

Review

He Ding, Yuxin Zhang, Yu Mao, Yan Li, Yan Shen, Jingyi Sheng* and Ning Gu*

Modulation of macrophage polarization by iron-based nanoparticles

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Abstract: Macrophage polarization is an essential process involved in immune regulation. In response to different microenvironmental stimulation, macrophages polarize into cells with different phenotypes and functions, most typically M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages. Iron-based nanoparticles have been widely explored and reported to regulate macrophage polarization for various biomedical applications. However, the influence factors and modulation mechanisms behind are complicated and not clear. In this review, we systemically summarized different iron-based nanoparticles that regulate macrophage polarization and function and discussed the influence factors and mechanisms underlying the modulation process. This review aims to deepen the understanding of the modulation of macrophage polarization by iron-based nanoparticles and expects to provide evidence and guidance for subsequent design and application of iron-based nanoparticles with specific macrophage modulation functions.

Keywords: influence factors; iron-based nanoparticles; macrophage polarization; mechanism; modulation.

He Ding and Yuxin Zhang contributed equally to this study.

***Corresponding authors: Jingyi Sheng**, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210009, Jiangsu Province, China, E-mail: shengjingyi@hotmail.com.

<https://orcid.org/0000-0001-9254-5240>; and **Ning Gu**, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210009, Jiangsu Province, China; Medical School, Nanjing University, Nanjing 210093, Jiangsu Province, China, E-mail: guning@seu.edu.cn. <https://orcid.org/0000-0003-0047-337X>

He Ding, Yuxin Zhang and Yan Li, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu Province, China

Yu Mao, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu Province, China

Yan Shen, School of Biomedical Engineering and Informatics, Nanjing Medical University, Nanjing, Jiangsu Province, China

Introduction

Macrophages are efficient effector cells of the innate immune response, secreting various molecules that regulate the inflammatory response, host defense, and immune homeostasis [1]. The diversified immune effect of macrophage is achieved by cell polarization, during which process cell subsets with different phenotypes are produced in response to microenvironmental stimulations. Currently, the most commonly studied macrophage polarization mode is the M1/M2 dichotomy, which means primary macrophages are polarized into the M1 phenotype with pro-inflammatory functions or the M2 phenotype with anti-inflammatory functions. The plasticity of macrophage polarization allows them to adapt to various physiological and pathological conditions. Therefore, understanding the regulation of macrophage polarization provides an important rationale for the development of immune therapeutic strategies for many diseases.

Iron-based nanoparticles are mainly divided into iron monomer, alloy, iron oxide and iron complex [2], which are widely used in industrial and medical fields because of their unique physicochemical properties. Among them, iron oxide nanoparticles (IONPs), including magnetite (Fe_3O_4), hematite ($\alpha\text{-Fe}_2\text{O}_3$) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$) nanoparticles, exhibit unique superparamagnetism and excellent biocompatibility and are widely used in the field of nanomedicine. Based on their superparamagnetic properties, iron oxide nanoparticles can be used in magnetic resonance imaging, separation of bio-molecules, magnetic hyperthermia, and magnetically targeted delivery of drugs [3–5].

Various iron-based nanoparticles have been shown to regulate the polarization and function of macrophages, thus holding the potential to be applied as an immune regulator for disease treatment. For example, the iron supplement ferumoxytol (2.73 mg Fe/mL) co-injected with cancer cells was found to promote the polarization of M1 macrophage in tumor microenvironment and inhibit the tumour growth and metastasis of subcutaneous adenocarcinoma in mice [6]. While a 34 nm-sized Prussian blue (PB) nanozyme (50 $\mu\text{g}/\text{mL}$

or 500 µg/mL) applied to skin wounds could promote the anti-inflammatory phenotype of macrophages, relieving skin inflammation and accelerating wound healing and tissue regeneration [7]. The regulation of iron-based nanoparticles on macrophage polarization is a complex process, with significant variation under different conditions, which can be attributed to the interaction of multiple regulatory factors. This review starts with the characteristics and mechanism of macrophage polarization, summarizes the existing studies on the regulation of polarization by iron-based nanoparticles, and focuses on the material properties and possible mechanisms affecting iron-based nanoparticle-mediated macrophage polarization. This review may provide informative insight for further studies to develop novel immune modulation strategies using iron-based nanoparticles.

Polarization of macrophage

Origin of macrophage polarization

Tissue macrophages have a dual origin. Most tissue-resident macrophages are established before birth from yolk sacs or fetal precursors, which are independent of monocytes and capable of self-renewal [8]. While other adult-derived macrophages are terminally differentiated from circulating monocytes that arise from bone marrow and are released into the peripheral blood [9]. Embryonic macrophages mainly participate in tissue remodeling, whereas differentiated mononuclear macrophages and their ancestors constitute the mononuclear macrophage system, which acts as short-lived effector cells in tissues and assists in host defense [10]. Differentiated macrophages have plasticity, which can be further polarized into different phenotypes to play their roles. Polarization is a process in which macrophages respond functionally by producing different phenotypes to microenvironmental stimuli and signals in specific tissues [11]. In the 1960s, Mackness [12] reported an antimicrobial macrophage activation state known as classically activated macrophages (CAM, also known as M1). In 1992, the alternatively activated macrophages (AAM, also known as M2) were first proposed [13]. Subsequently, studies on the polarization of macrophages were deepened continuously, and it was found that the polarization of macrophages was varied, among which M1 macrophages and M2 macrophages were two extremely activated states [14].

Phenotypes and functions of macrophage polarization

Macrophages are polarized to M1 or M2 macrophages upon stimulation by the local cytokine milieu, inducing a pro-inflammatory response or promoting immune regulation and tissue remodelling.

M1 macrophages are induced by bacterial lipopolysaccharide (LPS) or other cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α). The typical phenotype of M1 macrophages involves the secretion of high levels of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, IL-12, IL-23, IL-1 α), high levels of Th1-recruiting chemokines (CXCL9, CXCL10, CXCL11), high expression of major histocompatibility complex class II (MHC II) and co-stimulatory molecules (CD40, CD80, CD86) and low levels of IL-10 [15]. Under physiological and pathological conditions, M1 macrophages mainly play their roles in bactericidal, tumor killing, tissue damaging, and hindering tissue regeneration and wound healing. For example, when infected by pathogens, M1 macrophages could activate the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system and produce reactive oxygen species (ROS) to facilitate their antibacterial function [16]. Moreover, tumor-infiltrating M1 macrophages function as tumor killers by secreting tumor growth inhibitors, including TNF and nitric oxide (NO) [17].

On the other hand, M2 macrophages are polarized by the stimulation of Th2 cytokines, such as IL4, IL10, and IL13, etc. [10, 18]. Phenotypically, M2 macrophages express high levels of anti-inflammatory molecules (TGF β , IL-10), endocytic receptors (CD163, CD206, and CD301), and Th2 recruiting chemokines (CCL17, CCL18, CCL22, and CCL24) [19]. Functionally, M2 macrophages recruit Th2, basophils and eosinophils through the production of Th2-recruiting chemokines [20]. Besides, M2 macrophages scavenge debris and apoptotic cells with a strong phagocytosis capacity, and accelerate inflammation resolution, tissue regeneration and wound healing via anti-inflammatory molecules [10]. For instance, M2 macrophages effectively eliminate early apoptotic cells under the expression of Mer tyrosine kinase [21]. By Trem2 signaling, M2 macrophages promote epithelial proliferation, reduce mucosal damage, and accelerate wound healing [22]. In addition, M2 macrophages could promote angiogenesis and fibrosis by secreting vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) [23].

Regulatory factors of macrophage polarization

Macrophage polarization is a dynamic process that regulates the body in a balanced and integrated way [24]. Phenotypes of M1–M2 macrophage polarization can be reversed *in vivo* and *in vitro*. The M1/M2 axis balance of macrophages is the basis of maintaining body homeostasis. Once the balance is broken, chronic diseases and inflammation will be induced [25]. Therefore, understanding the regulatory factors affecting macrophage polarization is particularly crucial, which would provide an important rationale for developing disease treatment strategies.

Single regulatory factors

When macrophages encounter invading microorganisms or inflammatory microenvironment, their gene expression profiles change dramatically [26]. In this process, the direction of macrophage polarization can be regulated through the activation or inhibition of signaling pathways and downstream transcription factors. In addition, epigenetic mechanisms also mediate macrophage polarization by altering gene expression profiles [11]. Various single factor-mediated regulations at the transcription and post-transcription levels contribute to the dynamic and reversible polarization of macrophages.

