Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) in press
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Review

https://doi.org/10.1631/jzus.B2400183

How do mechanical forces impact macrophages in the processes of mechanosensing and mechanotransduction?

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Abstract: Macrophages are sensitive cells to various external mechanical forces in the environment, such as stretch, shear and pressure. Mechanical forces can be recognized by mechanical signal receptors on the cell surface, such as cell adhesion molecules and ion channels, and transformed into intracellular biological signals, in turn activating different signaling pathways and thereby regulating the phagocytosis, migration and polarization of macrophages. The phenomenon in which macrophages transform into different activated phenotypes and perform different functions under varying environmental stimuli is also known as macrophage polarization. In this review, we discuss the role of mechanically sensitive integrins and ion channels in the mechanical signal sensing of macrophages. We expound on several downstream signaling pathways closely related to integrins and ion channels, such as the Nuclear transcription factor kappa B (NF-κB) pathway, Mitogen-activated protein kinase (MAPK) pathway and Yes-associated protein (YAP)/Tafazzin(TAZ) pathway, that have made good research progress. In addition, we summarize some in vitro experiments on the regulation of macrophage polarization by external mechanical forces, some current cell models for macrophages in vitro, and some commonly used force application devices, with the aim to provide convenience for future in vitro research on macrophages. This paper offers a deep understanding of the mechanical sensitivity and conduction mechanisms of macrophages, which can provide new ideas for the treatment of human diseases.

Key words: Macrophage polarization; Mechanical forces; Mechanosensing; Mechanotransduction; Integrin; TRPV4; Piezo1; Signaling pathways

1 Introduction

Macrophages as important components of innate immunity exist in various organs in the body. They play the essential roles of recognizing and devouring microorganisms and maintaining the stability of the inner microenvironment (Nakai, 2021). As a heterogeneous cell group, macrophages are usually divided according to

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Received Apr. 7, 2024; Revision accepted Nov. 21, 2024;
Crosschecked xxx. xx, 20xx; Published online xxx. xx, 20xx

their functions into the classic activated M1 type and the alternative activated M2 type. M1 cells, which are activated by cytokines secreted by Th1 cells, such as interferon-γ (IFN-γ), tumor necrosis factor (TNF) and lipopolysaccharide recognition (LPS), often use CD80 and CD86 as surface markers and can phagocytose pathogens. They secrete reactive oxygen species, proinflammatory factors such as TNF and IL-6, and free radicals, often causing tissue inflammation. On the other hand, M2 cells, which are activated by cytokines secreted by Th2 cells, such as IL-4 and IL-13, often use CD163 and CD206 as surface markers. They remove tissue debris, phagocytose apoptotic cells and promote tissue repair by secreting anti-inflammatory factors such as IL-4, IL-10 and transforming growth factor-β (TGF-β) (Wang et al., 2019; Ross et al., 2021). M2 cells are classified into four subtypes: M2a, M2b, M2c and M2d (Harwani, 2018). Each subtype is activated by different factors and plays distinct roles (Fig. 1). M2a can typically be induced by IL-4 and IL-13. Immune complexes can activate several signaling pathways such as MAPK-, PI3K- and NK-κB-mediated pathways. TLR ligands then act as a second stimulus to fully activate MAPKs, PI3K/Akt and NK-κB, turning macrophages toward the M2b phenotype (Zhang et cl., 2023). Meanwhile, IL-10, TGF-β and IL-19 can trigger macrophages to transfer to the M2c phenotype, and TLR ligands such as LPS can switch macrophages to the M1 phenotype. However, in the presence of adenosine, M1 cells are switched to the M2d phenotype (Ferrante et al., 2013). A study on the microenvironment of human atherosclerotic plaques revealed the possible types of specific macrophage subgroups stimulated by different microenvironments. Such are the M(Hb) subtype activated by hemoglobin–haptoglobin complexes and the Mhem subtype induced by Haem and the M4 subtype expressing platelet factor 4 (CXCL4). In addition, a macrophage subtype, Mox, activated by oxidized phospholipids was found in mice, accounting for 30% of atherosclerotic plaques. However, the existence of this subtype has not been confirmed in the human body (Chinetti-Gbaguidi et al., 2015).

