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Review article

# Shaping the immune landscape: Multidimensional environmental stimuli refine macrophage polarization and foster revolutionary approaches in tissue regeneration

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ABSTRACT

In immunology, the role of macrophages extends far beyond their traditional classification as mere phagocytes; they emerge as pivotal architects of the immune response, with their function being significantly influenced by multidimensional environmental stimuli. This review investigates the nuanced mechanisms by which diverse external signals ranging from chemical cues to physical stress orchestrate macrophage polarization, a process that is crucial for the modulation of immune responses. By transitioning between pro-inflammatory (M1) and anti-inflammatory (M2) states, macrophages exhibit remarkable plasticity, enabling them to adapt to and influence their surroundings effectively. The exploration of macrophage polarization provides a compelling narrative on how these cells can be manipulated to foster an immune environment conducive to tissue repair and regeneration. Highlighting cutting-edge research, this review presents innovative strategies that leverage the dynamic interplay between macrophages and their environment, proposing novel therapeutic avenues that harness the potential of macrophages in regenerative medicine. Moreover, this review critically evaluates the current challenges and future prospects of translating macrophage-centered strategies from the laboratory to clinical applications.

## 1. Introduction

Within the vast expanse of the immune system, macrophages stand at the helm, orchestrating a diverse array of physiological processes that span from immune surveillance to tissue regeneration [1,2]. The phenomenon of macrophage polarization, where these

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cells adapt their functional states in response to environmental cues, highlights their unique plasticity and critical role in modulating adaptive immune regulation. This polarization transcends the simplistic binary classification of M1 (classically activated) versus M2 (alternatively activated), unfolding into a spectrum of activation states each characterized by distinctive functional and phenotypic attributes [3]. Investigating the multifaceted factors that drive macrophage polarization and their profound implications on immune responses is essential for unraveling the complex interactions between innate and adaptive immunity. An endeavor in exploring macrophage polarization involves delineating the myriad factors that dictate the transformation of macrophage identity. These range from physical signals to chemical signals (with/without biological activity), collectively influencing macrophages towards specific polarization states. The integration of these signals not only shapes immediate immune reactions but also decisively impacts inflammation resolution, tissue repair, and remodeling [4,5].

The remarkable adaptability of macrophages in responding to and modulating their microenvironment positions them as focal points in the investigation of therapeutic strategies aimed at enhancing immune responses, alleviating pathological inflammation, and fostering tissue regeneration. Particularly in tissue reconstruction and repair, the application of macrophage polarization presents expansive prospects, paving new therapeutic pathways that leverage the inherent plasticity of these cells to promote tissue healing and regeneration [6]. This includes the development of innovative biomaterials, pharmaceuticals, and cell therapy approaches that, grounded in a profound understanding of macrophage biology, are designed to modulate macrophage function *in situ*, guiding the effective repair and regeneration of damaged tissues [7].

In recent years, an expanding body of research has llustrated the mechanisms of macrophage polarization and its role in modulating immune responses, the factors influencing this dynamic process, and its application in tissue reconstruction and repair constitute a vibrant research direction in the fields of immunology, regenerative medicine, and therapeutic innovation [5,8,9]. This review systematically explores macrophage polarization and its immune responses to diverse stimuli, investigates regulatory mechanisms and intervention strategies, and aims to identify potential pathways for clinical translation in tissue repair and regeneration.

## 2. Macrophage polarization overview

The study of macrophage activation and polarization dates back to the 1960s. The concept of macrophage activation (classical activation) was first introduced by Mackaness in the 1960s, in the context of describing the antigen-dependent yet non-specific enhancement of macrophage bactericidal activity following secondary exposure to Bacille Calmette-Guérin (BCG) and *Listeria*, an enhancement later confirmed to be associated with the T helper (Th)1 response and interferon (IFN)-γ produced by antigen-activated immune cells [10]. At that time, the relationship between Th2 cells and macrophages, in the context of immunoglobulin (Ig)E production, protection against extracellular parasites, and allergic reactions, remained unelucidated. It was not until the 1990s that Stein and Doyle et al. proposed another activation phenotype induced by interleukin (IL)-4 and IL-13, distinct from the IFN-γ activated state yet far from being deactivated [11,12]. They discovered selective enhancement of mannose receptor in murine macrophages under the stimulation of IL-4 and IL-13 secreted by Th2, inducing heightened endocytosis for the clearance of mannosylated ligands, increased expression of major histocompatibility complex (MHC) class II antigens, and reduced pro-inflammatory cytokine secretion. In the 2000s, Mills et al. observed qualitative differences in the response of macrophages activated in Th1 and Th2 contexts to classical stimuli such as IFN-γ or lipopolysaccharide (LPS) or both, in studies investigating factors regulating arginine metabolism in macrophages, defining a significant metabolic distinction in the pathway: M1 macrophages produce toxic nitric oxide (NO), while M2 macrophages produce nourishing polyamines [13]. Against this backdrop, they proposed naming these responses as M1 and M2 macrophage responses. To integrate similarities and differences in phenotypes, Mantovani et al. began grouping stimuli into a continuum between two functional polarization states based on macrophage markers and types of stimuli, naming them M1 (IFN-γ, LPS, tumor necrosis factor [TNF]) and M2 (M2a: IL-4, M2c: IL-10 and glucocorticoids) [14,15]. Following closely, Mosser et al. further refined the M2 group, defining the activation pathway induced by Fc receptors and immune complexes as M2b [16]. Duluc et al. reported the identification of a novel subset within tumor-associated macrophages (TAMs), named M2d [17]. Discoveries regarding the influence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on macrophages led to these being independently included as M1 and M2 stimuli [18].

The differentiation of macrophages into distinct functional phenotypes is governed by a myriad of environmental cues, driving their evolution towards either the M1 phenotype, characterized by a pro-inflammatory response initiated in a classical manner, or the M2 phenotype, indicative of a traditionally elicited immunoregulatory response [19]. The transition to the M1 or M2 states encompasses a complex regulatory framework, entailing an array of signaling cascades, transcriptional and epigenetic mechanisms, alongside post-transcriptional regulatory dynamics [20]. The classic activation of macrophages and their subsequent immune responses are precipitated by diverse pathogen-associated molecular patterns (PAMPs) such as LPS, damage-associated molecular patterns (DAMPs), and the Th1 cytokine IFN-γ, which is pivotal in fostering pro-inflammatory (M1) outcomes [21,22]. Conversely, the Th2 cytokines, including IL-4 and IL-13, are instrumental in orchestrating the alternative, M2 immune responses. Furthermore, the reprogramming and polarization processes of macrophages are modulated by a broad spectrum of stimuli, highlighting the intricate interplay of factors influencing macrophage functionality [23].

### *2.1. M1/2 polarized macrophage*

M1 and M2 macrophages epitomize divergent polarization states within the spectrum of mononuclear phagocyte differentiation, each characterized by distinct phenotypic configurations and functional capacities. The expression profile of salient polarization markers in these macrophage subsets is intricately modulated by both the qualitative nature and temporal dynamics of inciting stimuli

[24]. Within the in vivo milieu, the delineation between M1 and M2 phenotypes becomes increasingly nuanced, influenced by a complex interplay of local environmental cues and intrinsic signaling mechanisms (Fig. 1) [25].

M1 macrophages, also known as classically activated macrophages, are induced by pro-inflammatory cytokines, notably TNF-α and IFN-γ, or through engagement with PAMPs, such as bacterial LPS [26]. The integral role of M1 macrophages in orchestrating antibody-dependent cellular phagocytosis (ADCP) is well-documented, particularly within the context of neoplastic and infectious disease states. This pro-inflammatory lineage is further characterized by its dependence on growth-promoting factors, including GM-CSF and M-CSF, and is distinguished by a prodigious output of inflammatory mediators—IL-1β, IL-6, TNF-α, and IFN-γ [27]. A hallmark of M1 macrophage functionality is the secretion of IL-12, pivotal for the induction of Th1 lineage differentiation, augmented by the generation of NO via inducible nitric oxide synthase (iNOS), which, in concert with reactive oxygen species (ROS), underpins their antimicrobial effector functions [28].

In contrast, M2 macrophages, often referred to as alternatively activated macrophages, are hallmarked by their production of anti-inflammatory cytokines, IL-10 and transforming growth factor (TGF)-β, playing instrumental roles in tissue repair, remodeling, and attenuation of immune responses [29]. The M2 macrophage population is inherently heterogeneous, encompassing various subsets delineated by specific functional roles and expression of surface markers. Despite the identification of distinct M2 subsets, there exists a continuum of activation states, reflecting a degree of functional and phenotypic overlap. The M2a subset, elicited by IL-4 and IL-13, is primarily involved in tissue reparative processes and wound healing, facilitated by enhanced expression of the mannose receptor (Cluster of differentiation (CD)206), which mediates the phagocytic clearance of apoptotic cells and cellular debris [30]. M2b macrophages, induced in response to immune complex engagement and Toll-like receptors (TLR) activation, are characterized by elevated production of anti-inflammatory cytokines IL-10 and C–C motif ligand (CCL)-1, with a notable capacity for antigen presentation, as evidenced by increased CD86 expression [31]. M2c macrophages, differentiated in response to IL-10 and glucocorticoids, are implicated in the resolution phase of immune responses and the de-escalation of inflammatory processes, marked by high expression levels of the scavenger receptor CD163, critical for the clearance of hemoglobin-haptoglobin complexes [32]. The M2d subset, differentiated through adenosine receptor activation and independent of Interleukin 4 receptor (IL-4R) signaling, has been associated with both reparative functions and the modulation of neoplastic cell invasiveness, underscoring the complex and multifaceted roles of

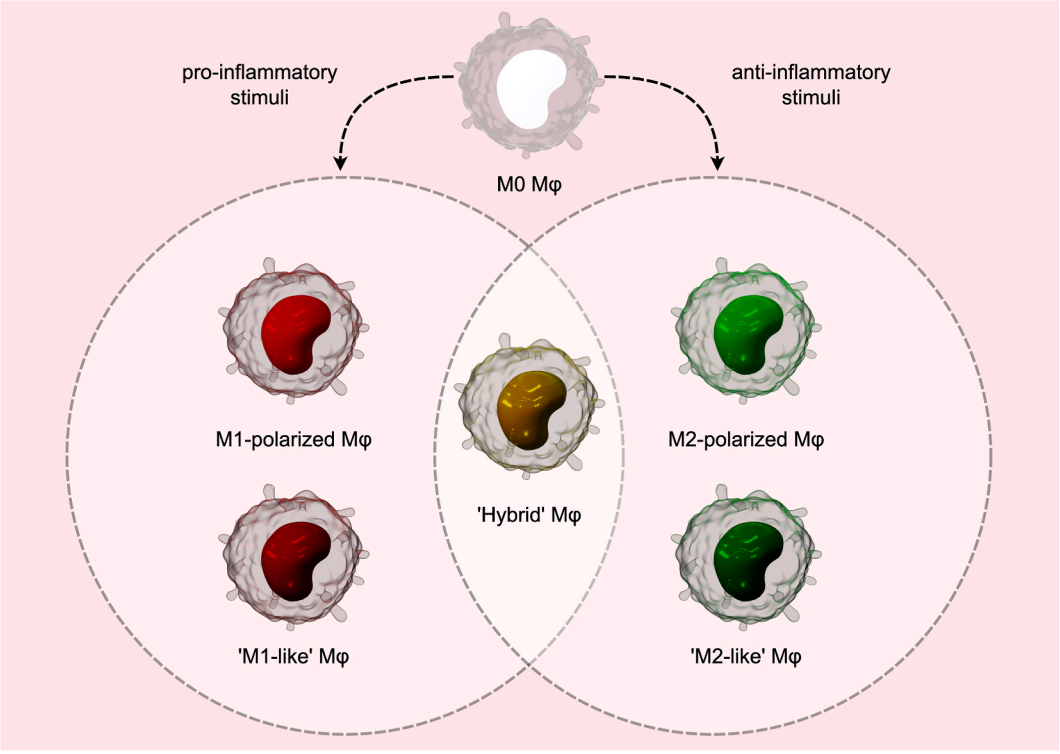


**Fig. 1.** Macrophage Polarization and Hybrid Phenotypes. Left cycle depicts macrophages polarized to the M1 phenotype in response to pro-inflammatory signals. This phenotype is induced by pro-inflammatory cytokines, such as TNF-α and IFN-γ, or by pathogen-associated molecular patterns (PAMPs), such as bacterial LPS. Right cycle shows macrophages polarized to the M2 phenotype under anti-inflammatory signals. This polarization is driven by anti-inflammatory cytokines such as IL-10 and TGF-β, and is associated with tissue repair, immune response attenuation, and anti-inflammatory activities. 'M1-like' and 'M2-like' macrophages denote a broader spectrum of macrophage states that may overlap or transition between these traditional M1 and M2 categories. Central section displays Hybrid macrophages, which combine features of both M1 and M2 phenotypes. For instance, the M1M2d hybrid phenotype is optimized for targeting neoplastic cells while minimizing collateral tissue damage, whereas phenotypes such as M2aM2d and M2cM2d are associated with tumor progression. (drawn by FigDraw software).

macrophages in the regulation of physiological and pathological processes [33,34].

