Macrophages were discovered and named by Elie Metchnikoff in 1882 during his studies on phagocytosis, which laid the foundation for the concept of innate immunity[19]. Subsequent studies revealed that macrophages are widely distributed as either tissue-resident or recruited immune cells, and that their function is strongly shaped by the local microenvironment[20]. In 1887, Metchnikoff further divided phagocytes into macrophages and microphages (the latter later termed neutrophils). In 1924, Aschoff proposed the reticuloendothelial system (RES). From the 1920s to 1950s, numerous scientists progressively established the fundamental functions of macrophages, including phagocytosis, antigen processing, and inflammatory regulation. In 1968, Van Furth et al. revised the RES concept and proposed the mononuclear phagocyte system (MPS), clarifying that macrophages originate from bone marrow monocytes[21]. After decades of research, by 2013, macrophages had become recognized as regulators of development, tissue homeostasis, inflammation resolution, and repair, rather than merely as terminal phagocytes[22,23]. This concept is particularly relevant to the dental pulp, where macrophages operate within a confined tissue space rich in innervation and dentinal tubules, and are exposed to rapidly changing microbial or damage-derived stimuli. During the 1990s, researchers gradually discovered that macrophages can polarize in response to microenvironmental stimuli, exhibiting diverse phenotypes and exerting distinct functions[24]. During this period, the two classic polarized phenotypes—M1 (pro-inflammatory) and M2 (anti-inflammatory)—were identified, along with some of their core regulatory factors: IFN-γ and LPS induce M1 polarization, whereas IL-4 and IL-13 induce M2 polarization. Research on dental pulp macrophages has since evolved from descriptive histology to functional and omics-based analyses. In 1977, Pulver et al. first reported macrophages in inflamed dental pulp[25]. Ten years later, Jontell M. et al. confirmed the presence of immunocompetent macrophages in healthy human dental pulp using monoclonal antibodies[26]. As the most abundant immune cells in the dental pulp, they display a distinct tissue-specific distribution, being concentrated mainly in the central pulp region around microvessels and capillaries. They secrete microbial enzymes, reactive oxygen species (ROS), and cytokines such as IL-1, IL-6, and tumor necrosis factor (TNF), as well as pro-healing factors including fibroblast growth factor and endothelial growth factor. Ito T. et al. demonstrated that M2 macrophages accumulate at mineral trioxide aggregate (MTA) capping sites and participate in reparative dentin formation[27]. In 2015, Gaudin A. et al. characterized the phenotype ($CD68^+$, $CD14^+$) of macrophages in healthy human dental pulp, showing that pulp-resident macrophages promote dental pulp angiogenesis, improve nutrient supply in inflamed pulp, and establish the basis for immune cell trafficking and restoration of immune surveillance[28].

The successful isolation and identification of human DPSCs in 2000 laid the groundwork for studying macrophage-DPSC interactions and facilitated the development of in vitro co-culture models[29]. Huang H. et al. established a rat pulpitis model and confirmed that macrophage polarization patterns differ significantly between types of pulpitis: M2-type macrophages predominate in reversible pulpitis, whereas M1-type macrophages dominate in irreversible pulpitis[30]. In 2020, Zheng J. et al. found that DPSC-derived exosomes contain miR-125a-3p, which induces M2 polarization of macrophages and promotes dentin regeneration through exosome-mediated action on macrophages[31]. The following year, studies revealed that $LYVE\text{-}1^+$ M2-like macrophages promote angiogenesis and participate in dental pulp development and repair, further advancing macrophage research in oral medicine[32].

Subsequently, researchers continued to explore the interaction mechanisms between macrophages and dental pulp cells. In 2023, Gong X. et al. confirmed that DPSCs can transfer mitochondria to macrophages via tunneling nanotubes (TNTs), inducing M2 macrophage polarization and alleviating inflammation. Wang Y. et al. further identified core targets including Gas6-TAM, CD200-CD200R, and PPARγ, providing a theoretical basis for novel therapeutic approaches such as immunomodulatory vital pulp therapy[33].

Taken together, these findings underscore the considerable potential of research on dental pulp macrophages, while also highlighting their yet-to-be-elucidated critical roles in dental pulp immunity and cellular crosstalk.

3 Macrophage phenotypes and their key functions

In traditional studies, macrophages are mostly divided into M0, M1, and M2[35]. The most widely used classification currently divides human tissue macrophages into three main subsets: M0, M1 (pro-inflammatory), and M2 (anti-inflammatory). M1 macrophages are classically activated, whereas M2 are alternatively activated. Immunohistochemical staining shows high CD80 expression in M1 and high CD206 in M2, reflecting their distinct functions and transcriptional profiles[36].

M0 macrophages possess strong phagocytic capacity and can rapidly polarize into pro- or anti-inflammatory states. M1 macrophages are typically induced by TLR ligands, Th1 cytokines (IFN-γ, TNF-α), or LPS[37]. Core regulators include STAT1, IRF5, and NF-κB (p65/p50), with HIF-1α significantly activated. During infection, M1 cells activate the NADPH oxidase system to produce ROS and clear pathogens. Their surface markers include CD80, CD86, MHC II, TLR2/4, Fc receptors (CD16, CD32, CD64), and CCR7[38]. They secrete IL-6 and TNF-α, activating immune defense against pathogens and tumors. This pathway is the primary activation route when PAMP/DAMP and Th1 signals coexist[39], contributing to clearance and early warning in acute inflammation. However, M1 activity can impair wound healing[40]; thus, late chronic inflammation is primarily regulated by anti-inflammatory M2 macrophages.