Signaling molecules and transcription factors coordinate to regulate macrophage polarization. Different molecules have various effects on changing the polarization phenotype of macrophages. For example, alterations in AKT1/2, SHP-1/2, and TNF can regulate the polarization of macrophages. Different Akt kinase isoforms regulate macrophage polarization differentially, with Akt1 ablation promoting M1 phenotype and Akt2 knockout causing M2 phenotype [27]. By decreasing the production of TNF mRNA, which can block M2 gene expression in macrophages, the amount of M2 macrophages is enhanced [28]. In addition, the lack of STAT6, IRF4, JMJD3, PPAR δ , and PPAR γ blocks the M2 polarization phenotype and inhibits their anti-inflammatory effects. Studies have shown that PPAR γ is required for alternatively activated macrophage maturation. Destruction of PPAR γ impairs macrophage activation towards M2 [29]. Various genes associated with the mouse M2 macrophage phenotype are modulated by STAT6, such as arginase 1 (Arg1) and macrophage mannose-receptor 1 (Mrc1) [26]. Furthermore, the molecular switches of certain factors can completely reverse the polarization phenotype, such as RBP-J, Btk1, KLF4/6, let-7c, and DAB2 [30]. In particular, RBP-J

inhibits the expression of the M2 macrophage characteristic genes and induces the activation of the M1 phenotype [31]. KLF6 promotes the M1 phenotype by cooperating with NF- κ B and suppresses M2 targets by inhibiting PPAR- γ expression [32].

Epigenetics changes gene function by inducing or modifying the information encoded in DNA [33]. The epigenetic mechanisms involved in the modulation of macrophage polarization mainly include MicroRNA (miRNA), DNA methylation (DNAm), and histone modification. MiRNAs are short non-coding RNA molecules regulating gene expression at the post-transcriptional level. For instance, increased expression of miR-155 induces the M1 subtype with the secretion of TNF- α , whereas macrophages shift to M2 phenotype after miR-155 knockout [34, 35]. Besides, the abnormalities in DNAm patterns, especially the chemical modification of DNA cytosine residues, significantly affect the behavior of macrophages. Modification modes include DNA hypermethylation and DNA hypomethylation [36]. Various research proved that DNA hypermethylation was a determinant of macrophage polarization, leading to the development of inflammatory diseases [37]. Furthermore, the genes encoding enzymes catalyze post-translational modification of histones, which induce gene activation and gene silencing and are differentially expressed in M1/M2 macrophages [37]. For example, the promoters of pro-inflammatory cytokines TNF- α and IL-6 have a histone 3 lysine 36 (H3K36) dimethylation effect under the modification of specific methyltransferase SET, which inhibits NF- κ B and ERK signaling and leads to decreased M1 polarization [38].

Systematic regulatory factors

There are significant metabolic differences between M1 and M2 macrophages, which make metabolic modulation a powerful factor in regulating the polarization of macrophages. In glycometabolism, M1 macrophages enhance the absorption of glucose, accelerating the aerobic glycolytic pathway and pentose phosphate pathway with the production of lactic acid and NADPH. At the same time, reactive oxygen species (ROS) and NO are produced intracellularly, which provide the cells with rapid energy and bactericidal activity which regulates [39, 40]. Under low oxygen levels, macrophages modulate polarization by changing the level of glucose metabolism at the site of inflammation. In this process, transcription factor HIF is the key mediator for macrophages to adapt to hypoxic conditions. HIF-1 α regulates glycolysis through the NF- κ B pathway, resulting in the production of pro-inflammatory cytokines and M1

phenotypes [41]. On the opposite, M2 macrophages up-regulate glucose metabolism to meet the energy demand. Apart from glycometabolism, M2 macrophages also obtain fuel through fatty acid oxidation, enhancing anti-inflammatory function [42]. In addition, the differential metabolism of arginine is one of the most accurate differentiators between M1 and M2 macrophages. Arginine is the common substrate of Arg1 and inducible nitric oxide synthase (iNOS). According to different activation states, different enzymes responsible for arginine metabolism induce different phenotypes [43]. In M1 macrophages, iNOS upregulation leads to the breakdown of arginine into citrulline and NO, which resists bacterial infection. On the contrary, in M2 macrophages, Arg1 is induced to produce polyamines and ornithine, which promote wound healing [44]. In addition, serine, glycine, and glutamine are also vital metabolic regulators of macrophage polarization [45]. As for iron metabolism, M1 macrophages express proteins connected with iron absorption and storage (ferritin, natural resistance-associated macrophage protein 1, and divalent metal transporter-1), restricting the utilization of iron in bacteria growth. While, M2 macrophages up-regulate molecules relevant to iron circulation and release (transferrin receptor, hemoxygenase-1, and ferroportin), contributing to cell proliferation and wound healing [46].

In addition to metabolic modulation, physical factors also systematically regulate macrophage polarization. Changes in the physical microenvironment, including the structure, morphology, and stiffness of the extracellular matrix, affect the polarization phenotype of macrophages. Matrix stiffness affects cell adhesion, contraction, migration and differentiation by changing the elastic modulus of substrate and density of surface adhesion ligand and indirectly regulates cell polarization [47]. Furthermore, substrate pattern and surface roughness also affect cell phenotypes. Previous studies have reported that macrophages showed biphasic polarization in response to the size of substrate microgrooves [48], and the surface roughness synergically up-regulated the secretion of all inflammatory cytokines [49].

Iron-based nanoparticles modulate macrophage polarization

Numerous studies reported the role of iron-based nanoparticles in modulating macrophage polarization and function. Macrophage polarization is a dynamic and reversible process and involves the changes of a series of markers and signals. Macrophage polarization is not a dualistic model, while M1 and M2 represent the two extremes of the phenotype. By means of phenotypic loss, phenotypic induction, and phenotypic reversal, iron-based nanoparticles polarize macrophages toward either a pro- or anti-inflammatory phenotype (Figure 1).

Polarization towards the pro-inflammatory phenotype

A variety of iron-based nanoparticles are found to promote a pro-inflammatory phenotype of macrophages by means of phenotype induction and phenotype reversal (Table 1). In most cases, IONPs promote the pro-inflammatory polarization of macrophages, while in rare cases PBNPs with specific coating materials [50], can also induce a M1-like phenotype. For example, the polyethylenimine-coated superparamagnetic iron oxide nanoparticles (SPION) induced M1 polarization dramatically, characterized by notable upregulation of typical M1-related genes such as CD80, IL-1 β , TNF- α and so on [51]. Likewise, ferucarbotran and ferumoxytol, the two kinds of clinically used SPION, were reported to promote M1-like inflammatory response both *in vitro* and *in vivo* [52]. Furthermore, PBNPs coated with low molecular weight hyaluronic acid (LMWHA molecular weight [MW] < 5 kDa) have been shown to induce the pro-inflammatory phenotype and inhibit tumor growth [50, 53].

For diseases that are attributed to the lack of active pro-inflammatory M1 macrophages, reprogramming

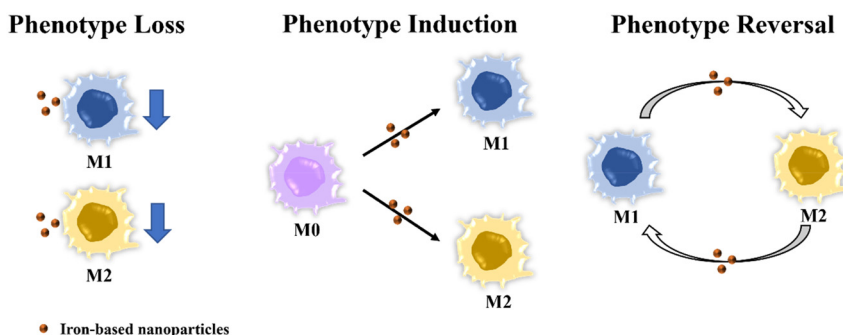


Figure 1: Iron-based nanoparticles modulate macrophage phenotype through phenotype loss, induction and reversal.

Table 1: Iron-based nanoparticles inducing polarization towards pro-inflammatory phenotype.