### 2.2. M1/2-like macrophage

While the traditional dichotomy of M1/M2 classification offers a foundational framework for understanding macrophage polarization, its application is increasingly recognized as somewhat reductive, particularly in light of the nuanced and complex nature of macrophage function within diverse immunopathological contexts [35]. This dichotomy, however, retains a degree of relevance, particularly in delineating macrophage responses within specific settings such as tumor-immune interplay and the dynamics of host-pathogen interactions during microbial pathogenesis [36]. In these environments, the distinct signaling pathways activated by M1 and M2 macrophages substantiate the utility of the M1/M2 paradigm to a certain extent [37].

Advancements in immunological research have unveiled the remarkable plasticity and heterogeneity characterizing macrophage polarization, especially in the face of complex pathophysiological challenges such as cancer, type 2 diabetes, atherosclerosis and liver diseases [38]. These insights have led to an evolving understanding that macrophage polarization extends along a continuum rather than residing within fixed states [39]. In response to this evolving understanding, the scientific community is increasingly adopting the 'M1-like' and 'M2-like' nomenclature (Fig. 1) [40,41]. This refined classification acknowledges the dynamic spectrum of macrophage polarization and accommodates the transitional and mixed phenotypes observed, particularly in vivo, where macrophages are subject to a multitude of converging signals [42].

The introduction of 'M1-like' and 'M2-like' descriptors represents a significant conceptual shift, moving away from a binary classification towards a more fluid and nuanced characterization of macrophage states [43]. This shift not only reflects a deeper appreciation for macrophage plasticity but also aligns with the contemporary understanding of their roles in various pathologies. The 'M1-like' and 'M2-like' terms serve to capture the essence of macrophage behavior that straddles traditional classifications, embodying a spectrum of functional states influenced by the complex interplay of environmental cues and intrinsic cellular mechanisms [44]. This lexicon, therefore, provides a more accurate and flexible framework for articulating the diverse roles of macrophages in health and disease, embracing the complexity inherent in their biological functions and interactions [45].

### 2.3. Hybrid phenotype macrophage

The actual phenotypic landscape of macrophages is characterized by a continuum of states that exhibit considerable overlap, contingent upon the specific microenvironmental context and the nature of the signaling cues encountered [46]. Empirical evidence, particularly from studies employing Boolean network models, has elucidated the existence of several hybrid macrophage phenotypes (Fig. 1). These phenotypes assume critical functional significance in nuanced biological scenarios [47]. Notably, within the oncological milieu, the M1M2d hybrid phenotype has emerged as an optimally balanced effector profile, capable of effectively targeting neoplastic cells whilst minimizing ancillary tissue damage. In contrast, phenotypic amalgamations such as M2bM2d, M2aM2d, and M2cM2d have been associated with a propensity to foster tumor progression, with the M2aM2d configuration identified as particularly deleterious in this regard, underscoring the complex interplay between macrophage plasticity and disease pathogenesis [48]. Elena Mitsi et al. elucidated that the manifestation of a hybrid phenotype in alveolar macrophages may endow these cells with the capacity for rapid functional transitions between M1-and M2-associated modalities [49]. This phenotypic flexibility facilitates tailored responses to diverse stimuli and the specific requisites of the surrounding tissue microenvironment, thereby optimizing the immunological efficacy and adaptability of macrophages [50].

## 3. Macrophage polarization and its immune responses to diverse stimuli

### 3.1. Chemical stimuli with or without biological activity

Chemical substances can modify the physical and chemical conditions of the cellular microenvironment, which, in turn, influences macrophage behavior. Changes in biologically active substances and non biologically active substances, pH or the presence of reactive oxygen species can shift macrophage polarization towards either an M1 or M2 phenotype [51,52]. These substances directly or indirectly interact with biological molecules, they can cause cellular stress or damage that indirectly activates signaling pathways relevant to macrophage polarization. Chemical substances can also affect the metabolic pathways of macrophages, which is closely linked to their polarization. Chemicals affecting mitochondrial function, for instance, could tip this balance by affecting the energy supply of the cells [53]. Substances might alter the expression of surface molecules on macrophages or other immune cells, affecting how these cells interact with each other. This can lead to a change in cytokine production profiles, favoring the development of either M1 or M2 macrophages. Some chemicals can cause epigenetic changes that affect gene expression in macrophages without directly interacting with the genome. These changes can influence the polarization of macrophages by upregulating or downregulating genes associated with either the M1 or M2 phenotype [54]. This definition can be somewhat broad, and these typically include PAMPs and DAMPs, cytokines and chemokines, growth factors, hormones, nutrients and metabolites, complement proteins, and bioactive irons, gases in the environment (Fig. 2).

#### 3.1.1. PAMPs & DAMPs

PAMPs are molecular signatures associated with pathogens, including LPS in Gram-negative bacteria, peptidoglycans in both Gram-positive and Gram-negative bacteria, flagellin in bacterial flagella, unmethylated CpG motifs in bacterial and viral DNA, double-

stranded RNA (dsRNA) associated with viral infections [21]. DAMPs are endogenous molecules that normally are sequestered and become released in response to cell injury or endogenous stress signals, such as proteins (high-mobility group box [HMGB]-1, heat shock protein [HSP]s), nucleic Acids (mitochondrial DNA [mtDNA], cell-free DNA [cfDNA]), lipids (oxidized phospholipids, Sphingolipids), metabolites (Uric Acid, Adenosine triphosphate [ATP]) contributing to either pro-inflammatory responses or initiating repair and resolution processes [22]. PAMPs and DAMPs are recognized by a number of Pattern recognition receptor (PRRs) expressed on macrophage, which can trigger inflammatory and adaptive immune reactions. PRRs such as TLRs are specialized in recognizing bacterial components [55]. Specifically, TLR4 is activated by microbial ligands like LPS. Upon activation, TLR4 initiates two distinct signaling pathways mediated by the TIR-domain-containing adapter-inducing interferon-β (TRIF) and the Myeloid differentiation primary response 88 (MyD88) [56,57]. The TRIF-regulated pathway triggers kinase cascades leading to the activation of interferon regulatory factor (IRF)-3, which induces the secretion of IFNs such as IFN-α and IFN-β. In the TLR4 pathway mediated by MyD88, another adapter molecule, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, including its subunits p65 and p50, is activated. NF-κB is a crucial transcription factor involved in the polarization of M1 macrophages, promoting inflammation. Activator protein 1 (AP-1) is also activated by MyD88 via mitogen-activated protein kinase (MAPK) pathway [58,59]. These signaling cascades facilitate the expression of various inflammatory genes, including proinflammatory cytokines like TNF, IL-1β, and IL-12, as

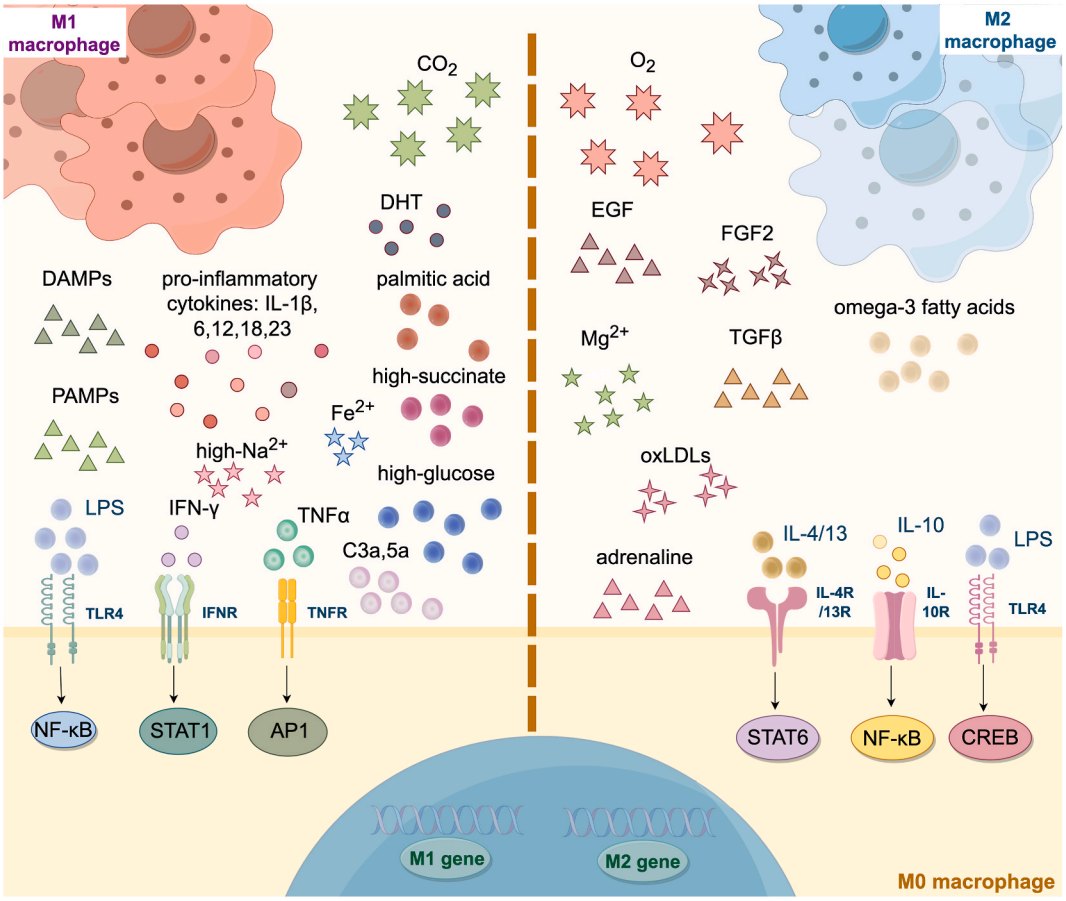


**Fig. 2.** Various factors influencing the polarization of macrophages into M1 and M2 phenotypes. The diagram distinguishes between Direct Stimuli and Associated Stimuli that drive macrophage polarization. Direct Stimuli include factors such as pro-inflammatory cytokines (e.g., TNF-α, IFN-γ, LPS). These stimuli directly induce M1 polarization by enhanced inflammatory pathways (e.g., AP-1, STAT1, NF-κB). Direct Stimuli for M2 polarization involve anti-inflammatory cytokines such as IL-4, IL-13, IL-10, and LPS. These stimuli promote M2 polarization through the activation of specific signaling pathways (e.g., STAT6, NF-κB, and CREB). Associated Stimuli encompass a broader range of signaling molecules and environmental cues that can influence M1 or M2 polarization. This includes pro-inflammatory factors (e.g., PAMP, DAMP, IL-1β, IL-6, IL-12, IL-18, IL-23, omega-3 fatty acids, C3a, C5a, DHT, $CO_2$, high-glucose, high-succinate, high-$Na^{2+}$) and anti-inflammatory factors (e.g., adrenaline, oxLDLs, TGF-β, omega-3 fatty acids, EGF, FGF2, $O_2$, $Mg^{2+}$), which promote M1/M2 polarization associated with inflammation, tissue repair and immune modulation. DHT, dihydrotestosterone; IL, interleukin; PAMP, pathogen-associated molecular pattern; DAMPs, damage-associated molecular pattern; LPS, lipopolysaccharide; TNF, tumor necrosis factor; IFN, interferon; C3a, complement 3a; C5a, complement 5a; TLR, Toll-like receptor; TNFR, tumor necrosis factor receptor; IFNR, interferon receptor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; AP-1, Activator protein 1; STAT, signal transducer and activator of transcription; EGF,epidermal growth factor; FGF, fibroblast growth factor; oxLDLs, oxidized low-density lipoproteins; TGF-β, transforming growth factor; CREB, cAMP-response element binding protein. (drawn by FigDraw software).

well as chemokines hemokine (C-X-C motif) ligand (CXCL)-10, CXCL11, co-stimulating proteins, and antigen-processing proteins [60].