M2 polarization is induced via STAT6 activation following IL-4/IL-13 binding to IL-4Rα. IL-10 can also promote M2 via STAT3[41-43]. M2 macrophages have strong phagocytic capacity, clear debris and apoptotic cells, promote tissue repair, and exhibit pro-angiogenic and pro-fibrotic traits[44]. They secrete IL-10 and TGF-β to suppress inflammation and support regeneration. Based on external stimuli and transcriptional changes, M2 macrophages are further subdivided into M2a, M2b, M2c, and M2d[45]. M2a macrophages are considered the primary subset involved in tissue regeneration. Triggered by IL-4/IL-13 and supplemented by M-

CSF, IL-33, and others, they exhibit enhanced fatty acid oxidation, restored OXPHOS as the main energy source, and reduced glycolysis. STAT6, IRF4, and PPARγ are core regulators, while NF-κB is suppressed. Markers such as Arg1, CD206, CD163, and FIZZ1/Ym1 are highly expressed, whereas iNOS and CD86 are low. M2a macrophages promote cell growth and tissue repair and participate in anti-inflammatory and immunoregulatory processes[46]. M2b macrophages regulate immune responses and exert strong anti-inflammatory effects. They are the only subtype that co-expresses M1-like markers (CD80/CD86) and high IL-10, balancing pro- and anti-inflammatory activities as a transitional subtype connecting M1 and M2. Their polarization is triggered by immune complexes combined with LPS/IL-1; ICs cross-link FcγR, and LPS/IL-1 activate TLR4/IL-1R[39]. STAT3 and IRF4 are core regulators: STAT3 promotes IL-10 secretion, and IRF4 inhibits M1 tendency. Surface markers CD80, CD86, CD14, and MHC-II are highly expressed, while CD206/CD163 are low; both glycolysis and fatty acid oxidation coexist[39]. M2c macrophages, also known as inactivated macrophages, specialize in clearing apoptotic cells and do so more rapidly than other subsets[47]. As an immunosuppressive subtype, they are commonly used for in vitro M2 studies[48]. Core induction signals include IL-10, TGF-β, glucocorticoids, and IL-27[49]. Surface markers CD163, CD206, and MerTK are highly expressed, whereas CD80 and CD86 are low[47]. Key pathways include TGF-β/Smad and IL-10R/JAK/STAT3, with STAT3, STAT6, and NF-κB (p50/p50) as primary transcription factors. M2d macrophages promote angiogenesis and tumorigenesis[50]. Research on M2d is relatively recent; they are also known as $Tie2^+$ or adenosine-activated macrophages[51]. M2d can be activated by adenosine combined with TLR agonists, IL-6, TNF-α, or hypoxia. Through pathways such as A2aR/A2bR-AMPK, STAT1/STAT3, PI3K/Akt, and HIF-1α, they drive angiogenesis and matrix remodeling under hypoxic or metabolically disordered conditions[52,53].

Owing to their high plasticity, macrophages exhibit overlapping expression profiles in vivo and form a continuous spectrum with spatial heterogeneity. Pure M1 or M2 states are rare; intermediate, mixed, and tissue-specific subsets predominate. Traditional in vitro classification cannot reflect the true dental pulp microenvironment, where macrophages interact intricately with blood vessels, odontoblasts, stem cells, and other cells.

Recent single-cell RNA sequencing, spatial transcriptomics, and multi-omics have further challenged the M1/M2 dichotomy[52,53]. These technologies allow subset definition by functional modules such as chemokine production, phagocytosis, antigen presentation, angiogenesis, ECM remodeling, and tissue repair. In dental pulp research, this shift is critical because macrophage states change along the continuum from homeostasis, early injury, and reversible inflammation to irreversible pulpitis and repair failure. In 2022, Ren H. et al. constructed the first single-cell atlas of human dental pulp, defining homeostatic subsets[54]. Using scRNA-seq on tissues at four developmental stages, they identified $CD68^+/CD163^+$ mononuclear phagocytes as the main immune cells, further divided into Mφ1 ($CD163^{high}/LYVE1^+$; perivascular residence, pro-angiogenesis, homeostasis), Mφ2 ($CD14^{high}/MHCII^{low}$; immune surveillance, mild inflammation), and Mφ3 ($MHCII^{high}/CD80^{low}$; antigen presentation, immune regulation). Huang H. et al. (2023) combined scRNA-seq with animal models and confirmed that reversible pulpitis is dominated by M2-like ($CD206^{high}/IL10^+$) macrophages with intermediate states, whereas irreversible pulpitis shows elevated M1-like ($CD80^{high}/TNFα^+$) macrophages, although $CD206^+$ repair subsets remain. Thus, macrophages do not exist in absolute M1 or M2 states but form a continuous polarization spectrum[30]. Sun T. et al. (2024) identified senescent pulp-specific $Ccrl2^+$ macrophages that highly express CCRL2/CMKLR1 and are recruited/activated via the RARRES2/CMKLR1 axis, promoting pulp fibrosis and inflammatory imbalance. This subset cannot be defined by traditional M1/M2 classification[55]. Gong Q. performed scRNA-seq with proteomics on chronic apical periodontitis, identifying prothymosin-α as a novel target in infected macrophages, and found that the $CD163^+/MHCII^+$ mixed subset exhibits both anti-inflammatory and antigen-presenting functions[56]. In 2026, a Visium spatial transcriptomics study localized macrophage subsets in intact dental pulp tissue for the first time and proposed the concept that "spatial location determines phenotype." Specifically, M1-like ($CD80^+/CXCL8^+$) macrophages in the coronal region adjacent to caries served as the inflammatory frontline; intermediate ($CD206^+/MHCII^+$) macrophages in the central pulp participated in immune regulation; and $LYVE1^+/CD163^+$ macrophages in the root pulp and perivascular regions mediated repair and angiogenesis[57].