Polarization	Properties of nanoparticles					Experimental procedures			Marker changes in polarization	Ref.
	Iron-based nanoparticles		Core			Cell types /Animal models	Methods	Marker changes in polarization		
	Core	Coating	Surface charge	Size (nm)	Methods					
Phenotype Induction (M0 to M1)	PMA@Fe ₃ O ₄	PMA	-	5	RAW264.7	5 nmol/L, 24h	M1: TNF-α CD86 NF-κB ↑ M2: CD206 ↓	[63]		
	PEI-coated SPION	PEI	+	139–228	RAW264.7, THP-1, mouse peritoneal macrophages	6.25–25 μg/mL, 6h	M1: CD40 CD80 CD86 IL-1β TNF-α IL-6 IL-12 ↑	[51]		
	Resovist (ferucarbotran)	Dextran	+	/	RAW264.7, BMDM, Ana-1	Fe 0–100 μg/mL, 24h	M1: IL-1β IL-12p40/70 TNF-α IL-2 IL-10 ↑ M1: IL-1β TNF-α ↑	[52]		
	Feraheme (ferumoxytol)	PSC	/	/	Balb/c mice RAW264.7, BMDM, Ana-1	Fe 25mg/kg Fe 0–100 μg/mL	M1: IL-12p40/70 IL-4 IL-13 ↑	[52]		
Phenotype Reversal (M2 to M1)	PEG-Fns	PEG	/	20	Balb/c mice RAW264.7	Fe 25mg/kg Fe 320 μmol/L, 12h	M1: IL-1β TNF-α ↑ M1: TNF-α ↑ M2: IL-10 ↓	[64]		
	Iron-dextran	Dextran	/	/	IL-4 treated BMDMs	5 mg, 7d	M1: CD16/32 ↑ M2: CD206 ↓	[65]		
	Fe ₃ O ₄ -PLGA	PLGA	-	283.8±24.1	IL-4 treated RAW264.7	10 mg/mL	M1: TNF-α IL-1β iNOS ↑ M2: TGF-β IL-10 Arg1 ↓	[66]		
	Fe ₃ O ₄	/	-	453	Lps treated BMDMs	25 μg/mL, 24h	M2: IL-10 SOCS1 SOCS3 ↓ M1: TNF-α IL-12 ↑	[67]		
Phenotype Reversal (M2 to M1)	Fe ₃ O ₄ @D-SiO ₂	Dendritic silica	-	490x320	IL-4 treated RAW264.7/BMDMs	15 μg/mL, 24h	M1: CD80 CD86 CD64 ↑ M2: CD206 Arg-1 ↓	[68]		
	CD206-Fe ₃ O ₄ -PLGA	PLGA	-	294.3±17.8	Subcutaneous tumor-bearing mouse model	/	M1: CD86 ↓	[66]		
	PHNPs@DPA-S-BSA-MA@3-MA	/	-	20	MDA-MB-231 co-cultured RAW264.7/THP1	200 μg/mL, 24h	M1: CD86 iNOS IL-12 TNF-α ↑ M2: CD206 IL-10 Arg-1 ↓	[69]		
	SPION	Carboxy-dextran	-	/	MDA-MB-231 tumor-bearing mouse model	20 mg/kg	M1: CD86 iNOS IL-12 TNF-α ↑	[70]		
Phenotype Reversal (M2 to M1)	Feraheme (ferumoxytol)	PSC	/	/	IL-4/IL-13 treated THP1	Fe 20 μg/mL, 24h/48h	M1: CD86 TNF-α ↑ M1: ROS TNF-α CD86 ↑ M2: CD206 IL-10 ↓	[6]		
	PEI-SPION	PEI	+	/	Mammary tumour model PMA treated THP1	Fe 2.73/8.37mg/mL 4 μg/mL	M1: IL-1β IL-6 ↑ M2: IL-12 ↓	[71]		
	Fe@PDA-PEG	mPEG-SH	/	162	Highly vascularized MDA-MB-231 xenograft tumors model IL-4 treated RAW264.7	1.2 mg/kg	M1: Mac3 iNOS ↑ M2: Arg-1 ↓ M1: iNOS IL-12 ↑ M2: IL-10 ↓	[72]		
	Subcutaneous model of colon	30 mg/kg, 24h					M1: CD80 ↑ M2: CD206 ↓			

Table 1: (continued)

Polarization	Properties of nanoparticles			Experimental procedures		Ref.		
	Iron-based nanoparticles	Core	Coating	Surface charge	Size (nm)		Cell types /Animal models	Methods
PEG-Fns	Fns	PEG	/	/	20	SCC-7 co-cultured RAW264.7	Fe 320µmol/L, 12h	M1: TNF-α ↑ M2: IL-10 ↓
LMWHA-MPB	PBNPs	LMWHA	-	-	127	Subcutaneous tumor-bearing mouse model IL-4 treated RAW264.7	Fe 800µmol/kg, 200 µL 50 µg/mL, 3h	M1: iNOS ↑ M2: CD163 ↓ M1: CD86 IL-12 ↑ M2: IL-10 ↓
HA-PB/ICG	PBNPs	HA	-	-	295	4T1 tumor-bearing mouse model IL-4 treated RAW264.7	10 mg/kg 1, 5, 20 and 50 µg/mL, 24h	M1: CD86 ↑ M2: CD206 ↓ M1: CD86 ↑ M2: CD206 ↓
HION@Macs	/	HA	/	/	/	4T1 tumor-bearing mouse model IL-4 treated RAW264.7	20 µg/mL, 8h 50 µg/mL, 24h	/ M1: CD80 iNOS ↑ M2: Arg-1 ↓
Man-HMPB/HCQ	PBNPs	Mannose	-	-	247	4T1 breast tumor bearing mouse model IL-4 treated RAW264.7	5 mg/kg, 24h 10h	M1: TNF-α CD86 ↑ M2: CD206 ↓ M1: CD86 IL-6 ↑ M2: CD206 IL-10 ↓
IMSN-PEG-TI	Iron manganese silicate nanoparticles	PEG	/	/	122	CT26 co-cultured mice abdominal macrophages CT26-tumor-bearing mouse models	2.5 mg/kg 100 µg/mL, 24h 10 mg/kg	M1: CD86 IFN-γ TNF-α ↑ M2: CD206 IL-10 ↓ M1: i6C ↑ M2: CD206 ↓ M1: i6C ↑ M2: CD206 ↓

PMA, Amphiphilic polymer; PEI, Polyethyleneimine; PSC, Polyglucose sorbitol Carboxymethyl ether; Fns, Ferrihydrate nanoparticles; PEG, Polyethylene glycol; PLGA, Poly(lactic-co-glycolic) acid; PDA, polydiacetylene; mPEG-SH, thiol-terminated polyethylene glycol monomethyl ether; PHNPs@DPA-S-BSA-MA@3-MA, mannose (MA) modified porous hollow iron oxide nanoparticles (PHNPs) loaded with 3-methyladenine (3-MA) and blocked with bovine serum albumin (BSA); LMWHA-MPB, Mesoporous Prussian blue NPs with low molecular weight hyaluronic acid surface modification; HA-PB/ICG, Hyaluronic acid modified mesoporous Prussian blue nanoparticles loaded photosensitizer indocyanine green; HA, Hyaluronic acid; HION, Hyaluronic acid-decorated superparamagnetic iron oxide nanoparticles; Man-HMPB/HCQ, hollow mesoporous Prussian blue (HMPB) nanosystem with mannose decoration and hydroxychloroquine (HCQ) adsorption; IMSN-PEG-TI, TGF-β inhibitor (TI)-loaded PEGylated iron manganese silicate nanoparticles (IMSN); ROS, reactive oxygen species.

macrophages from immunosuppressive M2 to the killing mode of M1 could be an ideal strategy. It is widely reported that most of the tumor-associated macrophages (TAMs) in the tumor microenvironment are the typical M2 phenotype [54–56]. By turning macrophages from M2-like TAMs to M1, iron-based nanoparticles can reverse the immunosuppressive status and promote effective tumour killing. For example, ferumoxytol inhibited tumor progression effectively by the promotion of M1 macrophages and inhibition of TAMs in mice [6]. Likewise, Hou et al. [57] designed and synthesized biological membrane-coated hollow mesoporous Prussian blue nanomaterials (PBNPs) for cancer therapy. This biomimetic nanosystem demonstrated dramatic effects on turning TAMs into M1 macrophages and suppressing tumor growth both *in vitro* and *in vivo*.