### 3.1.2. Cytokines and chemokines

Proinflammatory cytokines like TNF-α, IL-1β, IL-6, IL-12, IL-18 and IL-23 promote M1 polarization, while anti-inflammatory cytokines such as IL-4/IL-13, low IL-12, IL-23, high Interleukin 1 receptor antagonist (IL-1Ra), IL-10 drive M2 polarization (chemokines in Table 1) [5]. Proinflammatory cytokines bind to their respective receptors on the surface of macrophages, lead to conformational changes in the receptor, activate transcription factors such as NF-κB and Signal Transducer and Activator of Transcription (STAT)-1, which drive the expression of genes associated with the M1 phenotype [61]. While, anti-inflammatory cytokines activate transcription factors such as STAT6 and peroxisome proliferator-activated receptor gamma (PPAR-γ), which promote the expression of genes associated with the M2 phenotype [62]. Typically, signaling pathways mediated by Janus Kinase (JAK)-1 and JAK3 culminate in the activation of STAT6. Upon activation, STAT6 undergoes nuclear translocation, thereby influencing the modulation of antiviral innate immune responses through the regulation of interferon gene expression [63]. Further, the transcriptional landscape is complexified by the involvement of additional transcription factors such as IRF4 and PPAR-γ. These transcriptional regulators orchestrate the expression of a diverse array of proteins, including Arginase 1 (Arg1), Ym1 (also known as Chitinase 3-like 3 Protein [Chi3l3]), Resistin-like molecule alpha (Retnla) (also known as found in inflammatory zone 1 [Fizz1]), CCL17, and CD206 [64,65]. The regulatory purview of STAT6, IRF4, and PPAR-γ encompasses a broad spectrum of immunomodulatory and effector proteins. Furthermore, the IL-10 signaling cascade plays a pivotal role in immune regulation, wherein IL-10 interacts with its receptor complex, comprising IL-10 receptor (IL-10R)1 and IL-10R2 subunits [66]. This interaction precipitates receptor autophosphorylation and subsequent activation of the STAT3. Engagement of STAT3 with its target promoters modulates gene expression profiles, notably inducing the expression of suppressor of cytokine signaling (SOCS)3, which serves as a critical inhibitor within proinflammatory cytokine signaling pathways, thereby contributing to the attenuation of inflammatory responses [67].

### 3.1.3. Growth factors

Growth factors are a diverse group of proteins capable of stimulating cellular growth, proliferation, healing, and cellular differentiation [68]. Epidermal growth factor (EGF) signaling pathways can intersect with those activated by other growth factors and cytokines involved in macrophage polarization via activation of epidermal growth factor receptor (EGFR)/protein kinase B (Akt)/-cyclic adenosine monophosphate (cAMP)-responsive element binding [69,70]. This crosstalk could potentially influence the balance between M1 and M2 polarization in response to different stimuli [71]. Studies have demonstrated that either pharmacological inhibition or genetic ablation of EGFR results in diminished activation of M1 and regulatory macrophages (Mreg) during bacterial infections, both in vitro and in vivo contexts [72]. Such interventions lead to a substantial reduction in the signaling via the NF-κB pathway, affecting both upstream components such as MyD88, phosphorylated inhibitor of nuclear factor kappa B (IKB) kinase (p-IKBK), and phosphorylated NF-κB inhibitor alpha (p-NFKBIA), and downstream processes including the nuclear translocation of RELA Proto-Oncogene and subsequent NF-κB transcriptional activity. It is pertinent to mention that mice deficient in MyD88 ($MyD88^{-/-}$) exhibit reduced gastritis and an increased bacterial load during *Helicobacter pylori* infections, suggesting that MyD88 may serve as a critical intermediary linking EGFR signaling to the NF-κB pathway in macrophages. Mechanistically, activation of EGFR is found to initiate the NF-κB and MAPK1/3 pathways, culminating in enhanced cytokine production and macrophage activation [73]. In cancer, EGF is often overexpressed, contributing to tumor growth and progression. TAMs, which are predominantly of the M2-like phenotype, play critical roles in tumor progression by promoting angiogenesis, immunosuppression, and metastasis [74]. Conditioned medium derived from colon cancer cells with a knockout of the EGFR was observed to suppress the expression of markers associated with M2 macrophages with upregulation of markers pertinent to M1 macrophages, including inducible iNOS, IL-12, TNF-α, and C-C chemokine receptor (CCR)-7 [75].

Fibroblast growth factors (FGFs) comprise a family of growth factors involved in angiogenesis, wound healing, and embryonic development. FGF2 plays a pivotal role in shifting TAMs towards a pro-tumourigenic, M2-like phenotype in the tumour microenvironment [76]. Li et al. demonstrated that in colorectal cancer, FGF2, as macrophage-regulating factor, drived M2 macrophage polarization. They showed that FGFR2 activates plasminogen activator inhibitor-1 (PAI-1) via the JAK2/STAT3 signaling pathway, enhancing immune activity and tumor suppression [77]. In gastric cancer, M2 macrophage/TAM was activated through the FGF2/fibroblast growth factor receptor (FGFR)1/phosphoinositide 3-kinase (PI3K)/AKT axis [78]. While, macrophages treated with FGF20 exhibited a reduced pro-inflammatory phenotype upon stimulation with LPS and IFN-γ [79]. This was evidenced by reduced expression of M1 macrophage markers and decreased secretion of pro-inflammatory cytokines. Mechanistic investigations indicated that FGF20 binds to the FGF receptor 1 isoform on macrophages, leading to enhanced stability of β-catenin through the phosphorylation of glycogen synthase kinase (GSK)3β. This process ultimately attenuates the polarization of macrophages towards the M1 phenotype [79]. Meanwhile, Mechanistic studies have shown that FGF12 stimulated the pro-inflammatory activation of macrophages,

**Table 1**
Cytokines and chemokines influencing macrophage polarity.

| | M1 macrophage | M2 macrophage |
|---|---|---|
| Cytokines<br>Chemokines | IL-1β, IL-6, IL-12, IL-18, IL-23, TNFα<br>CXCL1, CXCL3, CXCL5, CXCL8, CXCL9, CXCL10, CXCL11, CXCL13, CXCL16, CX3CL1, CXCR3, CCL2, CCL3, CCL4, CCL5, CCL8, CCL11, CCL15, CCL19, CCL20; NOS2, CD64, IDO, SOCS1 | IL-1RA, IL-4/IL-13, IL-10, IL-12, IL-23<br>CCL1, CCL2, CCL5, CxCL10, CCL13, CCL14, CCL17, CCL18, CCL22, CCL23, CCL24, CCL26, CXCL16, CCR2, CCR3, CCR4 |

leading to the activation of hepatic stellate cells via the monocyte chemoattractant protein-1 (MCP-1)/CCR2 signaling axis, and additional experiments revealed that FGF12-mediated regulation of macrophage activation predominantly occurred through the Janus kinase–signal transducer of activators of transcription pathway [80].

TGF-β primarily can drive macrophage polarization toward M1 or M2, sometimes referred to as "Mreg" or "M2-like" macrophages by binding to and activating the TGF-β/Smads signaling pathway depending on different situation [25,81]. TGF-β acts through the type II receptor, which then complexes with the type I receptor to form a receptor complex, leading to the phosphorylation of the type I receptor domain. This, in turn, activates downstream signaling molecules to regulate the expression of Smad2 and Smad3 genes. Furthermore, the influence of Smad-dependent pathways on M2 polarization is modulated by Smad7, which interferes with TGF-β receptor type I (TGF-βRI) and obstructs the formation of the Smad2/3/4 complex, thus curbing the synthesis of anti-inflammatory M2 cytokines [82]. In contrast, through Smad-independent pathways, TGF-β stimulates the MAPK, c-Jun N-terminal kinase (JNK), p38, and NF-κB pathways via the TGF-β-activated kinase 1 protein, leading to macrophage reprogramming toward the M1 phenotype, particularly when the Smad-dependent pathway is inhibited [83]. Additionally, TGF-β is reported to promote an M2-like phenotype by enhancing SNAIL expression and disruption of TGF-β/SNAIL signaling, resumed the generation of pro-inflammatory cytokines [84]. TGF-β secreted by Mesenchymal stem cells (MSCs) could skew LPS-stimulated macrophage polarization towards the M2-like phenotype, reduce inflammatory reactions, and improve the phagocytic ability via the Akt/forkhead box protein O1 (FOXO1) pathway, providing potential therapeutic strategies for sepsis [85].

### *3.1.4. Hormones*

Steroid hormones, including the vast majority of sex hormones are derived from cholesterol. The mechanism through which hormones exert their influences on macrophage polarization is multifaceted, involving intricate intracellular signaling cascades that ultimately lead to altered gene expression profiles conducive to the M1/M2 phenotype depending on the external environmental conditions, receptor expressions, and concentration of hormones. Taking dihydrotestosterone (DHT) as an example, androgen binding to classical and non-classical androgen receptors (ARs) mediates genomic and non-genomic androgen effects, respectively. Androgens play a pivotal role in regulating the expression of proinflammatory molecules, such as TNF-α, within mouse monocytes and macrophages [86]. Lai et al. demonstrated that LPS-induced TNF-α production was diminished in bone-marrow-derived macrophages (BMDMs) lacking classical ARs, while observing enhanced TNF-α promoter activity upon DHT stimulation [87]. Nonetheless, divergent findings have been reported, indicating contrasting effects of DHT on TNF-α output in various contexts. For instance, DHT was found to mitigate TNF-α secretion in LPS-stimulated splenic macrophages from hemorrhaged mice and to inhibit TNF-α and nitric oxide production in macrophage cell lines, concurrently elevating IL-10 expression levels. Moreover, DHT exhibited the capacity to augment markers associated with M1 macrophage polarization and to induce apoptosis through the fas ligand (FasL) pathway and caspase activation [88]. Recent investigations have revealed that pre-exposure to DHT amplifies markers indicative of M2 macrophage polarization, such as Chi3l3, Arg1, and Ym1, subsequent to IL-4 stimulation in BMDMs, with analogous results observed in RAW264.7 cell lines. This effect was linked to diminished TLR4 expression and reduced sensitivity to its ligands [89]. Cumulatively, these studies delineate the intricate and context-dependent impact of androgens and ARs on macrophage inflammatory responses, with an overarching tendency towards the suppression of M1 macrophage polarization. This underscores the pivotal role of androgens in modulating inflammatory pathways, particularly in male individuals. The variability in outcomes underscores the necessity for comprehensive exploration of androgen/AR-mediated immune modulation, particularly in light of their significance in immune response dynamics and cancer progression. Additionally, discrepant results may stem from differential activation of classical versus non-classical AR pathways [90].