Technological innovations have driven a paradigm shift: from a dichotomous classification to a continuous spectrum with distinct subsets, from *in vitro* studies to *in situ* spatial exploration, from single markers to transcriptome-defined subsets, and from static descriptions to dynamic trajectories. Current research has therefore moved beyond the rigid M1/M2 framework. Based on microenvironmental initiation signals, monocyte-derived macrophage activation can be categorized into several functional pathways, including inflammatory cytokine production, oxidative stress and antimicrobial responses, phagocytic clearance, and extracellular matrix (ECM) remodeling (Fig. 1)[56,58]. In dental pulp, these pathways may be activated sequentially or simultaneously, depending on factors such as caries depth, bacterial load, tissue hypoxia, vascular changes, and signals derived from DPSCs or odontoblasts. The inflammatory cytokine production pathway (primarily the TLR4-NF-κB/IRF axis) employs LPS and IFN-γ as core triggers, supplemented by TNF-α and GM-CSF. This pathway is activated in the presence of PAMP/DAMP and Th1-type immune signals, exhibiting characteristics similar to M1 phenotype. Excessive activation of this pathway can lead to tissue damage and chronic inflammation. The oxidative stress and antibacterial pathway (mainly the JAK-STAT axis) serves as a molecular bridge for macrophage polarization. With IL-4/IL-13 as the core trigger, this pathway is dominated by Th2 signals and tissue repair demands. Different STAT branches exert antagonistic effects: STAT1 and STAT6 inhibit each other, while STAT3 suppresses STAT1 and NF-κB, enabling fine-tuned regulation. The phagocytosis pathway (primarily the PI3K-AKT-mTOR/HIF-1α axis) is induced by M-CSF, IL-4, high glucose/lactic acid, hypoxia, or oxidized low-density lipoprotein (OxLDL). It links metabolism with immune activation, providing energy and biosynthetic materials for monocyte-derived macrophage activation, and exhibits extensive crosstalk with the JAK-STAT and TLR4-NF-κB pathways[39]. The extracellular matrix remodeling pathway (mainly the TGF-β-Smad axis) is initiated by TGF-β, IL-4 and IL-10, and is predominantly activated at the late stage of inflammation or during tissue repair. Using the Smad2/3/4 heterotrimer as a core transcription factor complex, it cooperates with STAT6, PPARγ, and KLF4 to potently suppress inflammation and enhance efferocytosis. These pathways cross-talk with one another to coordinate inflammatory cytokine production, oxidative stress responses, phagocytosis, and matrix remodeling. Therefore, in the

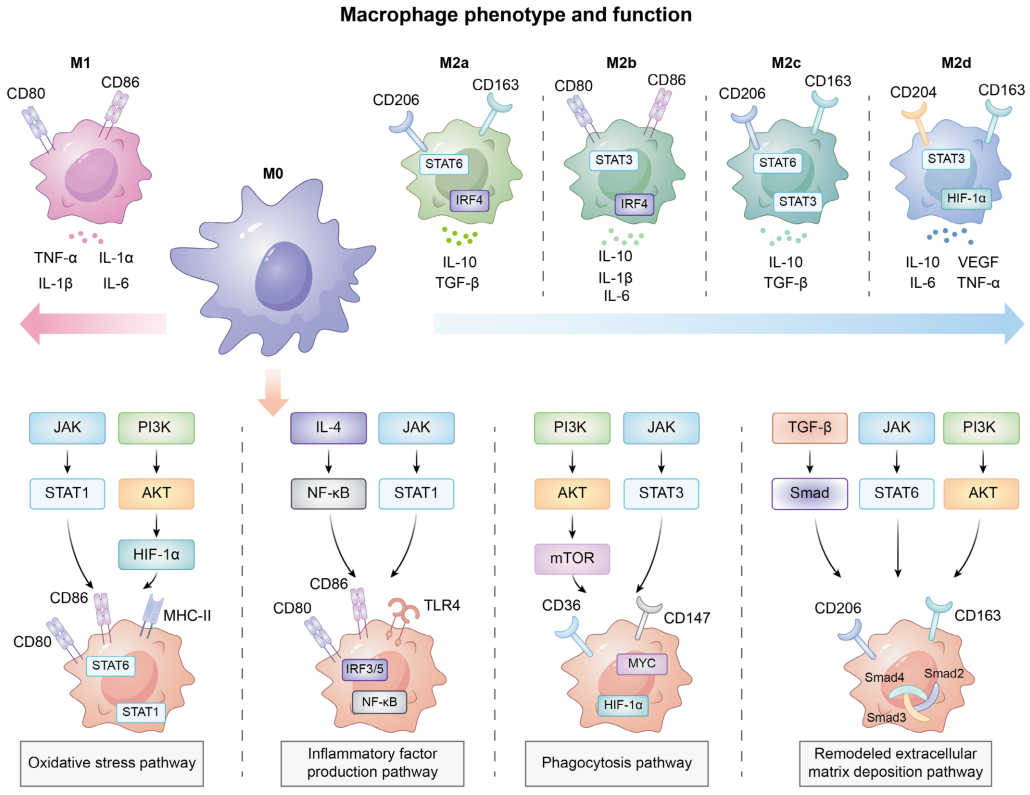

Figure 1 Schematic representation of macrophage phenotype and key function. The figure was created by Adobe Illustrator (Adobe Inc., California, USA).

following sections, macrophage function in the dental pulp is discussed in the context of physiological homeostasis, non-infectious injury, and bacterial infection, rather than solely on the basis of static polarization markers.