Polarization towards the anti-inflammatory phenotype

On the other hand, some iron-based nanoparticles are capable of inhibiting the inflammatory response and inducing M2 polarization, which has great potential to be applied for damaged tissue repair, wound healing, anti-inflammation, etc (Table 2). Iron-based nanoparticles can induce an anti-inflammatory phenotype of macrophage in three ways, loss of M1, phenotype induction from M0 to M2, and phenotype reversal from M1 to M2. Loss of M1 could be achieved by inhibiting pro-inflammatory cytokines release or reducing the numbers of M1 macrophages. For example, Park et al. reported that hyaluronan-coated PBNPs significantly suppressed both the function and population of M1 macrophage in an LPS-induced murine model, with great therapeutic effect for murine peritonitis [58]. Similarly, Chen et al. found that both 10 and 30 nm PEG-coated SPIONs inhibited the expression of LPS-induced pro-inflammatory factor, IL-6 and TNF- α , in a dose-dependent way [59]. However, in these models, whether the loss of M1 phenotype is associated with increased numbers and function of M2 macrophages remains unknown.

In addition to phenotypic loss, treatment of iron-based nanoparticles could directly induce the M2-like phenotype from either M0 or M1 phenotype. Liu et al. [60] developed self-assembled Fe³⁺-catechin nanoparticles (Fe-cat NPs) with a strong ability to facilitate M2 polarization from M0. By secreting anti-inflammatory cytokines, the Fe-cat NPs-treated macrophages contributed to efficient bone repair. For inflammation-related diseases characterized by massive accumulation of M1, the shift from M1 to M2 by

iron-based nanoparticles could strongly diminish inflammation and reverses disease conditions. Fan et al. developed hollow-structured manganese prussian blue nanozyme with the ability to convert macrophages from M1 to M2, resulting in effective treatment of osteoarthritis [61]. In addition, Huang et al. also applied polyvinylpyrrolidone (PVP)-coated PBNPs to eliminate inflammation in the hepatic ischemia-reperfusion injury model, mainly by promoting M2 macrophages [62].

Influence factors of macrophage polarization by iron-based nanoparticles

As we summarized in Tables 1 and 2, different types of iron-based nanoparticles exert different influences on macrophage polarization. The modulation of iron-based nanoparticles on macrophage polarization is a complex process relying on the coordination of various factors [82–85]. The inherent physicochemical properties of designed nanoparticles, such as composition, size, and surface characteristics, largely affect the interactions between nanoparticles and macrophages and are attributed to the differences in polarization (Figure 2).

Composition

The different compositions and structures of iron-based nanoparticles notably affect their functions on macrophage polarization. In general, IONPs tend to induce a pro-inflammatory M1 phenotype, while PBNPs tend to induce an anti-inflammatory M2 phenotype. For example, one of the FDA-approved IONPs ferumoxytol strongly induced the M1 phenotype at a dose of 2.73 mg Fe/mL *in vivo* for effective inhibition of the growth and metastasis of mammary mouse tumor [6]. On the other hand, polyvinylpyrrolidone-coated PBNPs with an average size of 80 nm exhibited a notable induction of M2 phenotype in LPS-treated macrophage and was successfully applied for alleviating hepatic ischemia-reperfusion injury in mouse model [62]. However, the general principle is not always followed when other influencing factors become dominant, such as size, surface modifications, heterogeneous compositions, and external factors. Therefore, exceptions were found in special cases where PBNPs triggered M1 polarization and Fe₃O₄ nanoparticles induced M2 polarization. For instance, the PMA-coated mesoporous hollow Fe₃O₄ nanoparticles induced M0 to M2, which is largely affected by the addition of an alternating

Table 2: Iron-based nanoparticles inducing polarization towards anti-inflammatory phenotype.

Polarization	Properties of nanoparticles				Experimental procedures		Marker changes in polarization	Ref.	
	Iron-based nanoparticles	Core	Coating	Surface charge	Size, nm	Cell types/Animal models			Methods
Loss of M1 phenotype	Fe-cat NPs	Fe-cat complex	/	+	40–150	Lps treated RAW264.7	10–50 µg/mL	M1: TNF-α IL-1β IL-6 iNOS ↓	[60]
	MelLiON	Fe ₃ O ₄	Melittin	-	202.4	Rat femur defect models Intracranial arterial dolichoectasia model	20 µg/rat 2.5 mg/kg	M1: iNOS ↓ M1: CD86 TNF-α ↓	[75]
	PBNPs	PBNPs	Hyaluronan	-	180	Lps treated RAW264.7	31.25 µg/mL, 24 h	M1: H ₂ O ₂ CD86 ↓	[58]
Phenotype induction (M0 to M2)	PBNPs	PBNPs	PVP	-	60	Colitis mouse model	40 mg/kg	M1: ROS TNF-α IL-6 IL-1β ↓	[76]
	HPBZs	PBNPs	PVP	-	78	Lps treated RAW264.7	/	M1: iNOS IL-1β NO ↓	[77]
	Fe-cat NPs	Fe-cat complex	/	+	40–150	Middle cerebral artery occlusion model RAW264.7	40 µg/mL, 10 µL 50 µg/mL	M1: TNF-α IL-1β ↓ M2: Arg-1 CD163 CD206 ↑	[60]
Phenotype reversal (M1 to M2)	MHFPs	Fe ₃ O ₄	PAM	-	185	RAW264.7	40 µg/mL, alternating magnetic field (1.5 mT, 30 Hz, 2 h/day), 2 days	M1: CD32 iNOS ↓ M2: Arg-1 CD206 IL-10 ↑	[78]
	Prussian blue nanozyme	PBNPs	PVP	-	67	Lps treated RAW264.7	0–40 µg/mL, 24 h	M1: IL-6 TNF-α iNOS ROS CD16/32 ↓ M2: CD206 IL-10 Arg-1 ↑	[79]
	Prussian blue nanozyme	PBNPs	/	-	34	Lps treated RAW 264.7	6 weeks	M1: CD16/32 ↓ M2: CD206 ↑	[7]
HMPB@curcumin@PDA	PBNPs	PBNPs	PDA	-	188	Lps treated RAW264.7	50 µg/mL, 24 h	M1: TNF-α IL-1β ROS ↓ M2: Arg-1 ↑	[80]
	MPBzyme	PBNPs	NCM	-	160.7 ± 6.08	Inflammation skin wound model Glucose deprivation treated microglia	50/500 µg, 14d 24 h	M1: TNF-α ↓ M1: ROS CD80 TNF-α IL-1β ↓ M2: CD206 TGF-β IL-10 Arg-1 ↑ M1: ROS TNF-α IL-1β ↓	[81]
	HMPBzyme	Manganese prussian blue	BSA	-	210	Lps treated BMDMs	80 µg/mL, 24 h	M1: IL-1β TNF-α HIF-1α ROS ↓ M2: Arg-1 IL-10 ↑	[61]
PBNPs	PBNPs	PBNPs	PVP	-	80.2	Lps treated RAW264.7	80 µg/mL, 24 h	M1: HIF-1α iNOS TNF-α IL-1β ROS ↓	[62]
	PBNPs	PBNPs	PVP	-	80.2	Lps treated RAW264.7	50 µg/mL	M1: TNF-α IL-1β ↓ M2: IL-10 Arg-1 ↑	[62]
	PBNPs	PBNPs	PVP	-	80.2	Lps treated RAW264.7	1 mg/kg, 24 h	M1: TNF-α IL-1β ↓ M2: IL-10 ↑	[62]

Fe-cat NPs, Fe³⁺-(+)-catechin nanoparticles; MHFPs, Mesoporous hollow Fe₃O₄ nanoparticles; PAM, Polyacrylamide; PVP, Polyvinylpyrrolidone; HMPB, Hollow-structured manganese prussian blue; MPBzyme, Mesoporous Prussian blue nanozyme; NCM, Neutrophil-like cell membrane; HMPBzyme, Hollow-structured manganese prussian blue nanozyme; BSA, Bovine serum albumin; HPBZs, Hollow prussian blue nanozymes; MelLiON, Melittin-loaded L-arginine-coated iron oxide nanoparticle.