Peptide Hormones, composed of amino acids, include hormones such as insulin, growth hormone, and prolactin. Insulin, a central regulatory hormone in glucose metabolism, also exerts significant effects on the immune system, including the modulation of macrophage polarization. Macrophages express insulin receptors (IRs) that are integral to downstream signaling networks, which exhibit considerable overlap with the signaling pathways of pattern recognition receptors and cytokine receptors. These shared signaling nodes are pivotal in shaping the polarity of macrophages. The primary action of insulin on cells, including macrophages, is mediated through the IR, a transmembrane tyrosine kinase receptor [91]. The IR, once activated, functions as a crucial hub for downstream signaling molecules such as insulin receptor substrates (IRS) and key kinases including MAPK, PI3K, and protein kinase B (PKB)/Akt [92]. These molecules influence the regulation of significant pathways such as the target of rapamycin kinase complex (TORC)1 and GSK3. Notably, the insulin signaling pathway shares common elements with the signaling pathways initiated by TLRs. TLRs are critical in recognizing PAMPs and DAMPs. Upon ligand engagement, TLRs engage various adaptor proteins, including MyD88, TIR domain containing adaptor protein (TIRAP), TRIF, and TRIF-related adaptor molecule (TRAM). These proteins subsequently activate a cascade involving interleukin-1 receptor-associated kinases (IRAKs), TNF Receptor Associated Factor 6 (TRAF6), ubiquitin E3 ligases, and a complex consisting of TGF-beta activated kinase (TAK) and TAK binding protein (TAB). This pathway culminates in the activation of the inhibitor of nuclear factor-κB kinase (IKK) complex, leading to the stimulation of the transcription factor NF-κB, which then upregulates the transcription of pro-inflammatory cytokines and IFN-γ. Furthermore, the signaling mechanisms utilized by TLRs overlap with those of the insulin receptor, particularly in the activation pathways involving NF-κB, MAPK, and Akt2, which are pivotal in promoting the transcription of pro-inflammatory mediators. The IFN-γ signaling pathway, mediated through the activation of the interferon receptor γ, also engages JAKs and STAT1, leading to MAPK activation and the pro-inflammatory polarization of macrophages. Moreover, the interaction between MAPK pathways and insulin signaling may represent a significant point of synergistic crosstalk. In addition to these pathways, IL-4-mediated signaling through STAT6 plays a crucial role in the differentiation of M2 macrophages. The synergy between insulin and IL-4 is further evidenced by the Akt1-dependent activation of TORC1 and the inhibition of GSK3, which collectively deliver anti-apoptotic and anti-inflammatory signals. IL-4 also triggers the

activation of IRS2, suggesting a potential crosstalk between IL-4 and insulin signaling pathways that could drive M2 macrophage differentiation, highlighting the interconnected nature of these signaling networks in cellular responses [93].

Amino Acid-Derived Hormones are derived from amino acids, and Catecholamines, specifically noradrenaline and adrenaline, engage with α and β adrenergic receptors present on macrophages, thereby influencing the functional responses of these cells across various tissues including the small intestine, lung, and adipose tissue. Specifically, α adrenergic receptors are predominantly linked to pro-inflammatory activities, whereas β adrenergic receptors, particularly the β2 subtype, are associated with anti-inflammatory responses and facilitation of tissue remodeling [94]. The mechanism by which adrenaline promotes macrophage polarization involves the modulation of adrenergic receptors expressed on these cells. Studies have shown that beta-2 adrenergic receptor (β2AR) activation by adrenaline or synthetic agonists can stimulated macrophages firmly to some M2 side categories of the M1-M2 spectrum [95]. M2-promoting effects were mediated specifically through β2-adrenergic receptors and were associated with cyclic AMP response element binding protein (CREB), CCAAT-enhancer-binding protein (C/EBP)β, and activating transcription factor (ATF) transcription factor pathways but not with established M1-M2 STAT pathways [96]. Examination of global transcriptional profiles revealed the identification of twenty supplementary M2 genes exhibiting regulation patterns consistent with β-adrenergic and C/EBPβ signaling pathways [97]. Subsequent promoter-centric bioinformatics assessments substantiated the prevalence of C/EBPβ transcription factor binding motifs within these M2 genes [97].

### *3.1.5. Fats, lipids, glucose, amino acid and their metabolites*

Fatty acids and lipids play an indispensable role in the functionality of immune cells, crucially maintaining membrane integrity, fluidity, and enabling biochemical processes. In the dynamic milieu of the immune system, environmental lipids orchestrate macrophage responses through direct receptor interactions and the modulation of signaling cascades, thereby exerting profound influence over their functional activities [98,99]. Specifically, saturated fatty acids (SFAs), prevalent in high-fat diets and certain microbial profiles, prompt macrophages to adopt a pro-inflammatory M1 phenotype. For instance, palmitic acid, a common SFA, activates pattern recognition receptors such as TLR4 and TLR2, which engage the NF-κB signaling pathway to upregulate pro-inflammatory gene expression. This activation spearheads the release of pro-inflammatory cytokines and chemokines, hallmarks of M1 macrophages, also facilitated by the MAPK pathways triggered by specific environmental lipids. Conversely, unsaturated fatty acids, particularly vv like eicosapentaenoic acid and docosahexaenoic acid, generally promote an anti-inflammatory M2 macrophage phenotype conducive to tumor suppression [100]. This is achieved through their interaction with G-protein coupled receptors and subsequent activation of anti-inflammatory pathways mediated by STAT3 and PPAR-γ. Among the lipids that influence M2 polarization, oxidized low-density lipoproteins (oxLDLs) are notably potent, alongside the metabolites of arachidonic acid—an omega-6 essential fatty acid—such as prostaglandin E2, which also fosters an M2 phenotype [101]. Furthermore, other lipids can trigger activation of STAT6, PPARs, and Liver X receptors (LXRs), enhancing gene expression associated with M2 macrophages and promoting processes such as tissue repair, fibrosis, and the resolution of inflammation [102]. In the metabolic activities of macrophages, while M1 macrophages primarily accumulate lipids and engage in phagocytosis via lipogenesis, M2 macrophages predominantly rely on fatty acid β-oxidation as their chief energy source. Additionally, cholesterol metabolism, regulated by sterol regulatory element-binding proteins (SREBPs), PPARs, and LXRs, plays a critical role in cholesterol efflux capacity and the development of foam cells, which are reminiscent of M2-like macrophages [102]. The dynamics of lipolysis and fatty acid uptake, marked by proteins such as CD36, further contribute to the cytokine production landscape within macrophage populations [103].

Glucose availability and the concentration levels of glucose in the environment, epecially glucose metabolism plays a crucial role in dictating macrophage polarization through various biochemical pathways and signaling mechanisms [104–106]. A high-glucose environment not only directly induces M1 polarization via overproducing ROS but also promotes this process by increasing the sensitivity of macrophages to LPS, commonly observed in vivo diabetic conditions [107,108]. Mechanistically, M1 macrophages are distinguished by a primarily glycolytic metabolic process [109]. Glycolysis involves the decomposition of a six-carbon glucose molecule—visually represented by blue circles, turning white upon phosphorylation—into three-carbon sugars, which are subsequently converted into pyruvate, ATP, nicotinamide adenine dinucleotide hydrogen (NADH), and hydrogen ions ($H+$). The transcriptional framework facilitating glycolysis is regulated by hypoxia-inducible factor (HIF)-1 and, to some extent, by IRF5. This process is supported by the augmented expression of the glucose transporter 1 (GLUT1), providing an increased glucose substrate. Additionally, various glycolytic enzymes perform non-traditional roles to bolster M1 effector functions. Disruption of the mitochondrial tricarboxylic acid (TCA) cycle in M1 macrophages leads to the accumulation of citrate and succinate, further augmenting M1 effector functions. Conversely, M2 macrophages exhibit a fully functional TCA cycle, enhanced oxidative phosphorylation (OXPHOS), and increased mitochondrial biogenesis [110]. Activation of ATP citrate lyase (ACLY), triggered by IL-4 signaling, augments M2 effector functions through epigenetic modifications and the generation of substrates for lipogenesis. The enzyme sedoheptulose kinase inhibits the pentose phosphate pathway, and the transcriptional regulation of M2 macrophage metabolism is mediated by PPAR-γ and LXR [111].

Amino acids and their metabolic pathways critically influence the activation of M1 macrophages [112,113]. The electron transport chain and mitochondrial complexes undergo remodeling in response to LPS and IFN-γ stimulation, which enhances mitochondrial ROS production and reduces respiration. This mitochondrial ROS is pivotal in driving the inflammatory response of M1-polarized macrophages. Arginine metabolism through inducible iNOS results in the production of citrulline and NO, which inhibit mitochondrial complexes I and II and facilitate the loss of these complexes during the later stages of M1 polarization [114]. Concurrently, the TCA cycle experiences rapid modifications during M1 polarization. Initially, itaconate production leads to succinate accumulation via succinate dehydrogenase inhibition, stabilizing HIF-1α and enhancing the inflammatory response. NO further inhibits aconitase 2 and isocitrate dehydrogenase, reducing carbon flux from citrate to α-ketoglutarate (α-KG), which in turn triggers increased carbon input

from glutamine into the TCA cycle, supporting α-KG and succinate anaplerosis. The balance between succinate and α-KG regulates M1 polarization through prolyl prolyl hydroxylase domain (PHD)-dependent proline hydroxylation of IKK-β, crucial for the activation of NF-κB signaling [114]. High levels of succinate early in M1 polarization promote a robust inflammatory response. The later phase is marked by inhibition of the pyruvate dehydrogenase complex (PDHC) and oxoglutarate dehydrogenase complex (OGDC), drastically reducing citrate, itaconate, and succinate levels. PDHC inhibition has been observed through NO-mediated nitrosation of cysteine residues in dihydrolipoamide dehydrogenase, which is also associated with OGDC. This suggests that NO likely mediates the reduced flux from α-KG to succinate observed in the late phase [115]. Furthermore, glutamine-derived α-KG is crucial for inducing endotoxin tolerance, and inhibition of glutaminase increases mortality following repeated LPS exposures. The serine synthesis pathway (SSP) also contributes to M1 polarization by generating glycine, which drives the folate cycle essential for glutathione and s-adenosyl methionine (SAM) production. Glutathione is vital for optimal IL-1β expression, and SAM enhances the expression of inflammatory genes through H3 lysine 36 (H3K36) trimethylation [116].

#### *3.1.6. Complement proteins*

The complement system encompasses a repertoire of more than 35 cell-associated and soluble molecules. Among these, macrophages express various receptors that recognize complement components. Notably, globular C1q receptor (gC1qR) and C1q collagen-like domain binding calreticulin (cC1qR) interact with the globular heads and collagen-like tail domains of C1q, respectively. However, it is postulated that additional C1q receptors exist, some of which may also recognize the collagen-like domains of related defense collagen family members such as mannan-binding lectin and ficolins. Moreover, macrophages express receptors complement receptor (CR)1, CR3, CR4, and CRIg, which recognize C3b-opsonized targets either as intact C3b or its degradation fragment iC3b. Additionally, receptors for complement activation fragments C3a (C3aR) and C5a (C5aR1 and C5aR2) are also present on macrophages. Consequently, macrophages serve as pivotal effectors in executing the functions of complement activation. Complement components such as C3a, C5a, and C5b-9 influence cytokine production in macrophages, promoting an inflammatory (M1-like) phenotype [117]. Conversely, apoptotic cells and targets opsonized with complement components C1q or C3b enhance clearance processes and modulate cytokine production towards an anti-inflammatory, resolving (M2-like) phenotype in macrophages, potentially inhibiting pro-inflammatory signaling mediated by PAMPs [118]. C3a and C5a, known for their pro-inflammatory properties, activate macrophages through diverse signaling mechanisms [119]. For instance, C3a induces NLRP3 inflammasome activation via extracellular signal-regulated kinase 1/2 (ERK1/2)-mediated ATP release upon LPS stimulation in human monocytes. Samstad et al. observed that C5a generated during complement activation by cholesterol crystals contributes to inflammation by activating the NLRP3 inflammasome and promoting the release of IL-1β, TNFα, and ROS [117]. In contrast to other phagocytic receptors such as Fcγ receptors, the engulfment of iC3b-coated particles via CR3 has traditionally been regarded as anti-inflammatory. CR3-dependent engulfment does not trigger the arachidonic acid cascade or the release of toxic oxygen products. However, regardless of macrophage phenotype, significant levels of TNFα secretion were not observed following CR3 ligation, unlike ligation of Fcγ receptors, which induced TNFα release across all macrophage subsets [119].

#### *3.1.7. Bioactive ions*

Cumulatively, early investigations suggest that heightened extracellular concentrations of salt, induced either in vivo or in vitro through NaCl-induced hyperosmolarity, create an environment conducive to the differentiation of pro-inflammatory macrophages. Specifically, elevated salt levels (sodium chloride (NaCl)) promote the activation of pro-inflammatory macrophages via the p38 MAPK-dependent activation of the transcription factor nuclear factor of activated T cell 5 (NFAT5), subsequently leading to NO production by inducible nitric oxide synthase 2 [120]. Furthermore, elevated salt levels induce p38-dependent activation of the heterodimeric transcription factor AP-1, resulting in the expression of pro-inflammatory target genes [119]. Additionally, high salt triggers the activation of the NLR family pyrin domain containing 3 (NLRP3) and NLR family caspase activation and recruitment domain-containing 4 (NLRC4) inflammasomes, leading to caspase 1 activation and subsequent secretion of IL-1β [121]. The augmentation of pro-inflammatory macrophage activation facilitated by high salt is advantageous for pathogen clearance and promotes Th17 cell differentiation through IL-1β secretion. Conversely, heightened salt concentrations diminish alternative macrophage activation and suppressive function by downregulating the expression of STAT6 and inhibiting AKT–mammalian target of rapamycin (mTOR) signaling. These effects result in decreased expression of anti-inflammatory macrophage marker genes [122]. The compromised function of alternatively activated macrophages under conditions of elevated salt has been associated with delayed wound healing [120].