4 Pathophysiological effects of macrophages in dental pulp

4.1 The role of macrophages during physiological conditions

The establishment of comprehensive single-cell transcriptome atlases of mouse and human teeth has become an important resource for research in stomatology. For instance, the observation of a relatively high proportion of macrophages in mouse teeth may be attributed to the young developmental age of the sampled tissues, as studies have suggested that macrophages play a significant role in both dentin and enamel formation. In 1987, Jontell, Gunraj, and Bergenholtz used monoclonal antibodies to demonstrate that the human dental pulp contains cells capable of initiating immune responses under normal conditions, providing the first confirmation of the presence of macrophages in healthy dental pulp[26]. Similar to macrophages in other parts of the body, dental pulp macrophages originate from blood monocytes and subsequently proliferate and differentiate in situ, without requiring a supply of precursor cells from the blood[59]. One year later, the same research group found that both macrophages and dendritic cells in the dental pulp express MHC class II antigens, indicating that dental pulp tissue possesses the ability to process and present foreign antigens[60]. However, macrophages and dendritic cells expressing MHC class II antigens only enter the tissue and mature during the late stages of dental pulp development. Compared with alveolar bone and oral mucosal tissues, the appearance of immunocompetent cells in the dental pulp is delayed, suggesting that the dental pulp acquires immune defense capacity later than other tissues in the body[61].

Subsequently, Okiji and colleagues employed immunohistochemical double staining and identified that dental pulp macrophage-like cells can be OX35$^+$, ED1$^+$, or ED2$^+$. They found that nearly all such cells are ED1$^+$ and can be divided into OX6$^-$/ED2$^+$ and OX6$^+$/ED2$^+$ subsets. Among these, the OX6$^-$/ED2$^+$ cells are the most abundant and are predominantly distributed in the coronal pulp[34]. In addition, CD14 and CD68 are surface markers highly expressed on macrophages[62]. Among these, F4/80$^+$ macrophages constitute the main immune cell population in healthy dental pulp, accounting for approximately 68% of total immune cells[63]. Macrophages and dendritic cells are both major immune cells in the dental pulp, concentrated around blood vessels and near the odontoblast layer of the coronal pulp. They are positioned at the forefront of the pulp exposed to external stimuli, acting as the first line of defense in the dental pulp immune system to monitor foreign antigens[64]. Apart from morphological characteristics, macrophages can be distinguished from dendritic cells by their higher phagocytic capacity and greater acid phosphatase activity[65]. Generally, dendritic cells lack phagocytic capacity; they represent a distinct cell lineage from macrophages and lack the intracellular lysosomes and phagocytic vacuoles typically found in macrophages[26]. Consequently, macrophages are more effective than dendritic cells at taking up and processing antigens[66].

Under normal conditions, tooth tissue wear resulting from long-term chewing or age-related changes can induce the formation of tertiary dentin[67]. Previous studies have shown that LYVE-1$^+$ resident macrophages in the dental pulp colonize the dental papilla

at the embryonic bell stage (E18). These cells are mainly localized around capillaries, co-localizing with the endothelial marker CD146. Their expression is bidirectionally regulated by inflammatory signals from the microenvironment, and they participate in the repair and regeneration of dental pulp tissue through pro-angiogenic mechanisms[32]. Furthermore, studies have demonstrated that macrophages are key regulators of local dental pulp stem cell activation and inflammatory balance during tertiary dentin formation[68]. They release regulatory factors such as BMP2, TGF-β1, and TGF-β3, actively participating in the odontogenic differentiation of dental pulp stem cells (DPSCs) and regulating the physiological formation of tertiary dentin[31].

4.2 Responses of macrophages to noninfected exogenous injuries

Dental trauma is a common cause of tooth defects and pulpal injury[69]. Exogenous injury to the tooth induces multicellular responses in the local immune system of the pulp, including apoptosis, proliferation, migration, and differentiation. For example, cavity preparation during iatrogenic procedures and tooth replantation can cause destructive changes in odontoblasts in the affected area, leading to early neutrophil aggregation, followed by macrophage and fibroblast responses. Subsequently, medullary mesenchymal cells replace degenerated odontoblasts and differentiate into odontoblast-like cells to form reparative dentin[70,71]. The role of macrophages in pulp injury is illustrated below using examples of dental trauma and tooth replantation. Several studies have used mouse dental trauma models to investigate macrophage function. Paganelli et al. performed pulpotomy on the occlusal surface of the left upper first molar in rats using a round bur, exposed the pulp with a sterile probe, applied direct pulp capping with MTA, and sealed with glass ionomer[72]. On days 1 and 2 post-treatment, the number of $CD86^+$ M1 macrophages increased, whereas $CD206^+$ M2 macrophages increased significantly on day 5. Reparative dentin formed 14 days later. These results indicate that M1 macrophages mediate the early inflammatory response after pulp exposure, whereas M2 macrophages contribute to reparative dentin formation. Further research by the same team showed that conditioned medium from M2 macrophages significantly upregulated the odontogenic differentiation effect of MTA on DPSCs, suggesting that M2 macrophages secrete factors that promote DPSC odontogenic differentiation[36]. Previous studies have reported that M2 macrophages strongly induce mesenchymal stem cell mineralization[73,74]. M2 macrophages have been shown to induce DPSC odontogenic differentiation and reparative dentin formation, and the MSC-mediated inhibitory effect also influences monocytes and activated macrophages, promoting M0-to-M2 polarization[72].