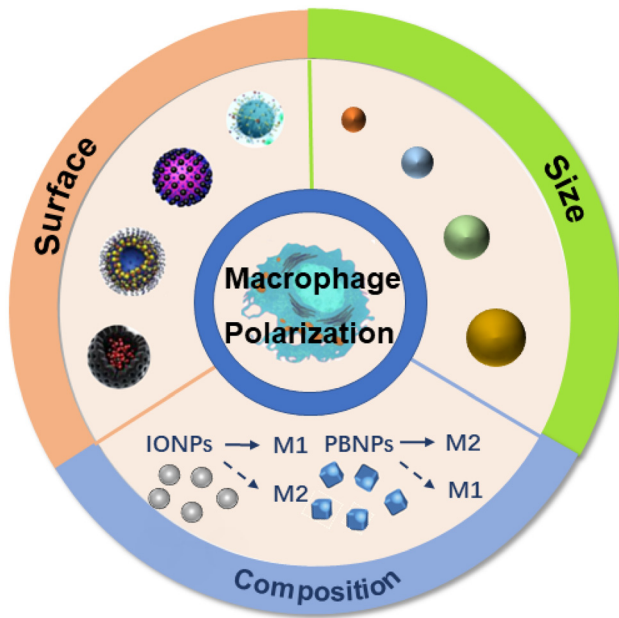


Figure 2: Internal influence factors of iron-based nanoparticles affecting macrophage polarization.

magnetic field [78]. In addition, the PB-based nanoparticles composed of hollow mesoporous Prussian blue, hydroxychloroquine (HCQ), and mannose decoration (Man-HMPB/HCQ) are reported to induce TAM to M1, probably due to the strong autophagy suppression effect of HCQ since inhibition

of autophagy is known to contribute to the phenotypic reversal of TAMs [57].

Furthermore, the differences in valence states of iron ions in nanoparticles also influence whether and how they induce macrophage polarizations. It was generally believed that magnetite IONPs (Fe_3O_4) are much more effective in inducing M1 polarization than hematite IONPs (Fe_2O_3) [68]. To compare the impact of states of iron (II and III) on macrophages polarization, Yu et al. synthesized the $\text{Fe}_2\text{O}_3@D\text{-SiO}_2$ and $\text{Fe}_3\text{O}_4@D\text{-SiO}_2$ nanoparticles with similar size (about 40 nm), parallel core-shell structures, identical ellipsoidal morphology, as well as the same surface modification by large-pore dendritic silica shell ($D\text{-SiO}_2$). They found that only $\text{Fe}_3\text{O}_4@D\text{-SiO}_2$ achieved significant M1 polarization from M0 or M2 macrophages, resulting in an excellent tumor suppression effect in the melanoma mouse model. Compared with $\text{Fe}_2\text{O}_3@D\text{-SiO}_2$, $\text{Fe}_3\text{O}_4@D\text{-SiO}_2$ induced much higher intracellular iron accumulation and thus triggered the interferon regulatory factor 5 (IRF-5) pathway, which is one of the key transcriptional factors that promoted the M1 polarization (Figure 3A). The reason for these different intracellular iron levels largely depends on their diversity in the dynamic process of iron endocytosis, intracellular degradation, and export rate from cells. In this model, $\text{Fe}_3\text{O}_4@D\text{-SiO}_2$ exhibited a relatively higher uptake ability than $\text{Fe}_2\text{O}_3@D\text{-SiO}_2$, which is probably due to the magnetism-induced aggregation [86].

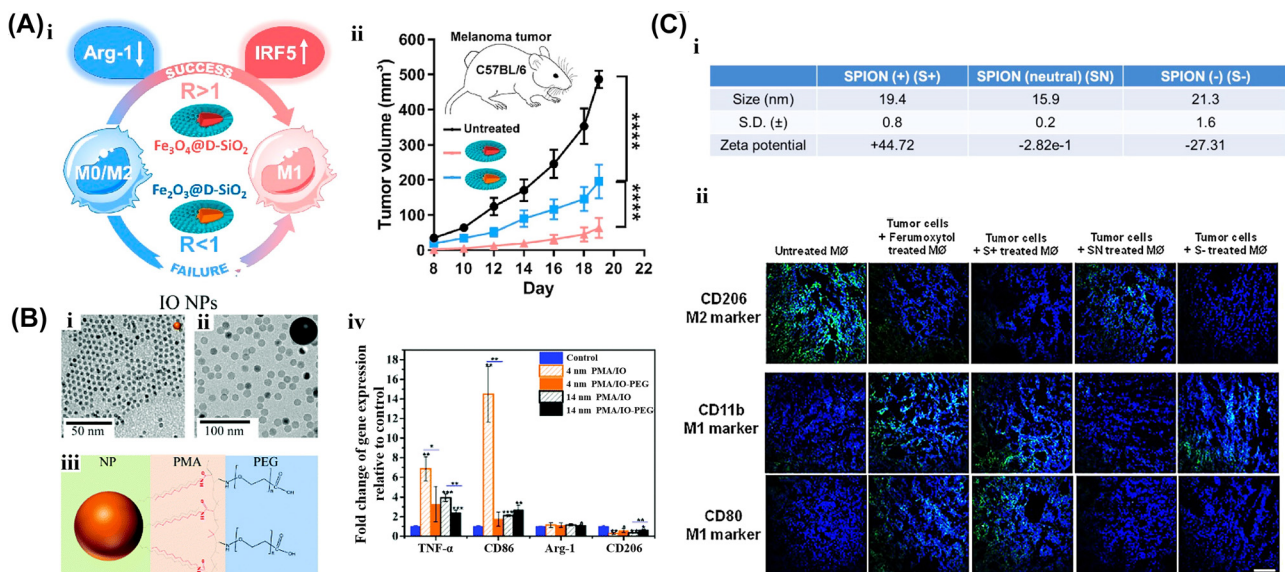


Figure 3: The effect of composition, size and surface of iron-based nanoparticles on the macrophage polarization. A. (i) Schematic diagram of the macrophage polarization effects of $\text{Fe}_2\text{O}_3@D\text{-SiO}_2$ and $\text{Fe}_3\text{O}_4@D\text{-SiO}_2$ with different the ratio (R) of intracellular iron accumulation. (ii) Antitumor properties of IONPs with different components in the melanoma tumor model. Reproduced with permission [68]. Copyright © 2019 American Chemical Society. B. TEM images (i, ii) and schematic diagram (iii) of nanoparticles with a polymer coating and surface PEGylation. (i. PMA/IO NPs; ii. PMA/IO-PEG NPs). (iv) Gene expression in RAW264.7 cells after IONPs treatment for 24 h. Reproduced with permission [63]. Copyright 2019, RSC Pub. C. (i) Characteristic of three differently charged SPION. (ii) Immunohistochemical staining of different treatment groups. The green area reflects the expression of CD206, CD11b and CD80. Reproduced with permission [90]. Copyright © 2020 Zhang, Cao, Liang, Tan, Luo, Xu and Saw.

Size

Iron-based nanoparticle-induced macrophage polarization is typically affected by the size properties of the materials. In particular, nanoparticles with similar composition but different sizes could exhibit different functions on macrophage polarization [87, 88]. For instance, Cheng et al. synthesized two groups of amphiphilic polymers (PMA)-modified Fe_3O_4 NPs and Au NPs with two different sizes, 4 and 14 nm, to investigate the influence of nanoparticles' size on macrophage polarization [63]. They demonstrated that the 4 nm Fe_3O_4 NPs triggered M1 polarization more effectively than 14 nm Fe_3O_4 NPs, which might be due to the higher cellular uptake rate and intracellular accumulation of small nanoparticles. Meanwhile, the same trend was found with Au NPs (Figure 3B). Besides, Dalzon et al. compared the effects of two carboxymaltose-modified Fe_2O_3 nanoparticles with different sizes (20 and 100 nm) on the macrophages. They found that at a concentration of 1 mg/mL, the 100 nm Fe_2O_3 nanoparticles significantly inhibited the secretion of pro-inflammatory cytokines such as IL-6, TNF- α , and NO, as compared to smaller ones [89]. These results demonstrated that the size of iron-based nanoparticles indeed has an essential influence on the degree of macrophage polarization. However, whether and how the size factor contribute to the direction of macrophage polarization is unclear and requires further exploration in the future.

Surface

As another important characteristic of nanoparticles, the surface property is of great significance in influencing

macrophage polarization, including surface charge, morphology, hydrophilicity, etc. In general, nanoparticles with a charged surface induce the polarization of M1 or M2 more easily than neutral ones. For example, Saw et al. explored the impact of the surface charge of nanoparticles on macrophage polarization by modifying ferumoxytol with different charges. They demonstrated that both positively and negatively charged SPIONs established a strong effect to repolarize macrophage from M2-like TAM to M1 and significantly suppressed tumor growth in the mouse model, while the neutral ones failed to induce polarization of macrophage [90]. It was presumed that the neutral surface limited the nanoparticles' internalization by cells, and thus restrained the M1 polarization of ferumoxytol (Figure 3C). Consistently, a previous study has reported that PMA-coated IONPs significantly induced M1 polarization in RAW264.7, while further modifying IONPs with PEG outside the original layer dramatically reduced the effect of M1 polarization [63]. This is likely due to the limited uptake and endocytosis of PEGylation of nanoparticles by macrophages, resulting in less amount of particle accumulation and decreased activation of cellular signal and immune response [91]. The difference in cellular uptake of distinct surface modified-nanoparticles is supported by another research, since polyethyleneimine (PEI)-coated ultrasmall superparamagnetic IONPs induced much higher cellular uptake than PEG-modified IONPs (Figure 4) [87, 91].