Iron plays a pivotal role in modulating the fate and function of macrophages [123]. Specifically, acute iron deprivation significantly diminishes the expression of ATF4 in quiescent human macrophages and suppresses the expression of interleukin IL-1β and TNF-α in human macrophages treated with LPS. Conversely, iron supplementation distinctly influences macrophage phenotypes. The impact of iron on pro-inflammatory macrophages exhibits variability. Increased endogenous iron levels stimulate TNF production in macrophages [124]. Additionally, exogenous iron supplementation augments the expression of M1 markers (iNOS, TNF-α, and IL-1β) while reducing the expression of M2 markers (IL-10 and CD206) in RAW264.7 cells, BMDMs, and THP-1 cells [125]. Iron-induced mechanisms influencing macrophage polarization include the suppression of NF-κB activation due to iron deprivation, potentially promoting M1-like polarization by enhancing the activity of nicotinamide adenine dinucleotide phosphate oxidase (Nox), which facilitates p65 nuclear translocation and NF-κB activity. Iron treatment may activate the MAPK-phosphorylated mitogen-activated protein kinase-activated protein kinase 2 (MK2) pathway, enhancing TNF-α production and the generation of ROS and peroxynitrite, thereby promoting M1-like polarization. Acute iron deprivation can also inhibit ATF4 expression, suppressing M2-like polarization in tumorous tissues. Moreover, iron may facilitate M1-like polarization by activating MAPK and NF-κB pathways, enhancing intracellular

ROS production. In cases of iron overload, increased hepcidin expression can suppress STAT6 activation while enhancing IRF3 expression, leading to increased iNOS expression in M1-like macrophages. Iron can also activate the NLRP3 inflammasome, promoting M1-like macrophage polarization through ROS generation [123]. Conversely, iron may promote M2-like macrophage polarization by moderately activating mitochondrial ROS cascades in conjunction with the NF-κB pathway, mediated by the downregulation of glia maturation factor gamma [126].

Magnesium (Mg) emerges as a prominent divalent cation in cellular systems, occupying the second position in abundance. Its indispensability stems from its multifaceted involvement in biological processes, encompassing the regulation of energy metabolism, enzyme kinetics, signal transduction, and the synthesis of nucleic acids and proteins [127]. Over 300 enzymes crucially rely on Mg ions for catalytic activities, notably those engaged in ATP utilization or synthesis, as well as in DNA and RNA biosynthesis. Furthermore, $Mg^{2+}$ exerts a regulatory influence on calcium ($Ca^{2+}$) and potassium ($K^{+}$) channels, profoundly influencing intracellular ion dynamics and signaling modulation. In macrophages, magnesium orchestrates the modulation of ion channels and transporters, including calcium channels, potassium channels, and sodium-potassium pumps, thereby impacting intracellular signaling cascades, membrane potential, and cytokine production, pivotal in macrophage polarization processes [128]. Notably, the observed upregulation of CD206 alongside the downregulation of CCR7 and IL-1β in response to $Mg^{2+}$ stimulation hints at the promotion of a M2-like response [129]. Nevertheless, variations in intracellular $Mg^{2+}$ concentrations significantly dictate macrophage differentiation outcomes; elevated intracellular $Mg^{2+}$ levels favor M2-type polarization, whereas reduced levels tend to skew differentiation towards the M1 phenotype [130]. Moreover, Mg-dependent enzymes, such as protein kinase C (PKC) and MAPKs, exert regulatory control over macrophage activation and cytokine production, thereby modulating M1 or M2 polarization dynamics [131]. In the context of tendon–bone healing processes, Mg influences M2 polarization and facilitates fibrocartilage interface regeneration via the selective activation of AKT1 and the PI3K/AKT pathway [132].

### 3.1.8. Oxygen ($O_2$)

Oxygen plays a crucial role in macrophage polarization through its involvement in various metabolic and signaling pathways. The level of oxygen in the tissue environment can significantly influence the behavior and function of macrophages, leading to different polarization states. In hypoxic conditions, commonly found in inflammatory or tumoral tissues, HIF-1α is stabilized [133]. HIF-1α, a transcription factor pivotal in cellular response to hypoxia, promotes the polarization of macrophages towards a pro-inflammatory phenotype [134]. Mechanistically, HIF-1α facilitates the release of pro-inflammatory cytokines and antimicrobial peptides, enhances phagocytosis and NO production, inhibits apoptosis, and promotes intracellular oxygen redistribution and suppression of PHD activity, thus stabilizing HIF-1α protein and enhancing phagocytic activity to modulate macrophage immune response. HIF-1α significantly contributes to macrophage plasticity, during Th1 cytokine-induced macrophage polarization, HIF-1α binds to inducible iNOS, promoting the M1 phenotype conversion to maintain NO homeostasis during inflammation. Under hypoxic and inflammatory conditions, the association between HIF-1α and NF-κB is notably tight, as they share stimuli, regulatory factors, and target genes. Hypoxia activates HIF-1α and NF-κB through an inhibition of IKKβ and TAK1 dependent pathways [135]. Additionally, given NF-κB's role in regulating HIF-1α levels and activity under both normoxic and hypoxic conditions, the related pathways are evolutionarily conserved [134]. Comparative to hypoxic states, hyperoxic states are less extensively studied; however, they appear to also induce macrophage polarization towards the M1 phenotype. In a cohort study on hyperoxic exposure, researchers activated macrophages with high oxygen levels ($O_2 = 65\,\%$) followed by LPS (100 ng/ml) to investigate lung-related exposure factors. Post-activation with 65 % $O_2$, preterm macrophages exhibited a significant pro-inflammatory response (increased major histocompatibility complex, class II, DR [HLA-DR] expression and cytokine release) compared to LPS stimulation alone, suggesting their involvement in exacerbated inflammation, potentially mediated by downregulation of early growth response 2 (EGR2) and growth factor independent 1 (Gfi1) in developing lungs [136].

### 3.1.9. Carbon dioxide ($CO_2$)

The mechanisms by which $CO_2$ affects immune cell function remain under investigation, and it is unclear whether these effects are due to changes in cellular and tissue pH or if they follow a pathway akin to cellular oxygen sensing via hypoxia-inducible factors, with no definitive evidence yet established for $CO_2$ sensing [137]. Using monocytes and macrophages as examples, which play roles in inflammation and promoting wound healing, circulating monocytes are exposed to approximately 5 % $CO_2$ in the bloodstream, while macrophages recruited to inflamed sites may encounter environments with up to 10 % $CO_2$ concentrations, or even higher. Elevated $CO_2$ levels can lead to acidification of the local environment. This acidification may affect macrophage polarization. In certain contexts, acidic pH can enhance the production of pro-inflammatory cytokines, whereas in others, it may promote anti-inflammatory or tissue regeneration phenotypes. $CO_2$ is converted by carbonic anhydrase into bicarbonate ($HCO_3^-$) and protons ($H^+$), where enzyme activity is crucial for maintaining tissue acid-base balance and can influence macrophage function [138]. The availability of bicarbonate and changes in intracellular pH may affect macrophage polarization by regulating the activity of transcription factors and signaling pathways involved in inflammation and tissue repair [139]. Additionally, $CO_2$ levels may also indicate hypoxic conditions, particularly in tumor microenvironments. Hypoxia and $CO_2$ accumulation can synergistically affect macrophage behavior, promoting phenotypes that support tumor growth and suppress immune responses. Recent studies have shown that $CO_2$ reduces autocrine inflammatory gene expression, thereby inhibiting macrophage activation in a manner dependent on the reduction of intracellular pH [140].

### 3.2. Physical stimuli

The influence of physical cues on the modulation of macrophage functionality, encompassing their polarization towards inflammatory and reparative phenotypes, constitutes a subject of considerable interest [141]. Intriguingly, the underlying mechanisms governing these responses appear distinct from those elucidated in the context of conventional mechanobiological investigations, owing primarily to disparities in the molecular apparatus implicated in mechanotransduction, notably adhesive and cytoskeletal components. In this part,we will delineate investigations highlighting the impact of temperature, space size, time, mechanical forces, electrical fields and magnetic fields, substrate stiffness on macrophage behavior (Fig. 3).

#### 3.2.1. Temperature

Temperature, as one of the crucial physical parameters governing cellular life cycles, plays a pivotal role in maintaining cellular homeostasis and regulating cellular functions. At the cellular level, temperature appears to play a significant role in modulating the immune response of macrophages. To investigate the impact of temperature on LPS-activated macrophages, RAW 264.7 cells were cultured with LPS under different temperature conditions, and the secretion of three cytokines was measured. Compared to 37 °C, incubation at 31 °C increased the secretion of TNF, while exposure at 39 °C resulted in decreased TNF secretion. Secretion of IL-6 was lower at 31 °C compared to 37 °C, remaining unchanged at 39 °C. Secretion of white cell protein-10 was inhibited on both sides of 37 °C. Only IL-6 secretion was sensitive to the pre-culture temperature effect [142]. Dynamics of cytokine secretion and steady-state messenger RNA analysis suggest that the temperature regulation mechanisms of TNF and IL-6 may differ. Mouse-derived RAW 264.7 macrophages stimulated with LPS exhibit distinct functional characteristics at different temperature conditions. Secretion of HMGB1, IL-1β, IL-6, and TNF-α, as well as NF-κB dimer (p50 and p65) levels, were higher when cultured in a 37 °C environment compared to

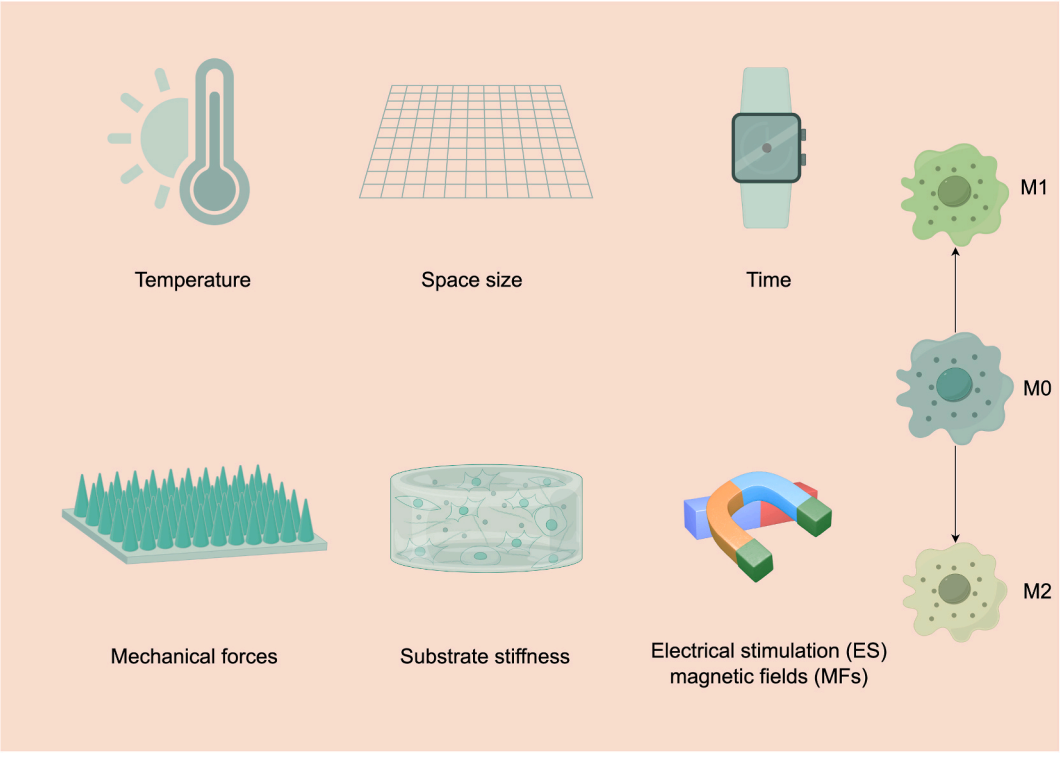


**Fig. 3.** The influence of various physical stimuli on macrophage polarization into M1 and M2 phenotypes. The depicted stimuli include temperature, space size, time, mechanical forces, electrical fields, magnetic fields, and substrate stiffness. Elevated temperatures can induce heat shock proteins (HSPs), influencing cytokine production through pathways such as NF-κB and MAPK. Spatial constraints, achieved through micropatterning or confinement, inhibit macrophage spreading and alter polarization. The temporal dynamics of macrophage polarization are illustrated by the time-dependent expression of M1 and M2 markers. Mechanical forces such as shear stress and substrate stiffness modulate macrophage behavior. For instance, Piezo1 mechanosensitive channels influence macrophage polarization and function. Stiff substrates promote M2 polarization, while softer substrates encourage M1 polarization through pathways involving ROS and NF-κB. Electrical Fields (ES) can polarize macrophages towards M2 phenotypes, with specific frequencies and intensities impacting polarization. Exposure to magnetic fields affects macrophage polarization by altering ion currents and receptor aggregation. The aggregation of specific receptors under magnetic fields can lead to macrophage elongation and M2 polarization, influencing immune responses. Substrate Stiffness affects macrophage morphology and polarization. Rigid substrates generally promote M2 polarization, while softer substrates favor M1 polarization through mechanisms involving ROS production and integrin signaling. (drawn by FigDraw software).