Hara et al. established a similar model and detected Wnt10a in $F4/80^+$ macrophages on day 14 after direct pulp capping, indicating that these macrophages, like odontoblasts, also secrete Wnt10a. Given the established role of the Wnt signaling pathway in odontoblast differentiation and dentin formation[75], the observation of β-catenin in the nuclei of pulp cells surrounding macrophages further suggests that macrophage-derived Wnt10a contributes to odontoblast differentiation during dentin bridge formation[76]. In 2000, Shimizu et al. developed a tooth replantation model by extracting mouse first molars and immediately reimplanting them without treatment. They observed an increase in both the number and immunostaining intensity of $ED1^+$ cells. By day 7, $ED1^+$ cells were scattered in the odontoblast layer, reparative dentin began to form, and nerve regeneration appeared in the coronal pulp[77]. More recently, a mouse tooth transplantation model was established by extracting the right upper first molar, resecting the root and pulp chamber floor, and implanting the crown into the sublingual area. On days 1–3 post-surgery, macrophages highly expressed GM-CSF and osteopontin (OPN), with some aligning along the pulp-dentin border. Tubular dentin formation occurred at the exposure site on days 5–7, and osteoid tissue formed in the pulp cavity by day 14. Macrophages and dendritic cells were identified as the main OPN-secreting cells during pulp healing, consistent with Rothstein's report that OPN can be secreted by T cells, B cells, dendritic cells, and macrophages[78]. Functionally, OPN mediates cell activation and cytokine production during immune responses, and also acts as an adhesion protein to promote cell attachment and wound healing[79]. In the context of pulp regeneration after replantation, macrophages promote dendritic cell maturation and odontoblast differentiation by secreting GM-CSF and OPN, thereby facilitating reparative dentin formation[80]. Neves et al. used clodronate liposomes to deplete macrophages and found that reparative dentin formation was significantly reduced in the absence of these cells. Building on previous evidence that Wnt pathway activation promotes reparative dentin formation in carious pulpitis[81,82], they demonstrated that macrophages are essential for activating Wnt-responsive stem cells. Furthermore, increased Wnt signaling in pulp cells indirectly drives the transition of macrophages from a pro-inflammatory to an anti-inflammatory phenotype. Collectively, these findings indicate that the Wnt/β-catenin signaling pathway plays a dual role in reparative dentin formation: it both activates dental pulp stem cells and initiates an anti-inflammatory macrophage response[68].

Exogenous injury can act directly on macrophages to induce cytokine secretion and downstream signaling changes that affect dentin formation. Additionally, external stimuli can indirectly activate the dentinogenesis-promoting function of macrophages by acting on other pulp cells. Fibroblasts, the most abundant cell type in dental pulp, are mainly distributed in the coronal pulp, where they form and maintain the extracellular matrix, providing structural support. Fibroblasts also secrete cytokines, chemokines, and growth factors to maintain pulp homeostasis and participate in pulp repair and regeneration[83]. However, these models have limitations, including the inability of in vitro co-cultures to fully replicate the in vivo microenvironment, high heterogeneity of clinical samples, and insufficient dynamic temporal evidence, which require further validation and investigation.

4.3 Responses of macrophages to caries-related bacterial infection and pulpitis

4.3.1 Early pulp response and mild pulpitis

Dental caries is a chronic, progressive disease of hard tooth tissues primarily caused by bacteria. During caries progression, bacterial products can reach the pulp through dentinal tubules even before direct pulp exposure, thereby activating odontoblasts, fibroblasts, and immune cells. Le Fournis et al. physically damaged fibroblasts and then stimulated them with lipoteichoic acid (LTA) to simulate the post-bacterial pulp environment; their findings suggested that pulp fibroblasts influence macrophage differentiation and immune defense during pulpal inflammation[84]. Previous studies have also described pulpal immune responses and biomedical approaches for pulp-dentin healing[85-87]. In this context, macrophages should be viewed not only as antibacterial effector cells but also as regulators

of stromal cell behavior, extracellular matrix remodeling, and the early reparative dentin response. In carious teeth, oral pathogens and their products enter the pulp through exposed dentinal tubules, inducing an inflammatory response. During early inflammation, odontoblasts release chemokines such as CCL2 to recruit monocytes/macrophages[88]. Histological evidence from human pulpitis samples indicates that inflammatory cell infiltration and immune marker expression vary with clinical severity, although the precise relationship between clinical diagnosis and macrophage activation states remains poorly defined[89]. Thus, reversible or mild pulpitis may be best understood as a stage in which macrophage-mediated antimicrobial defense and inflammatory resolution coexist with the potential for dentin-pulp repair.

In 2020, Neves et al. clearly proposed that macrophages are key regulators of dental pulp inflammation and reparative dentin synthesis[68]. The dental pulp is richly innervated by trigeminal afferent fibers. Intrapulpal hypertension and tissue damage caused by inflammation can lead to acute peripheral nerve injury, which in turn induces M2 macrophage polarization and triggers anti-inflammatory and immunosuppressive responses[89]. Studies have shown that timely M1-to-M2 transition in the inflammatory microenvironment effectively regulates dentin formation, positioning this process as a potential modulator of parenchymal stromal tissue regeneration[90]. Macrophage inflammatory protein-3α (MIP-3α) is a 9 kDa CC chemokine expressed in macrophages, dendritic cells, lymphocytes, and other cell types[91]. At carious sites where bacteria invade and cause inflammation, macrophage-derived MIP-3α may play a defensive role by recruiting cells expressing CC chemokine receptor 6[92]. Additionally, MIP-3α promotes odontogenic differentiation of dental pulp stem cells by stimulating dentin sialophosphoprotein expression and exhibits bacteriostatic activity against cariogenic bacteria such as Streptococcus mutans and Lactobacillus[93]. In vitro experiments by Iejima et al. demonstrated that MIP-3α enhances the transcription and expression of odontogenic genes without affecting osteogenic differentiation-related proteins, further supporting the importance of macrophage-derived MIP-3α in dentin formation[94]. Recent *in vivo* pulpitis models have further elucidated macrophage responses to bacterial infection. Huang et al. established a carious pulpitis model in mice using a high-sugar diet and found that reversible pulpitis is dominated by M2 macrophages, whereas irreversible pulpitis is dominated by M1 macrophages. The spatiotemporal distribution of M1 and M2 macrophages also differs: M1 cells are mainly located in the pulp region adjacent to carious tissue, while M2 cells predominate in the surrounding inflammatory area. As pulpitis progresses, M1 macrophage numbers increase significantly during the exacerbation phase, M2 macrophages increase during the tissue repair phase[30], and total macrophage numbers rise with pulpitis severity[89]. In a rat caries-induced reversible/irreversible pulpitis model, reversible pulpitis is characterized by a low bacterial load confined to superficial dentin, a predominance of M2-like macrophages with a small number of intermediate states, and moderate TLR4-NF-κB activity, collectively favoring immune defense and repair[30].