In addition, certain coating materials are known to have a strong macrophage modulating effect, therefore iron-based nanoparticles coated with them are inclined to gain the same effect as the coating material. For example, LMWHA can induce macrophage polarization from M2 to M1 phenotype [92, 93]. Therefore, iron-based nanoparticles coated with LMWHA could promote M1 polarization macrophages.

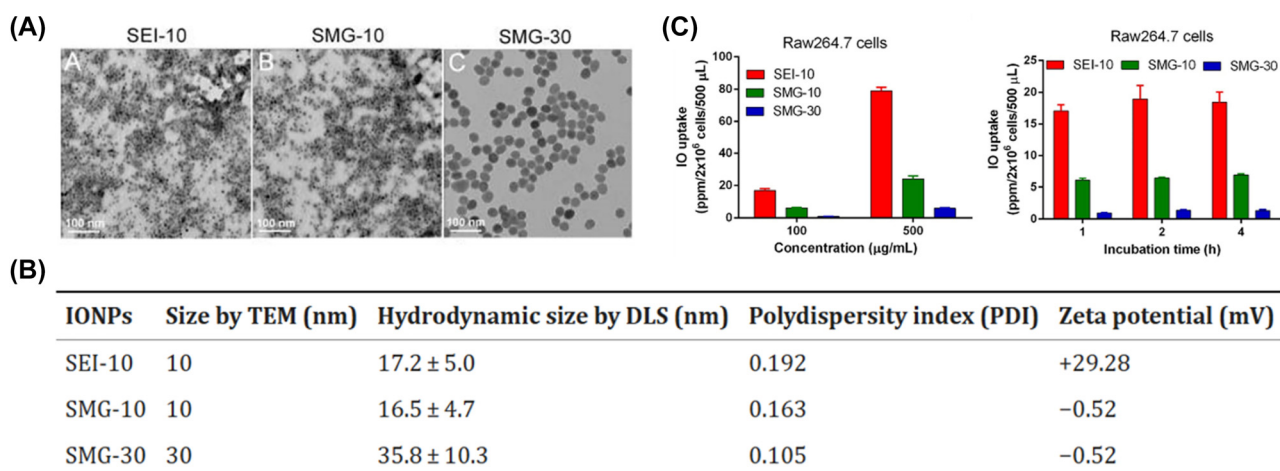


Figure 4: The effect of size and surface of iron-based nanoparticles on the cell uptake ability in macrophages. TEM images (A) and characteristics (B) of different IONPs. (C) Iron uptake in RAW264.7 cells measured by ICP-MS after co-culturing with different concentrations of IONPs for 1h or with the same concentration (100 $\mu\text{g/mL}$) for different incubation times. Reproduced with permission [87]. Copyright 2018, Springer Nature.

Zhang's group synthesized the LMWHA-modified mesoporous PBNPs (LMWHA-MPB) with an average size of 127 nm and further loaded them with photosensitizer indocyanine green (ICG) to form LMWHA-MPB/ICG. Both LMWHA-MPB and LMWHA-MPB/ICG promoted a M2 to M1 phenotype reversal in RAW264.7 cells [50, 53], which are opposite to most PBNPs reported by other studies [7, 62, 79], suggesting that the functional modifications on the surface of iron-based nanoparticles largely shift their effect on macrophage polarization.

Mechanisms of macrophage polarization by iron-based nanoparticles

The interactions between macrophages and iron-based nanoparticles involve multiple procedures and diverse modulation pathways (Figure 5), including cellular signaling sensing and transduction, intracellular redox level rebalancing, lysosomal-autophagy modulation and iron metabolism alteration, etc. [84, 94].

Membrane receptors-mediated modulation

Surface receptors are the first-line sensors by which macrophages identify and respond to external signals. Various macrophage surface receptors including pattern

recognition receptors (PRRs), scavenger receptors, complement receptors, cytokine receptors, etc., are responsible for phagocytosis and immunomodulation, mainly through the modulation of macrophage polarization. As important group members of PRRs, the Toll-like receptors (TLRs) family consists of several subtypes and is responsive to different danger signals. Among them, the activation of TLR-4 by lipopolysaccharide (LPS) strongly triggers M1 polarization to facilitate the killing of invaded bacteria, while blockage of TLR-4 limits its function effectively [95].

Importantly, iron-based nanoparticles are reported to interact with membrane receptors, such as TLR-4, to regulate macrophage polarization. For example, PEI-coated SPIONs initiated the TLR-4 cascade signaling pathway and promoted M1 polarization in both mouse and human-derived macrophages [51]. However, pre-treatment of RAW264.7 cells with the TLR-4 inhibitor CLI-095 strongly inhibited SPION-induced expression of M1 genes, suggesting that SPION-mediated M1 polarization is partly through inducing the TLR-4 pathway [51]. In addition, Ai et al. studied the interaction between two commercial SPIONs with macrophages, Resovist (ferucarbotran) and Feraheme (ferumoxytol). They found that both of the SPIONs interacted with TLR-4 and facilitated the M1-like phenotype through TLR-4-mediated p38-Nrf2 signaling pathway, with the secretion of pro-inflammatory cytokines like IL-12, TNF- α , and IL-1 β (Figure 6B) [52]. Furthermore, inhibition of TLR-4 by CLI-095 greatly limited the expression of SPION-induced M1-like phenotype (Figure 6C) [96]. Taken

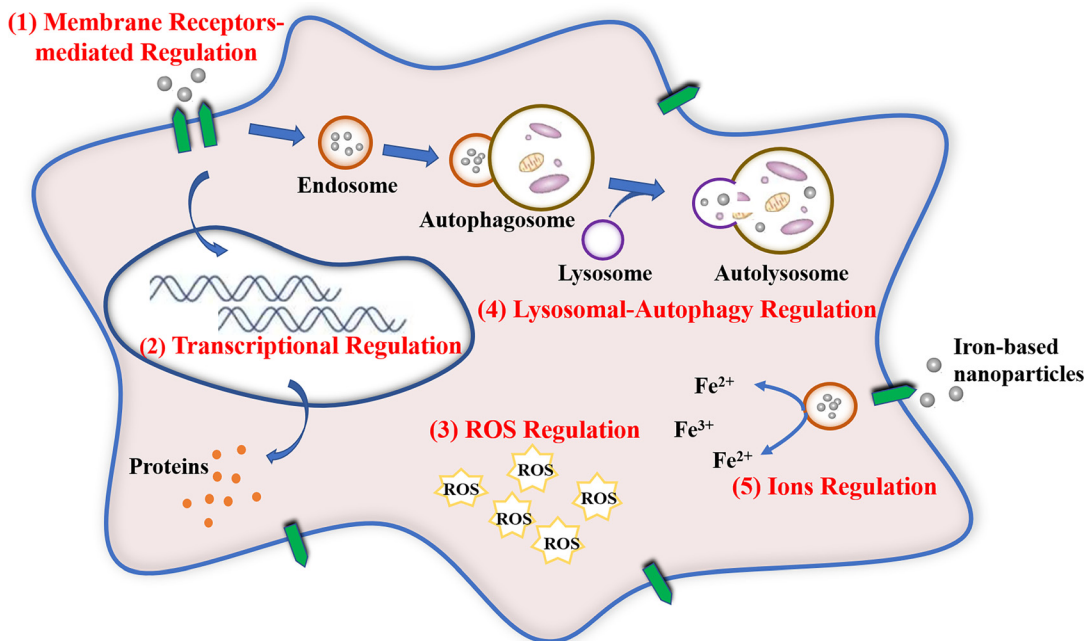


Figure 5: Modulation mechanisms of macrophage polarization by iron-based nanoparticles. ROS, reactive oxygen species.