34 °C and 40 °C. However, levels of HSP70 and heat shock factor (HSF) increased in the 34 °C and 40 °C environments, potentially leading to differential immune responses [143]. In vivo studies suggest that low temperatures are a risk factor for stimulating alveolar macrophages to produce pro-inflammatory factors. Research findings indicate that exposure to lower temperatures increases the release of TNF-α, macrophage inflammatory protein (MIP)-1α, and IL-6 in vivo [144]. Given that temperature is elevated during fever, a long-recognized cardinal feature of inflammation, it is possible that macrophage function is responsive to thermal signals. A study suggested that elevating body temperature of mice results in an increase in LPS-induced downstream signaling including enhanced phosphorylation of IKK and IκB, NF-κB nuclear translocation and binding to the TNF-α promoter in macrophages upon secondary stimulation. Mild heat treatment also induces expression of HSP70 and use of HSP70 inhibitors largely abrogates the ability of the thermal treatment to enhance TNF-α, suggesting that the induction of HSP70 is important for mediation of thermal effects on macrophage function [145].

Elevated temperatures can induce the heat shock response in cells, leading to the production of HSPs as direct effects. HSPs are known to play roles in protecting cells from stress and in modulating the immune response. The febrile temperature range can exert both stimulatory and inhibitory effects on the production of pro-inflammatory cytokines by macrophages. The review by Lee et al. concluded that these divergent outcomes may be contingent upon the activation state of macrophages and the subcellular localization of HSPs. During the initial phase of macrophage activation, febrile temperatures enhance the synthesis of TNF-α, IL-6, and NO, which are induced by LPS. This augmented cytokine production is correlated with the upregulation of HSP70/72 expression within these cells. Subsequently, HSP70 can be secreted into the extracellular milieu, where it exerts its cytokine-modulating effects via the CD14/TLR pathway. Consequently, this cascade culminates in the activation of NF-κB and the MAPK pathway, ultimately leading to the transcription of cytokine genes. Conversely, febrile temperatures can also dampen the production of TNF-α, IL-6, and IL-1β, as well as the release of HMGB-1 induced by LPS. This suppression is attributed to the direct binding of thermally-induced HSF-1 to the heat shock response element within the cytokine promoter region, thereby acting as a transcriptional repressor. Moreover, both LPS and heat treatment can induce the expression of HSP70. Subsequently, HSP70 interacts with TRAF6 or IKK, inhibiting subsequent NF-κB activation and cytokine production [146].

### *3.2.2. Space size*

The concept of "space size" influencing macrophage polarization refers to how the physical dimensions of the cell's environment or the geometry and spatial constraints of surrounding structures can affect macrophage behavior and function [147,148]. While the direct impact of physical factors on cellular physiology and differentiation is well-established, the precise influence of these mechanical aspects on macrophage activation and their functional outcomes remains predominantly elusive and has only recently garnered attention [149,150]. Spatial confinement, enforced through micropatterning, microporous substrates, or cell crowding, inhibits macrophage spreading and attenuates the late LPS-induced transcriptional programs, including biomarkers such as IL-6, CXCL9, IL-1β, and iNOS, through mechanomodulation of chromatin compaction and epigenetic modifications involving changes in histone deacetylase 3 (HDAC3) levels and H3K36 dimethylation. Mechanistically, confinement diminishes actin polymerization, thereby reducing the LPS-triggered nuclear translocation of myocardin-related transcription factor A-serum response factor (myocardin-related transcription factor-A, [MRTF-A]). Consequently, this diminishes the activity of the MRTF-A-serum response factor (SRF) complex, leading to the subsequent suppression of the inflammatory response [151].

In light of the impact of spatial considerations on macrophages and with the aim of better simulating in vivo structures, numerous studies have turned to employing 3D culture techniques for further investigation [152]. Under these conditions, such in vitro experiments, geared towards mimicking tumor microenvironments, infectious microenvironments, and tissue repair processes, predominantly explore the interactions between macrophages and other cells [153–155]. However, research specifically focusing on the influence of spatial factors on macrophage polarization remains relatively scarce. Of course, in the formation of 3D environments using experimental reagents, the inherent physical properties of the materials themselves, including hardness, configuration, connectivity, spatial dimensions, etc., may alter the polarization characteristics of macrophages, introducing a plethora of confounding factors.

### *3.2.3. Time*

The influence of time on macrophage polarization is a nuanced aspect of immune response dynamics, reflecting the changing needs of tissue environments during processes such as infection, wound healing, and chronic inflammation [156]. Macrophages are highly versatile immune cells capable of adopting a spectrum of functional states between the pro-inflammatory (M1) and anti-inflammatory or tissue-repairing (M2) phenotypes, in response to various stimuli. The temporal dynamics of macrophage polarization are crucial for the resolution of inflammation and the promotion of tissue repair, as well as in the pathogenesis of chronic inflammatory and fibrotic diseases [157]. In the initial stages of an immune response, such as after infection or injury, there's a predominant polarization towards the M1 phenotype. These macrophages produce pro-inflammatory cytokines, chemokines, and ROS to eliminate pathogens and clear dead cells. This phase is crucial for the initial clearance of infection and debris. Following the clearance phase, there is a transition towards M2 polarization, characterized by the expression of anti-inflammatory cytokines, growth factors, and enzymes involved in extracellular matrix (ECM) remodeling. This phase supports tissue repair, resolution of inflammation, and return to homeostasis. The production of certain cytokines and growth factors by M1 and M2 macrophages serves as feedback to modulate the polarization state. IL-10 produced by M2 macrophages can act in an autocrine or paracrine manner to suppress M1 polarization and promote M2 traits, facilitating the shift towards resolution and healing over time [157].

Macrophage polarization, a process requiring external stimuli, exhibits time dependency in vitro. The temporal expression profiles of these polarization markers at the RNA and protein levels vary depending on the duration of external stimuli [158]. In an in vitro study of human monocyte-derived macrophages (MDMs), it was observed that markers for M1 (CD64, CD86, CXCL9, CXCL10,

HLA-DR, indoleamine 2,3-Dioxygenase 1 (IDO1), IL1β, IL12, TNF), M2a (CD200R, CD206, CCL17, CCL22, IL-10, transglutaminase 2 [TGM2]), and M2c (CD163, IL-10, TGFβ) phenotypes appeared at different times after stimulation, ranging from 4 to 72 h. Generally, in vitro studies control stimulation durations within the range of 4–72 h, and the results overall demonstrate the time-dependent nature of macrophage polarization. The study comparing polarization states at different stimulation times found that a stimulation duration of 72 h yields better results compared to 24 h [158].

### 3.2.4. Mechanical forces

Mechanical forces within the body, such as shear stress, compression, and tensile strain, play crucial roles in influencing cellular behavior, including the polarization of macrophages. In response to heightened membrane tension, stretch-activated ion channels facilitate the passage of ions across the membrane. Significantly, piezo type mechanosensitive ion channel component 1 (Piezo1), a mechanically-sensitive, non-selective cation channel, plays a crucial role in various developmental processes and pathological conditions [159]. Recent investigations have unveiled the involvement of TLR4 signaling in augmenting macrophage bactericidal activity through the mechanosensitive Piezo1 sensor. Depletion of Piezo1 genetically abolishes these responses triggered by bacterial infection or LPS stimulation, which entails the formation of a Piezo1-TLR4 complex for the reorganization of F-actin architecture. Consequently, this reorganization enhances phagocytosis, mitochondrial phagosomal ROS production, and bacterial eradication. Furthermore, the investigation into the mechanically activated cation channel Piezo1 has extended to its impact on macrophage polarization and microenvironmental rigidity perception [160]. Studies indicate that Piezo1-deficient macrophages exhibit enhanced wound healing and reduced inflammation. Notably, calcium flux, regulated by Piezo1, is modulated by soluble cues and amplified on stiff substrates, as demonstrated by macrophages expressing a transgenic calcium reporter. These findings underscore Piezo1's pivotal role as a mechanosensor in macrophages, influencing responses to stiffness and polarization [161].

Another relevant mechanical signal transduction is integrin signaling mediating cell–cell and cell extracellular matrix adhesion [162]. Mechanical forces can alter integrin clustering and conformation, activating signaling pathways like focal adhesion kinase (FAK), which are implicated in macrophage polarization [163]. Structurally, integrins consist of heterodimeric α and β subunits, facilitating two primary roles: firstly, they serve as physical bridges linking the cytoskeleton and extracellular matrix, thereby preserving tissue morphology and function, subsequently they perceive external stimuli and modulate cellular responses [164]. Evidence indicates that matrix stiffness can directly influence macrophage cytoskeletal dynamics and phenotype via integrins. Notably, FAK predominantly associated with integrin signaling, has been implicated in this process. Studies have elucidated that a stiff matrix can induce macrophage M2 polarization through the integrin β5-FAK-mitogen-activated protein kinase kinase (MEK)1/2-extracellular signal-regulated kinase 1/2 (ERK1/2) pathway [165].

### 3.2.5. Electrical stimulation (ES) & magnetic fields (MFs)

Electric fields with intensities as modest as 5 mV/mm, along with magnetic fields, have been shown to modulate macrophage behavior and functional outcomes, primarily affecting processes such as migration, phagocytic activity, and the secretion of ROS and cytokines [166]. Electric stimulation (ES) has emerged as a viable approach for inducing macrophage polarization, offering versatility through variations in voltage, current, frequency, and waveform parameters. Particularly pertinent to wound healing, the transition from the inflammatory to the remodeling phase hinges upon achieving optimal macrophage polarization, a process amenable to controlled manipulation under ES [167]. Mccann et al. demonstrated the feasibility of polarizing macrophages towards the M2 phenotype using a direct current (DC) electric field ranging from 12.7 to 30.5 V/s for a duration of 2 h via glass microelectrodes. Notably, an incremental rise in intracellular calcium ions was noted post approximately 2 min of ES, characterized by repetitive calcium-dependent peaks, correlating with M2 polarization [168]. In a more recent study, electric fields characterized by capacitive properties were employed to modulate macrophage phenotype within a custom-designed system. Intriguingly, the frequency of the electric fields exhibited a significant impact on macrophage polarization, with frequencies of 10 and 60 Hz correlating with heightened expression of CD206 markers, thereby validating polarization [169]. Furthermore, Xu et al. introduced a noncontact electrical stimulation device utilizing capacitive coupling to investigate its effect on macrophage behavior during tissue repair. Under an electric field strength of 25 mV/mm, negligible alterations in the expression of CCR7 (M1 marker) and CD206 (M2 marker) were observed. However, an increased ratio of M2/M1 macrophages was evident, indicative of the successful modulation of macrophage polarization towards the M2 phenotype at a noncontact ES intensity of 53 mV/mm, thereby augmenting the immune response during the wound healing process [170].