4.3.2 *Severe or irreversible pulpitis*

Severe infection drives irreversible pulpitis, characterized by markedly elevated M1-type ($CD80^+$) macrophages and sustained NF-κB activation, which triggers a pro-inflammatory cytokine storm leading to pulp necrosis[30]. Immediate intervention is critical at this stage. Although persistent bacterial stimulation can maintain macrophages in a pro-inflammatory state, exacerbating cytokine release, tissue edema, and neurovascular dysfunction[27,95-96], severe pulpitis is not merely an amplification of early inflammation. It also involves impaired clearance of apoptotic cells, disrupted macrophage-DPSC communication, vascular compromise, and failure of reparative dentinogenesis. This distinction is clinically important, as therapies focused solely on suppressing inflammation may also compromise host defense or impair repair-promoting macrophage functions.

In summary, bacterial infection and pulpitis should be regarded as a continuous pathological process rather than discrete entities. Along this continuum, dental pulp macrophages initiate immune defense, communicate with odontoblast-lineage cells and DPSCs, and determine whether the dentin-pulp complex progresses toward repair or irreversible damage (Fig. 2).

5 Crosstalk between macrophages and dental pulp stem cells (DPSCs) in the pulpal inflammatory microenvironment

Cell communication between macrophages and DPSCs can be discussed through four partially overlapping categories: direct cell-to-cell contact, extracellular vesicle or organelle transfer, paracrine signaling via soluble mediators, and extracellular matrix-mediated regulation. This classification is useful because many cytokines

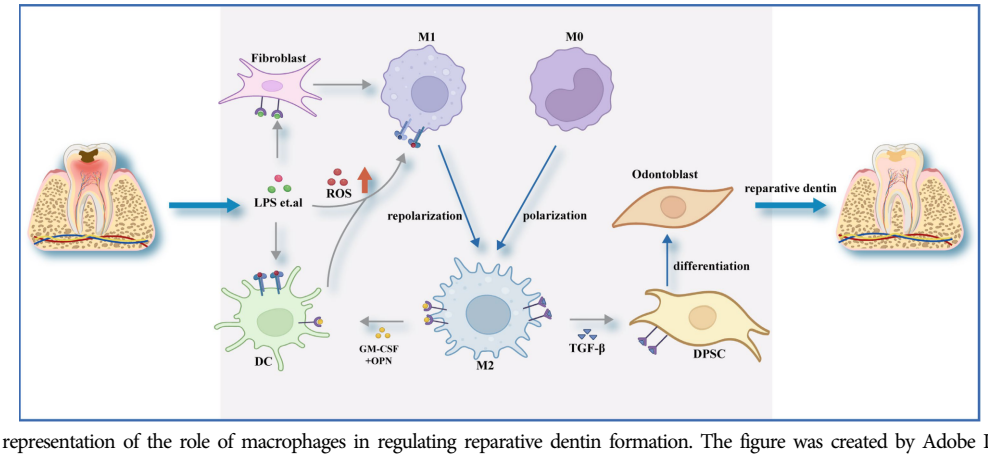

Figure 2 Schematic representation of the role of macrophages in regulating reparative dentin formation. The figure was created by Adobe Illustrator (Adobe Inc., California, USA).

previously attributed to "cell-extracellular matrix interaction" are more accurately considered paracrine mediators. Macrophages and mesenchymal stem cells may communicate through direct contact, including intercellular receptor-ligand interactions, tunneling nanotubes, and in some contexts, membrane hemifusion—an intermediate state where outer leaflets of two lipid bilayers merge while inner leaflets remain distinct[97]. However, several direct-contact mechanisms remain less validated in the dental pulp and should be interpreted cautiously. Although macrophages support stem cell behavior via close-range or niche-like signals in other tissues[98,99], their direct applicability to macrophage-DPSC crosstalk requires further confirmation.

Receptor-mediated interactions, such as the CD200-CD200R axis, regulate stem cell activity by maintaining immune homeostasis in the stem cell niche. This axis reduces TSG6 expression in macrophages, thereby decreasing $CD4^+$ T cell activation and promoting an anti-inflammatory state that enhances stem cell activity[100-103]. Notably, this immunosuppressive effect is lost in transwell indirect co-culture, indicating dependence on direct contact[102]. CD200 is expressed in DPSCs, but direct evidence that the CD200-CD200R axis regulates macrophage-DPSC interactions in the dental pulp remains lacking.

Mitochondrial transfer—horizontal movement of mitochondria from donor to recipient cells via tunneling nanotubes, extracellular vesicles, free mitochondrial release, or partial cell fusion[104,105]—has gained attention because it links intercellular communication with metabolic reprogramming[106-108]. Recent dental pulp studies suggest that mitochondrial transfer may participate in DPSC-macrophage communication and responses to pulp damage[109,110]. However, technical challenges remain: distinguishing intact mitochondrial transfer from fragments, tracing donor-recipient directionality in vivo, and proving functional necessity beyond mere association with inflammation.

In the inflammatory microenvironment, DPSCs secrete both pro- and anti-inflammatory factors, thereby modulating macrophage phenotypes[111,112]. Co-culture studies have shown that dental pulp cells alter macrophage inflammatory responses[113,114], and inflamed DPSCs regulate macrophage function via the TNF-α/IDO axis[115]. Additionally, DPSC-mediated IL-6/GP130/STAT3 signaling promotes anti-inflammatory macrophage transformation and enhances osteogenic potential[116]. These findings highlight soluble cytokines and metabolic enzymes as central components of macrophage-DPSC paracrine communication. Extracellular vesicles (EVs) represent another non-contact mechanism. In 2020, Zheng et al. reported that DPSC-derived small EVs participate in immune regulation during pulp inflammation. Subsequent studies showed that DPSC-derived exosomes or small EVs suppress pro-inflammatory macrophage polarization and regulate pathways such as ROS-MAPK-NF-κB p65 signaling[117,118]. Compared with direct cell transplantation, EVs offer potential advantages in safety, storage, and local delivery, but their cargo composition, dose-response relationship, and in vivo persistence require further investigation.