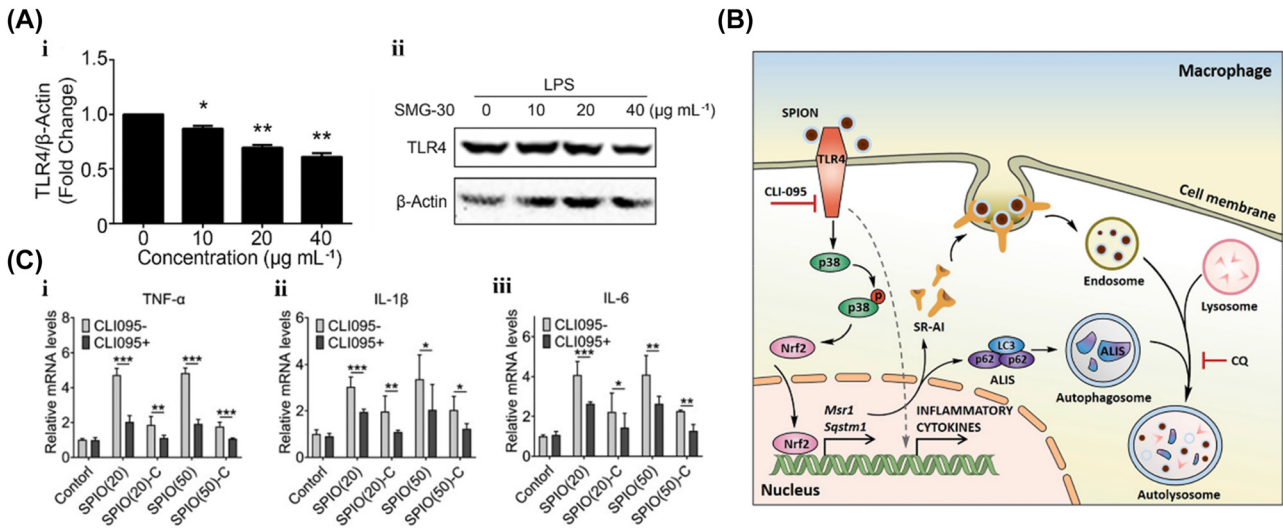


Figure 6: The membrane receptor-mediated modulation effect by iron-based nanoparticles. A. (i) The relative mRNA level and (ii) expression of TLR4 in RAW264.7 cells stimulated by different concentrations of SMG-30 and LPS ($1 \mu\text{g/mL}$). β -actin was used as a control. Reproduced with permission [59]. Copyright 2020, John Wiley and Sons. B. Schematic diagram of the mechanism of TLR-4 and autophagy pathway activated by SPION. Reproduced with permission [52]. Copyright 2019, Elsevier. C. The relative mRNA levels of (i) TNF- α , (ii) IL-1 β , and (iii) IL-6 in SPIO-treated RAW264.7 cells with or without CLI-095. Reproduced with permission. Copyright 2022, Oxford University Press.

together, these results demonstrated that TLR-4 acts as important membrane receptors for SPIONs to induce M1 polarization. On the other hand, blocking the expression or function of TLR-4 by iron-based nanoparticles is capable of limiting the LPS-induced M1 polarization. Chen et al. found that a 30 nm PEG-coated SPIONs decreased the transcription and expression of TLR-4 in a dose-dependent manner, which notably decreased the immune-activation function of LPS both in the mouse- and human-derived macrophages (Figure 6A) [97].

Transcriptional modulation

Several key transcriptional factors in the modulation of macrophage polarization are found to be selectively alternated upon IONPs treatment. A group of transcriptional factors is responsible for M1 polarization, including signal transducer and activator of transcription-1 (STAT-1), IRF-5, nuclear factor- κ B (NF- κ B), activator protein-1 (AP-1), etc. On the contrary, activation of STAT-3, STAT-6, peroxisome proliferator-activated receptor- γ (PPAR- γ), etc., are reported to facilitate M2 polarization.

Upregulation of M1-related transcriptional factors directly results in M1 polarization and the secretion of pro-inflammatory cytokines. For example, the hyaluronic acid-decorated SPIONs has a notable effect on shifting macrophages from M2 to M1 phenotype mainly through the activation of NF- κ B [73]. In addition, $\text{Fe}_3\text{O}_4@D\text{-SiO}_2$ was

found to promote the activation of IRF-5, thus inducing the polarization to M1 [68]. Similarly, Cheung et al. reported that IRF-5 was highly elevated by carboxymethylated IONPs, resulting in polarization from M2 to M1 [98]. Furthermore, bioinformatic gene analysis revealed that transcription factors like NF- κ B and AP-1 were significantly upregulated by DMSA-coated Fe_3O_4 magnetic nanoparticles, and thus M1-like pro-inflammatory reactions were induced [99]. On the other hand, suppressing key transcriptional factors restricted the ability of macrophage polarization and their specific functions. For instance, hollow Prussian blue nanozymes were able to inhibit NF- κ B and efficiently limited the M1 polarization induced by LPS, with down-regulated expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 and IL-1 β [77].

ROS modulation

During M1 macrophage-mediated anti-microbial process, reactive oxygen species (ROS) generate and act as the main weapon to destroy foreign pathogens. In addition, it is revealed that intracellular levels of ROS are involved in the modulation of macrophage polarization [100, 101]. In general, excessive intracellular ROS induces M1 polarization, mainly by activation of MAPK, STAT1 and NF- κ B signaling, while ROS scavenging suppresses M1-mediated pro-inflammatory effects and leads to an M2 phenotype. During the differentiation process of macrophages from monocytes, the existence

of ROS is essential for the subsequent M2 polarization, since the complete inhibition of ROS during differentiation strongly limited the later M2 or TAMs polarization [102]. As for the function of mitochondrial ROS on macrophage polarization, they could induce M1 or M2 polarization according to different reports, which are required for further studies to explain [103, 104].

It is well-known that IONPs can produce massive ROS by triggering Fenton reactions. Therefore, a variety of IONPs induces macrophage polarization through the modulation of ROS. For instance, Kim et al. demonstrated that the combination of dextran-coated IONPs with ascorbic acid was capable of eliciting vast ROS production and facilitating the pro-inflammatory phenotype of macrophages, which exhibited excellent bactericidal function against intracellular staphylococcus aureus [105]. In addition, Fan et al. found that alternating magnetic field further amplified ROS level in macrophages treated by ferrimagnetic vortex-domain iron oxide nanoring and graphene oxide (FVIOs-GO), and stimulated macrophage polarization from TAM to M1 [106]. Besides, the Prussian blue-modified ferritin nanoparticles (PB-Ft NPs) possessed ROS-inducing abilities by their peroxidase-like activity, particularly under 808 nm laser irradiation, and thus promoted M1 polarization. The enhanced peroxidase-like activity of PB-Ft NPs largely depends on the increased temperature under external laser irradiation [107].

In contrast, the elimination of excessive ROS by iron-based nanoparticles reverses the M1 phenotype and relieves the inflammatory condition. It is reported that a series of PBNPs exert ROS scavenging effects and suppress M1 polarization [7, 58, 79, 80, 108]. Due to their excellent anti-inflammatory ability, PBNPs have been widely studied for the treatment of peritonitis, maxillofacial infection, vascular restenosis and skin wound in different tissue. For example, Fan et al. reported that the hollow-structured manganese Prussian blue nanozyme (HMPBzymes) with intracellular ROS clearing activities induced M1 to M2 macrophage polarization under the hypoxia condition and suppressed inflammation in mice osteoarthritic model [61, 109].

Lysosomal-autophagy modulation

Nanoparticles' internalization, transportation, and degradation greatly relied on the intracellular membrane structure-based organelle system such as endosomes, lysosomes and autophagosomes, which also acted as important regulatory pathways for macrophage polarization. The co-localization of nanoparticles with lysosomes sometimes causes lysosome dysfunctions with high-permeable membranes. Thus, the

damaged lysosomes would release their containing enzymes like cathepsin-B and trigger NLRP3-mediated inflammation, which contributes to M1-like polarization (Figure 7A) [110]. For example, Song et al. demonstrated that PMA-coated IONPs caused M1 polarization mainly by inducing lysosomal damage. The same phenomenon was also observed in Au nanoparticles-treated macrophages [63]. Furthermore, the dysfunction of specific secretory lysosomes may block the transportation pathway of certain cytokines, thus inhibiting the function of macrophages. The commercially-used IONPs, Resovist, was reported to inhibit LPS-induced IL-1 β generation by hindering the secretory lysosome-mediated excretion, and thus partially reduced the inflammatory phenotype in murine microglial cells [111].