Magnetic-field forces induce changes in ionic currents and/or distort macrophage membranes, resulting in the aggregation of transient receptor potential cation channel subfamily M Member 2 (TRPM2) cation channel receptors in magnet-exposed macrophages [171]. This receptor aggregation may render them dysfunctional, disrupting calcium ion ($Ca^{2+}$) balance, consequently affecting actin polymerization and leading to macrophage elongation. Additionally, a similar aggregation pattern of CX3C motif chemokine receptor 1 (CX3CR1) receptors, responsible for guiding macrophages to transplanted organs, was observed in Ras homolog family member A (RhoA) knockout macrophages. Prior research has shown that, under controlled conditions, the polarization of M0 macrophages towards the M2 phenotype correlates with macrophage elongation and the activation of M2-specific gene expression. Therefore, the induction of macrophage elongation through magnetic field exposure may trigger the expression of M2-specific genes, such as Arg1 [172]. Adriana Vinhas and colleagues observed that magnetic stimulation exerted an influence on intercellular communication among tendon cells and macrophages, exhibiting immunomodulatory effects on macrophages and promoting the M2 phenotype. This phenomenon may involve the activation of FAK signaling pathways [173]. Jiang et al. reported that macrophages polarized from M0 to M2 phenotypes by blocking the Piezo1-AP-1-CCL2 signaling pathway under a rotating magnetic field [174].

#### 3.2.6. Substrate stiffness

Substrate stiffness can influence macrophage polarization through various mechanotransduction pathways and interactions with the extracellular matrix (ECM) [175]. The stiffness of the ECM profoundly impacts various aspects of macrophage biology, including morphology, cytoskeletal organization, functionality, and metabolic activity [176]. On rigid substrates, macrophages display an elongated, spindle-like morphology characterized by increased spreading and the formation of filamentous pseudopodia. In tumor micro-environment, elevated stiffness ECM also upregulates cancer cell secretion of M-CSF, consequently promoting macrophage polarization towards an M2-like phenotype. In contrast, flexible substrates trigger reactive oxygen ROS production in macrophages, initiating polarization towards the M1 phenotype through the ROS-mediated NF-κB pathway, thereby augmenting the secretion of cytokines such as IL-1β and TNF-α [177]. Furthermore, interactions between betaig-h3 and type I collagen result in the formation of denser fibers, impacting macrophage behavior. Specifically, macrophages cultured on these denser fibers exhibit morphological alterations indicative of an enhanced M2 phenotype and heightened immunosuppressive functions [178]. According to another research, on soft substrates, BMDMs undergo a shift towards classically activated macrophages via modulation of the ROS-initiated NF-κB pathway. This transition is beneficial for anti-tumor responses, facilitated by the secretion of pro-inflammatory cytokines [179].

## 4. Role of macrophage polarization in immune response and intervention strategies for clinical translation in tissue repair and regeneration

The polarization of macrophages is critical in orchestrating the immune response. M1 macrophages are key players in the early stages of the immune response, initiating the destruction of pathogens and affected tissues. They also stimulate other immune cells through the secretion of cytokines. Following the initial inflammatory response, the shift towards M2 macrophages helps in resolving inflammation, clearing debris, and repairing damaged tissues. This shift is vital to prevent chronic inflammation and associated diseases. The process of tissue repair following injury encompasses inflammation and post-inflammatory restoration, during which macrophages play a pivotal role by self-regulating their polarization transformations in response to changes in the microenvironment. Current research frequently capitalizes on this characteristic of macrophages, employing interventions aimed at accelerating tissue repair through the modulation of macrophage activity. Effective tissue regeneration necessitates the precise phenotypic transition of macrophages. The presence of any macrophage phenotype at an inappropriate time or location within the tissue invariably results in deleterious consequences. Once the wound is stabilized, macrophages undergo a phenotypic transition towards an M2-like state, which is conducive to tissue repair, cellular proliferation, and structural remodeling [180]. These macrophages facilitate angiogenesis and ECM remodeling by secreting a variety of growth factors, such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), FGF, along with proteases including serine proteases, matrix metallopeptidase (MMP)-2, and MMP-9. Additionally, they perform immunosuppressive functions through the secretion of IL-10 and TGF-β [180].

### 4.1. Pharmacological agents and small for macrophage reprogram

The strategic manipulation of macrophage polarization through pharmacological agents and small molecules stands as a cutting-edge approach in the field of tissue repair and regeneration. This perspective focuses on modulating the immune response to favor healing processes, leveraging the plasticity of macrophages to transition between pro-inflammatory (M1) and pro-healing (M2) states [181]. The sustained presence of M1 macrophages contributes to a persistent inflammatory response, detrimental to effective tissue repair and regeneration. A plethora of research has elucidated the mechanisms through which macrophages manifest anti-inflammatory and anti-fibrotic activities in the context of altered tissue homeostasis. Therefore, in therapeutic approaches, controlling appropriate M1 macrophage polarization and appropriately prolonging M2 polarization are currently therapeutic targets in the field of regenerative medicine [182].

In terms of macrophage polarization, the main strategies for tissue repair focus on two aspects: Pro-Healing Promotion and Anti-Inflammatory Strategies [25]. Studies have shown that activation of the PPAR-γ pathway can encourage macrophages to adopt an M2 phenotype, promoting anti-inflammatory responses and tissue repair mechanisms [183,184]. Mirza et al. observed a phenotypic transition in macrophages throughout the process of skin wound healing, which coincided with the upregulation of PPAR-γ and its downstream effectors, concomitant with an augmentation in mitochondrial content [185]. In diabetic conditions, the heightened activity of PPAR-γ was hindered due to the persistent expression of IL-1β in both murine and human wounds. Notably, experimental studies utilizing myeloid-specific PPAR-γ knockout mice revealed that the absence of PPAR-γ in macrophages alone was adequate to prolong the inflammatory phase of wound healing and impede overall tissue repair [186]. Furthermore, the administration of PPAR-γ agonists facilitated the emergence of a macrophage phenotype associated with wound healing, both in vitro and in vivo, even within the diabetic wound milieu. Of significance, the topical application of PPAR-γ agonists significantly ameliorated the healing process in diabetic murine models, thereby suggesting a promising therapeutic avenue for mitigating inflammation and enhancing the resolution of chronic wounds. Thiazolidinediones, a class of PPAR-γ agonists, have been examined for their potential to facilitate wound healing and mitigate fibrotic responses [187]. Interleukins 4 and 13 are key cytokines that drive M2 polarization. Research is exploring the delivery of these cytokines, either directly or through engineered systems, to injury sites to enhance tissue regeneration by encouraging a pro-healing macrophage phenotype [188].

NF-κB orchestrates the upregulation of genes associated with pro-survival and proliferative processes, thereby fostering tissue repair and regeneration in vivo [189,190]. However, NF-κB primarily functions as a central mediator in the augmentation of inflammation through its induction of inflammatory cytokines and chemokines [191]. NF-κB stands as the predominant transcription factor governing M1 macrophage polarization, with STAT1 assuming a consequential role thereafter. Pertinent literature documents

the existence of negative interplay between the pivotal IKKβ and STAT1 during instances of infection. This interplay serves to inhibit M1 polarization, possibly serving as a negative feedback mechanism conducive to inflammation resolution [192]. Inhibitors of the STAT1 and NF-κB pathways, which target signaling cascades critical in M1 polarization, have been demonstrated to mitigate chronic inflammation and fibrosis [193]. Such interventions may create an environment conducive to tissue repair and regeneration [194].

### 4.2. Drug delivery systems representing dual roles

Continuing the discussion on the aforementioned small molecules, the administration of these small molecules often requires drug delivery vehicles, which achieve repair objectives through the release of factors. Indeed, the design of these vehicles may inherently induce macrophage polarization due to their physicochemical properties, thereby representing dual roles of scaffolds [195]. The

**Table 2**
Responses of macrophage mediated by the physicochemical properties of biomaterials in vivo and vitro.

| properties of biomaterials | | macrophage responses | | reference |
|---|---|---|---|---|
| biomaterial size | poly (lactic-co-glycolic acid) (PLGA) microparticles (MPs) (5–7 μm), nanoparticles (PLGA-NPs) (389 nm) | in vitro | MPs > NPs: stimulated TNF-α and IL-1β in J774 macrophages. | [218] |
| | endotoxin-free intermediate chitin (40–70 μm), small chitin (<40 μm) | in vivo | intermediate chitin > small chitin: stimulated TNF production. Only small chitin particles stimulated IL-10 production in WT C57BL/6 mice lung macrophages. | [219] |
| geometric construction | endotoxin-free Ch powder in two geometries: ChO (with orthogonal struts), ChD (with diagonal and orthogonal struts) | in vitro | ChO > ChD: TNF-αand IL-12/23 in human monocytes isolated from healthy donors. | [220] |
| | subcutaneous implantation: 3D Poly ε-caprolactone (PCL) nanofiber, 2D nanofiber scaffolds | in vivo | 3D > 2D: macrophage infiltration, M2/M1 ratio after 4 weeks of implantation into 9-week old Sprague Dawley (SD) rats. | [221] |
| composition | gelatin methacryloyl (GelMA) and poly (ethylene glycol) diacrylate (PEGDA) hydrogels | in vitro | GelMA > PEGDA hydrogels: CD206 expression in THP-1 cells<br>PEGDA > GelMA hydrogels: CD86 expression in THP-1 cells | [222] |
| | coiled-coil (CC)- and $Ca^{2+}$-crosslinked alginate hydrogels | in vivo | $Ca^{2+}$- > CC-crosslinked gels: released IL-1β following LPS stimulation in female C57BL6/J mice peritoneal macrophage. | [223] |
| surface modification | amines (NH2), carboxylic acids (COOH) and phosphonic acids (PO3H2) modified poly (styrene) (PS) surface | in vitro | Substrates modified with COOH resulted in an increase in IL-10 secretion and CD206 expression but did not alter either TNF-α secretion or CD80 expression in THP-1-macrophage. | [224] |
| | polyetheretherketone (PEEK), sulfonated (SPEEK) and zinc-coated SPEEK scaffolds | in vivo | Zn-coated SPEEK > PEEK, SPEEK scaffolds: M2 (F4/80+CD206+)/M1 (F4/80+CCR7+) proportion subcutaneous implantation in C57BL/6 mice. | [225] |
| topography | smooth Ti and rough Ti surface | in vitro | smooth Ti > rough Ti: pro-inflammatory/anti-inflammatory phenotype in primary murine macrophages. | [226] |
| | bulk metallic glasses (BMGs) and BMGs with 55 nm nanorod arrays (BMG-55) | in vivo | BMG-55>flat BMG: the ratio of Arg-1/iNOS expression in macrophages of tissues surrounding after 2 weeks in C57BL/6 mice aged 9–12 weeks. | [227] |
| stiffness | transglutaminase cross-linked gelatins (TG-gels): degrees of matrix stiffness | in vitro | matrix stiffness (high > mid > low): M1-associated markers (IL-1β and TNF-α) in RAW264.7 cells | [228] |
| | methacrylate–gelatin (GelMA) hydrogel: low-, medium-, high-stiffness (2, 10, and 29 kPa) | in vivo | high-stiffness > medium- and low-stiffness hydrogel implants: M1/M2 proportion<br>low-stiffness > medium- and high-stiffness hydrogel implants: M2/M1 proportion in BALB/c 6-week-old male mice. | [229] |
| dynamic physicochemical changes | PEG hydrogels: homogeneous surface to a dynamic Arg-Gly-Asp (RGD)-patterned surface upon UV exposure | in vitro | After exposure to UV radiation: $iNOS^+$ $IL\text{-}10^{lo}$ M1 transformed to the Arg-$1^+$ $IL\text{-}10^{hi}$ M2 phenotype in mouse BMDMs. | [230] |
| | shape-memory PEG-modified gold nanorods (AuNRs)/PCL films (SMPs):flat surface to micro-grooved surface after near-infrared (NIR) irradiation | in vivo | upon exposure to NIR: $iNOS^+IL\text{-}10^{lo}$ macrophages to the Arg $^+$ $IL\text{-}10^{hi}$ phenotype in Sprague Dawley (SD) rats. | [231] |
| degradation products | Mg-based implants generate a range of bioactive substances, including degradation particles (DPs), 200, 400, 800 and 1600 μg/ml generated from Mg via electrochemical corrosion | in vitro | stimulated with DPs(1600 > 800>400 > 200 μg/ml): CD86+M1/CD206+M2 proportion in RAW264.7 cell line. | [232] |
| | Zn-based porous scaffolds (incorporating 0.8 wt% Li): isotropic minimal surface G unit and anisotropic BCC unit | in vivo | isotropic minimal surface G unit > anisotropic BCC unit: degradation rate and promoting macrophage polarization toward an anti-inflammatory phenotype in 12-week-old male Sprague-Dawley rats weighing 350–400 g. | [233] |

localized administration of active small molecular compounds, either through reservoirs or coating layers within scaffolds, presents a straightforward approach to modulating the behavior of monocytes/macrophages in tissue regeneration [196]. Given the mounting evidence underscoring the pivotal contributions of macrophages across various phases of tissue healing, researchers have devised diverse strategies for delivering bioactive substances aimed at regulating macrophage function during distinct stages of tissue regeneration. Biomaterials can be engineered to release cytokines, growth factors, or other bioactive molecules that directly influence macrophage polarization and function [197]. Present immunomodulatory delivery strategies can be categorized as follows: 1) augmentation of monocyte/macrophage recruitment to the site of injury; 2) induction of a pro-inflammatory phenotype during the initial phase of healing; 3) modulation of macrophage polarization toward an M2-like phenotype; and 4) sequential guidance of the M1-to-M2 macrophage phenotype transition to enhance tissue regeneration [104].