DPSCs induce macrophage polarization to regulate inflammation, and conversely, macrophages exert regulatory effects on DPSCs. Park et al. cultured human dental pulp cells (HDPCs) with conditioned medium from M1 or M2 macrophages. M2 conditioned medium enhanced alkaline phosphatase (ALP) activity, promoted dentin sialophosphoprotein (DSPP) mRNA expression, and induced matrix mineralization, whereas M1 conditioned medium had no effect on ALP activity[118]. Similarly, Jun Zhou et al. found that conditioned medium from M2 macrophages significantly increased DPSC migration, growth, and osteogenic differentiation compared to M0, M1, or control media. After five days, DPSC migration was higher in M1 than in M0 medium, but growth and osteogenic differentiation remained lower than in M2 medium[119].

In summary, macrophages and DPSCs interact bidirectionally to maintain or restore pulpal microenvironmental balance. Direct contact may provide close-range regulation, whereas paracrine mediators, EVs, and mitochondrial transfer may coordinate broader inflammatory and metabolic responses (Fig. 3). Future

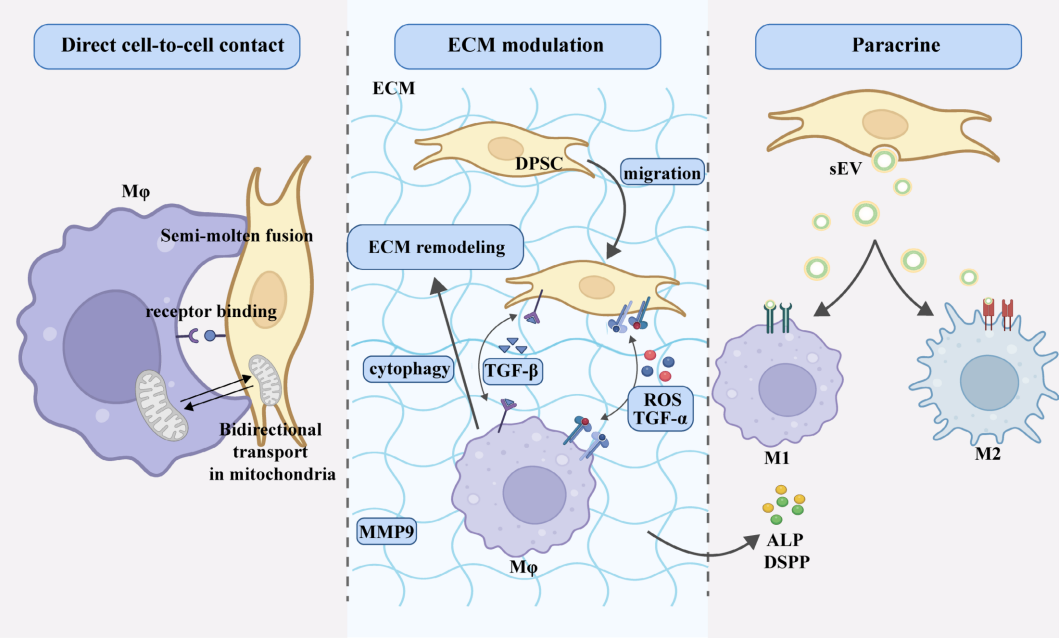

Figure 3 Schematic representation of the crosstalk between macrophages and dental pulp stem cells (DPSCs) in the pulpal inflammatory microenvironment. The figure was created by Adobe Illustrator (Adobe Inc., California, USA).

studies should clarify which communication mode dominates at different stages of pulpitis and which mechanisms are truly required for reparative dentinogenesis.

6 Potential macrophage-related therapeutic targets in pulpitis and regenerative endodontics

Precise regulation of macrophage numbers is essential for balancing pulp inflammation and repair. Studies have shown that macrophages rapidly accumulate during the initial inflammatory phase, whereas subsequent odontoblast-like differentiation and reparative dentin formation depend on timely inflammatory resolution[30,68]. Therefore, strategies targeting macrophage recruitment—such as those involving the CCL2/CCR2 or CX3CL1/CX3CR1 pathways—should be tailored to disease stage and the need to preserve host defense[120-128]. Macrophage phenotype determines their secretory profile and regulatory effects on stem cells. Multiple pathways contribute to macrophage activation, including PPARγ, IL-10/IL-10R, STAT6, TLR4/NF-κB, and miRNA-mediated regulation[129-133]. In the dental pulp, these pathways should not be viewed as simple "M1-to-M2 switching" targets. A more realistic therapeutic goal is to reduce destructive inflammation while preserving antibacterial activity, efferocytosis, and repair-supportive signaling. Recent work on macrophage-derived exosomes further suggests that immunomodulatory vesicles may promote odontogenesis, neurogenesis, and angiogenesis in regenerative endodontics[33].