As an essential intracellular process for energy recycles and stress handling, autophagy is commonly involved in the modulation of immune response including macrophage polarization. In general, the high level of autophagy induces macrophage polarization to the M1 phenotype. Ai's group has conducted a series of studies to clarify the modulation effect of autophagy on polarization. They found that both Resovist (ferucarbotran) and Feraheme (ferumoxytol), the two clinically applied SPION, can trigger the autophagy-mediated pro-inflammatory response *in vitro* and *in vivo*. Importantly, suppression of autophagy by the autophagy inhibitor, chloroquine, dramatically inhibited the SPION-mediated secretion of inflammatory cytokines, strongly suggesting that autophagy acted as one of the important modulation factors for M1-like polarization (Figure 7B) [52, 96]. Recently, further studies demonstrated that compared with bare IONPs, IONPs with carboxyl modification on the surface induced a much lower level of autophagy in macrophages, leading to reduced immune activation [96].

Ion modulation

Most iron-based nanoparticles ultimately undergo degradation by acid organelles and release iron ions into cells. These released ions would further lead to a series of physiological changes and serve as an important regulator of macrophage polarization [112, 113]. Studies have proved that, after the treatment with IONPs, intracellular iron levels were increased in a dose- and time-dependent manner [114]. Theoretically, endocytosis of iron-based nanoparticles with higher iron leaching tends to contribute to a higher level of intracellular irons and trigger M1 polarization. Notably, iron chelate desferrioxamine (DFO) blocked the polarization effect of SPIONs in macrophages [70, 112]. In addition, Resovist-induced polarization from M2 to M1 was strongly limited by the pre-chelation of cellular iron in human-derived

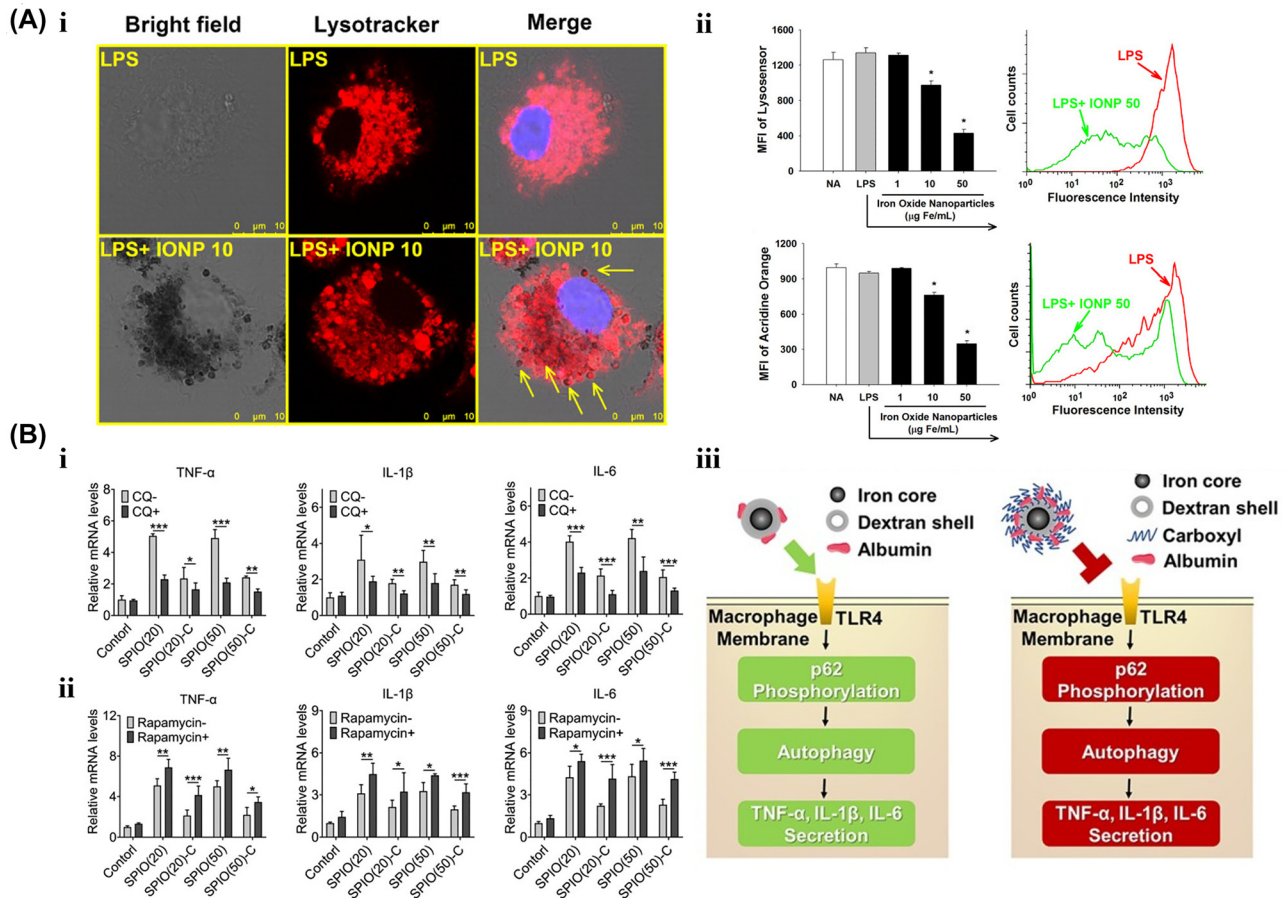


Figure 7: The Lysosomal-autophagy modulation effect by iron-based nanoparticles. A. After pre-treatment with IONPs (1–50 µg Fe/mL), (i) the morphology of lysosomes with Lysotracker (red) staining, (ii) lysosome acidity measured by lysosome sensor and (iii) cell permeability with acridine orange staining in microglia stimulated by LPS. IONPs treated cells had scattered dark brown dots. The arrows represent the colocalization of dark brown dots and lysosomes. Reproduced with permission [111]. Copyright 2013, Springer Nature. B. The relative mRNA levels of TNF-α, IL-1β, and IL-6 in SPIO-treated RAW264.7 cells (i) with autophagy inhibitor CQ and (ii) autophagy enhancer rapamycin, respectively. (iii) Schematic diagram of carboxyl-modified SPIOs inhibited macrophage autophagy and inflammatory cytokine secretion. Reproduced with permission [96].

macrophages. Also, with the addition of DFO, carboxyl-dextran-coated SPIOs failed to increase the expression of M1 makers, such as ferritin and cathepsin L in THP-1 [70]. These results strongly highlight the crucial roles of degraded iron ions in modulating macrophage polarization.

Summary and perspectives

Since the increasing studies on the design, synthesis, and biomedical applications of iron-based nanoparticles, their immune modulation effects have received much attention. After entering human bodies, most nanoparticles will be captured and metabolized by the reticuloendothelial system (RES), such as monocytes and macrophages. Upon uptake of iron-based nanoparticles, the polarization and function of macrophages can be modulated. In this review, we

systemically summarized different iron-based nanoparticles that could regulate macrophage polarization and function and discussed the influence factors and mechanisms underlying the modulation process, with the expectation of providing evidence and guidance for subsequent design and application of iron-based nanoparticles with specific macrophage modulation functions.

In summary, the effect of iron-based nanoparticles on macrophage polarization is a systemic result influenced by the intrinsic physicochemical properties of nanoparticles including composition, size, surface, and so on. Besides, the addition of various external factors such as magnetic fields, laser irradiation, and temperature can trigger specific properties of iron-based nanoparticles and influence macrophage polarization in unconventional ways. Multiple mechanisms are involved in the modulation of macrophage polarization by iron-based nanoparticles, including

membrane receptors interference, transcriptional modulation, ROS rebalancing, lysosomal-autophagy pathway, and iron ions release. Although some of the regularity has been revealed, there are still essential issues requiring further studies: (1) Among all the influencing factors and regulating mechanisms, which is dominant in macrophage polarization over others under specific conditions? (2) How do different factors and mechanisms coordinate with each other and determine the final direction of macrophage polarization? (3) Under different biological conditions, do the same iron-based nanoparticles present identical or different functions on macrophage polarization? (4) How to rationally design iron-based nanoparticles to achieve precise modulation and control of macrophage polarization? In the future, more in-depth research and studies considering the above issues are expected to gain a better understanding of iron-based nanoparticles' modulation on macrophages and promote their better applications to biomedicine.

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