Alterations in the physical, chemical, and biological attributes of biomaterials can modulate the host's specific response to them, consequently influencing the microenvironmental cues and the reciprocal communication between macrophages and biomaterials (Table 2) [198,199]. In the pursuit of enhancing tissue regeneration, researchers frequently aim to design and fabricate biomaterials with enhanced tissue compatibility and the ability to induce M2 polarization of macrophages [200–202]. This objective is typically pursued through the manipulation of specific physicochemical properties of the materials. Notably, a prevalent approach involves engineering biomaterials with tailored physical and chemical attributes capable of regulating macrophage behavior. Such materials, characterized by appropriate stiffness, surface morphology, and degradation kinetics, possess the ability to modulate macrophage phenotype, promoting M2 polarization while concurrently attenuating inflammation [110]. Alterations in the architecture of biomaterials, such as size, geometry, and porosity, as well as surface conjugations and mechanical properties, can significantly influence macrophage activation [203,204]. The response of macrophages to these implanted materials is pivotal in determining the success or failure of the device. As previously noted, macrophages serve as principal phagocytes that rapidly detect and internalize foreign entities. Smaller particles are easily assimilated; however, larger particles (exceeding 10 μm) may provoke macrophages, leading to an inflammatory M1-like phenotype characterized by the secretion of pro-inflammatory cytokines, proteases, and reactive oxygen species. In the case of larger implants (greater than 100 μm), a foreign body reaction (FBR) occurs, culminating in the formation of a foreign body giant cell (FBGC), which consists of a conglomerate of multiple macrophages surrounding the implant [205,206]. Additionally, researchers are actively involved in developing bioactive compounds and nanoparticles that can be integrated into biomaterial matrices to actively direct macrophage polarization. These compounds effectively target distinct signaling pathways implicated in macrophage activation, thereby steering their functional trajectory towards a regenerative phenotype [207]. Moreover, advancements in the field of biomedical devices, including scaffolds and implants, incorporate strategic interventions to promote M2 polarization and enhance tissue regeneration [208].

## 5. Future directions

Although autologous cell-based genetically engineered therapy is advancing rapidly in clinical trials, especially in the fields of cancer treatment, cell gene editing strategies like clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system for tissue regeneration treatment is still in very early stages of development [209,210]. The significance and intricacy of macrophages are further underscored by their involvement in the advancement of various diseases, including chronic wounds, diabetes and drug-induced liver injury (DILI) [211,212]. Emerging therapeutic approaches are being developed to modulate macrophage activity or ameliorate macrophage dysfunction [213]. In reality, the regulation of macrophage activity during tissue injury repair presents considerable challenges. The primary difficulty lies in the precise targeting and modulation of macrophages, which exhibit various phenotypes at different stages of the repair process. This often necessitates the modulation of multiple genes or pathways. Additionally, different tissue injuries involve distinct repair processes, or alternatively, variations in the repair process following medical intervention. Clinically, for infectious injuries, surgical debridement is frequently required to remove infectious agents. Subsequently, it can be posited that the repair enters a synthetically induced late phase, wherein macrophages can be maximally leveraged for their reparative functions. At this stage, the macrophages are engineered to adopt an M2-like phenotype, optimizing their healing capabilities. Theoretically speaking, the local genetically engineered macrophages contributed to accelerated tissue growth but not caused morbidity in animals. The versatility of genetically engineered macrophages is also highlighted by showing that they can be engineered to secrete proteins that either reduce excessive inflammatory response, such as IL-6, TNF, or promote tissue remodeling, by TGF, IL-10 [214]. The principal obstacle in applying genetic and epigenetic strategies to clinical contexts is the precise delivery to macrophages within the tissue microenvironment, which necessitates high specificity and minimal off-target effects [215]. Moreover, maintaining the long-term stability of these modifications and evaluating any unintended consequences are vital to ensuring their safe clinical use. Additionally, the deployment of genetic and epigenetic interventions in a clinical setting introduces a range of regulatory and ethical considerations, especially concerning modifications to the germline or the possibility of enduring alterations in gene expression [216]. These factors collectively underscore the complexity and the critical need for meticulous oversight in the clinical application of these advanced therapeutic technologies [217].

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## CRediT authorship contribution statement

**Jing-Dong Xue:** Writing – original draft, Resources, Conceptualization. **Jing Gao:** Writing – original draft, Resources. **Ai-Fang Tang:** Writing – review & editing, Supervision. **Chao Feng:** Writing – review & editing, Funding acquisition.

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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.

## Abbreviations

| | |
|---|---|
| BCG | Bacille Calmette-Guérin |
| Th | T helper |
| IFN | interferon |
| Ig | immunoglobulin |
| IL | interleukin |
| MHC | major histocompatibility complex |
| LPS | lipopolysaccharide |
| NO | nitric oxide |
| TNF | tumor necrosis factor |
| TAMs | tumor-associated macrophages |
| GM-CSF | granulocyte-macrophage colony-stimulating factor |
| M-CSF | gacrophage colony-stimulating factor |
| PAMPs | pathogen-associated molecular patterns |
| DAMPs | damage-associated molecular patterns |
| ADCP | antibody-dependent cellular phagocytosis |
| iNOS | inducible nitric oxide synthase |
| ROS | reactive oxygen species |
| TGF | transforming growth factor |
| CD | Cluster of differentiation |
| TLRs | Toll-like receptors |
| CCL | C–C motif ligand |
| IL-4R | interleukin 4 receptor |
| dsRNA | double-stranded RNA |
| HMGB | high-mobility group box |
| HSP | heat shock protein |
| mtDNA | mitochondrial DNA |
| cfDNA | cell-free DNA |
| ATP | Adenosine triphosphate |
| PRRs | pattern recognition receptors |
| TRIF | TIR-domain-containing adapter-inducing interferon-β |
| MyD88 | myeloid differentiation primary response 88 |
| IRF | interferon regulatory factor |
| NF-κB | nuclear factor kappa-light-chain-enhancer of activated B cells |
| AP-1 | Activator protein 1 |
| MAPK | mitogen-activated protein kinase |
| CXCL | chemokine (C-X-C motif) ligand |
| IL-1Ra | interleukin 1 receptor antagonist |
| STAT | signal transducer and activator of transcription |
| PPAR-γ | peroxisome proliferator-activated receptor gamma |
| JAK | Janus kinase |
| Arg1 | Arginase 1 |
| Chi3l3 | chitinase 3-like 3 protein |
| Retnla | Resistin-like molecule-alpha |
| Fizz | found in inflammatory zone |
| IL-10R | IL-10 receptor |

| | |
|---|---|
| SOCS | suppressor of cytokine signaling |
| EGF | epidermal growth factor |
| EGFR | epidermal growth factor receptor |
| Akt | protein kinase |
| BcAMP | cyclic adenosine monophosphate |
| IKB | inhibitor of nuclear factor kappa B |
| p-IKBK | phosphorylated inhibitor of nuclear factor kappa B |
| p-NFKBIA | phosphorylated NF-kB inhibitor alpha |
| CCR | C-C chemokine receptor |
| FGF | fibroblast growth factor |
| PAI | plasminogen activator inhibitor |
| FGFR | fibroblast growth factor receptor |
| PI3K | phosphoinositide 3-kinase |
| GSK | glycogen synthase kinase |
| MCP-1 | monocyte chemoattractant protein-1 |
| TGF-βRI | transforming growth factor-β receptor type I |
| JNK | c-Jun N-terminal kinase |
| MSC | mesenchymal stem cell |
| FOXO1 | Forkhead box protein O1 |
| DHT | dihydrotestosterone |
| AR | androgen receptor |
| BMDMs | Bone marrow-derived macrophages |
| FasL | fas ligand |
| IR | insulin receptor |
| IRS | insulin receptor substrates |
| PKB | protein kinase B |
| TORC | target of rapamycin complex |
| TIRAP | TIR domain-containing adapter protein |
| TRAM | TRIF-related adaptor molecule |
| IRAKs | interleukin-1 receptor-associated kinases |
| TRAF6 | TNF receptor-associated factor 6 |
| TAK | TGF-β-activated kinase |
| TAB | TAK binding protein |
| IKK | inhibitor of nuclear factor-κB kinase |
| β2AR | beta-2 adrenergic receptor |
| CREB | cAMP-response element binding protein |
| C/EBP | CCAAT-enhancer-binding protein |
| ATF | activating transcription factor |
| SFA | saturated fatty acid |
| oxLDL | oxidized low-density lipoprotein |
| LXR | liver X receptor |
| SREBPs | sterol regulatory element-binding proteins |
| NADH | nicotinamide adenine dinucleotide hydrogen |
| HIF | hypoxia-inducible factor |
| GLUT | glucose transporter |
| TCA | tricarboxylic acid |
| OXPHOS | oxidative phosphorylation |
| ACLY | ATP citrate lyase |
| α-KG | α-ketoglutarate |
| PHD | prolyl hydroxylase domain |
| PDHC | pyruvate dehydrogenase complex |
| OGDC | oxoglutarate dehydrogenase complex |
| SSP | serine synthesis pathway |
| SAM | s-adenosylmethionine |
| H3K36 | H3 lysine 36 |
| gC1qR | globular C1q receptor |
| cC1qR | C1q collagen-like domain binding calreticulin |
| CR | complement receptor |
| NFAT5 | nuclear factor of activated T cells 5 |
| NLRP3 | NLR family pyrin domain containing 3 |
| NLRC4 | NLR family caspase activation and recruitment domain-containing 4 |

| | |
|---|---|
| mTOR | mammalian target of rapamycin |
| MK2 | mitogen-activated protein kinase-activated protein kinase 2 |
| PKC | protein kinase C |
| HLA-DR | major histocompatibility complex |
| class II | DR |
| EGR | early growth response |
| Gfi | growth factor independent |
| HSF | heat shock factor |
| MIP | macrophage inflammatory protein |
| HDAC | histone deacetylase |
| MRTF-A | myocardin-related transcription factor-A |
| SRF | serum response factor |
| IDO | indoleamine 2,3-Dioxygenase |
| TGM | transglutaminase |
| Piezo1 | piezo type mechanosensitive ion channel component 1 |
| FAK | focal adhesion kinase |
| MEK | mitogen-activated protein kinase kinase |
| ERK | extracellular signal-regulated kinase |
| TRPM | transient receptor potential cation channel subfamily M Member |
| CX3CR1 | CX3C motif chemokine receptor 1 |
| RhoA | Ras homolog family member A |
| ECM | extracellular matrix |
| PDGF | platelet-derived growth factor |
| VEGF | vascular endothelial growth factor |
| MMP | matrix metallopeptidase |
| CRISPR | clustered regularly interspaced short palindromic repeats |

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