The interaction between macrophages and DPSCs also offers potential intervention points, with relevant pathways including Gas6-TAM, Annexin A1-FPR2, CD200-CD200R, and Sema3A-related signaling (Fig. 4)[134-141]. However, much of the evidence for these pathways derives from non-pulpal tissues or broader regenerative medicine studies. Thus, their application in pulpitis treatment should be considered hypothesis-generating until validated in dental pulp-specific in vitro systems, animal models, and clinical samples. Recent biomaterials research has shown that scaffold composition, surface properties, and degradation products can influence macrophage behavior. IL-4-loaded hydrogels, silicon-rich materials, calcium silicate-based biomaterials, and nanoparticle delivery systems have been reported to modulate macrophage polarization or tissue repair in various contexts[142-144]. For vital pulp therapy and regenerative endodontics, local delivery is particularly important because systemic macrophage modulation may cause off-target immunological effects. Accordingly, future materials should

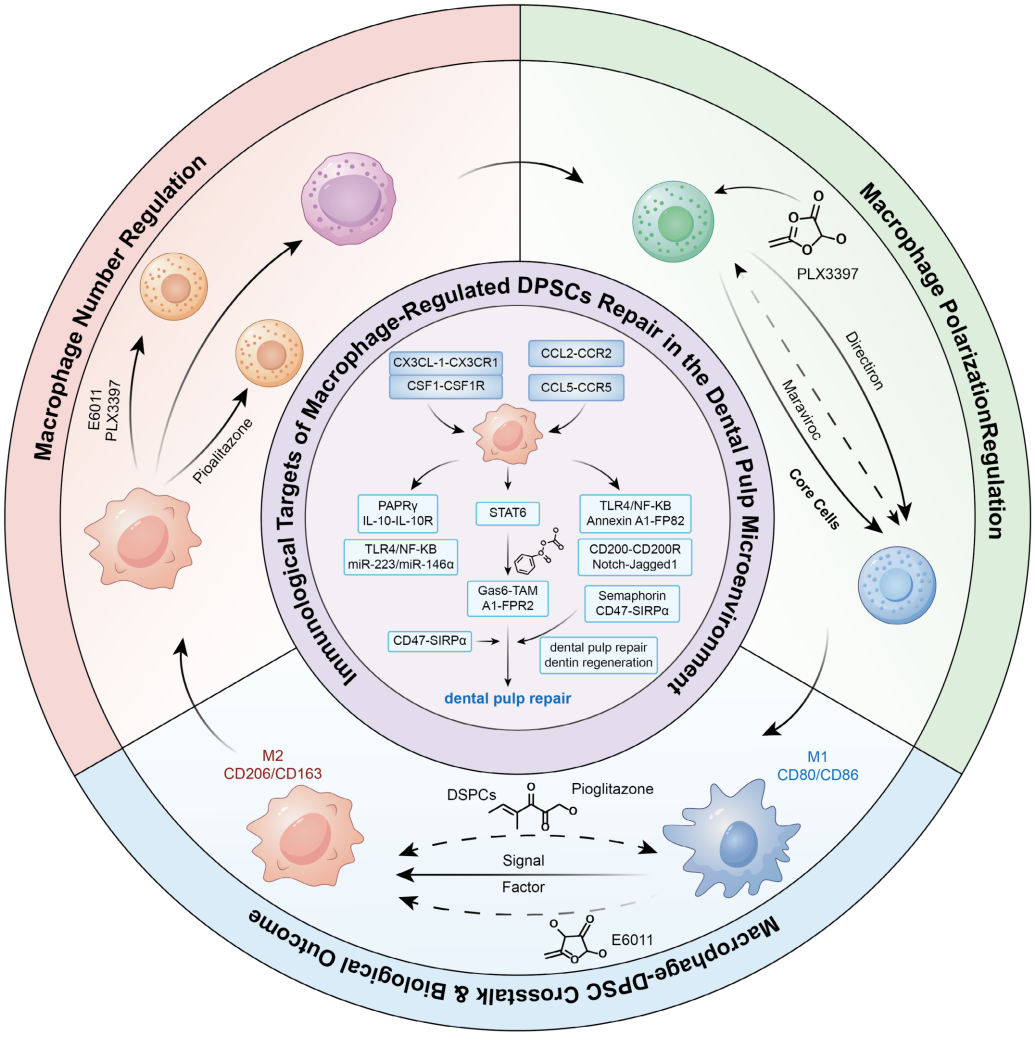

Figure 4 Schematic representation of the potential therapeutic targets related to macrophage. The figure was created by Adobe Illustrator (Adobe Inc., California, USA).

be designed to provide spatially and temporally controlled immunomodulation within the confined pulp space.

7 Concluding remarks and future perspectives

This review summarizes the potential roles of macrophages in physiological and pathological dentin formation within the dental pulp microenvironment, as well as the communication mechanisms between macrophages and DPSCs. Available evidence indicates that dental pulp macrophages participate in immune surveillance, inflammatory amplification, apoptotic cell clearance, extracellular matrix remodeling, angiogenesis, and reparative dentinogenesis. Rather than acting as a purely pro-inflammatory or anti-inflammatory population, they likely exist along a continuum of activation states shaped by microbial stimulation, tissue injury, vascular and neural signals, and stromal cell-derived cues.

Several limitations remain. First, the relative contributions of resident versus recruited monocyte-derived macrophages in human dental pulp are unclear. Second, the relationship between clinical pulpitis classification and macrophage functional states has not been systematically established. Third, many macrophage-DPSC communication mechanisms, particularly direct contact and mitochondrial transfer, require in vivo validation. Fourth, candidate therapeutic targets derived from other organs or systemic diseases need careful testing in dental pulp-specific models. Future studies should integrate clinical pulp samples, lineage tracing, single-cell and spatial transcriptomics, proteomics, metabolomics, and functional perturbation experiments. Recent studies on DPSC-mediated immune regulation, Sema3A-dependent sensory nerve-regeneration coupling, and bone-nerve crosstalk further suggest that pulp regeneration should be viewed as coordinated neuro-immune-mesenchymal regulation rather than isolated macrophage polarization[145-147].

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Data available statement

N/A

Author contribution

W.X. and B.W. guided and revised the manuscript. H.Z., J.C. and S.T. wrote the manuscript, L.C. and Z.W. revised the manuscript. The author(s) read and approved the final manuscript.

Ethics approval and consent

N/A

Consent for publication

All authors agree to publish.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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