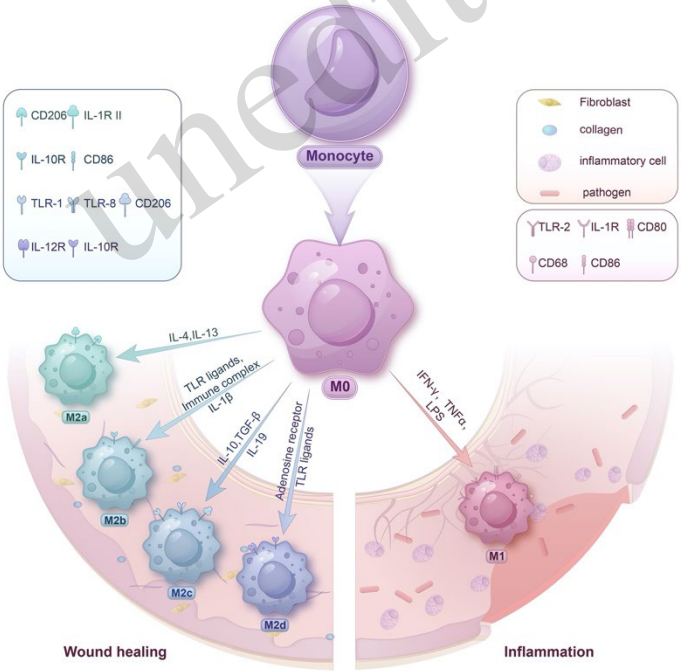

Fig. 1 Polarization of macrophages and their function in wound healing. CD206, cluster of differentiation 206; IL-1R II, Interleukin-1 receptor, type II; IL-10R, interleukin-10 receptor; CD86, cluster of differentiation 86; TLR-1, toll-like receptor 1; TLR-8, toll-like receptor 8; IL-12R, interleukin-12 receptor; TLR-2, toll-like receptor 2; CD68, cluster of differentiation 68.

Macrophages are mechanically sensitive cells, that is, they can sense mechanical forces in the microenvironment and transmit them as biological signals, and adjust their own functions accordingly, such as migra-

tion, phagocytosis and polarization (Vasse et al., 2021). Macrophages are subjected to various mechanical stimuli in the process of survival and migration, such as the shear force caused by blood flow and interstitial flow, cyclic stretching force in respiratory circulation, the compressive force of migration between the vascular endothelium, and the hardness of the extracellular matrix (Xia et al., 2010). These mechanical factors constitute the micromechanical environment in the body, which continuously affects the function and phenotype of macrophages. Because of the close proximity of cells, changes in the cytoskeleton caused by the mechanical stimulation of cells can be transmitted onto adjacent cells as slight mechanical stress. Besides, cells in the bone, cartilage, skin, heart, and lung are directly affected by macro-external forces. Macrophages are able to maintain the stability of the microenvironment under these external forces; however, changes in the external forces often cause pathological changes (Wells, 2013; Lee et al., 2020). For example, resident macrophages in the lung are often subjected to stretching. While macrophages can maintain microenvironment homeostasis under stable stretching, changes in external forces, such as ventilation imbalance, lead to the destruction of mechanical homeostasis, causing shifts in the phenotype and function of macrophages. In addition, hypertension and atherosclerosis are related to the destruction of shear stress homeostasis in vivo, which is often characterized by an increase in M1 macrophage polarization (Sager et al., 2016; Mennens et al., 2017).

2 Mechanosensing and macrophage polarization

As mechanoreceptor cells, macrophages exhibit various mechanoreceptors on their membranes, including cell adhesion molecules (CAMs), such as integrins and various ion channels (Dustin, 2019; Solis et al., 2019; Lee et al., 2022). These mechanoreceptors recognize external mechanical stimuli and convert them into intracellular biological signals. This in turn leads to the reorganization of the intracellular cytoskeleton and nuclear cytoskeleton, thus activating downstream signaling molecules to affect gene expression and transcription, ultimately determining the phenotype and function of macrophages (Zhang et al., 2020).

2.1 Integrin

Integrin has been shown to be associated with macrophage polarization. Zhang et al. (2012) reported that integrin-β3 is related to M2 phenotype polarization. Zhang and colleagues also proposed that α4β1 integrin could promote M1 phenotype polarization (Zhang et al., 2018). Integrin is a heterodimeric transmembrane protein that binds to the extracellular matrix (ECM) and thus connects the extracellular environment with the intracellular cytoskeleton (Hynes, 2002). Integrin has a α subunit and a β subunit. Two subunits constitute a structure consisting of an extracellular round head, an extracellular rod-shaped leg, a transmembrane helix, and an intracellular short tail. Eighteen α subunits and eight β subunits make up 24 kinds of integrins, and specifically binding α and β subunits can specifically recognize certain proteins in the ECM (Kolasangiani et al., 2022). The extracellular segment of integrin binds to the ligand of the ECM, while the β subunit tail of the intracellular segment is connected to the cytoskeleton through a series of adaptor proteins, such as talin, vinculin, paxillin, and focal adhesion kinase (FAK). Under mechanical stimulation, such as mechanical force and matrix hardness changes, integrins and adaptor proteins locally aggregate to form adhesive plaques, causing cytoskeleton remodeling, thereby activating downstream signaling pathways and regulating cell behavior (Sun et al., 2016).

When the contractility of intracellular actin increases or when cells are subjected to an external force, integrins are activated, then they combine with ECM ligands such as collagen, resulting in more integrins being expressed on the plasma membrane. Moreover, their intracellular tails combine with intracellular signal proteins such as talin, vinculin and paxillin, forming a complex called focal adhesions (FAs) (DuFort et al., 2011). Research has shown that the tension detected by a FRET-based tension sensor in the talin protein, a force transmission medium, has a single average force of approximately 5 pN (Kumar et al., 2018), whereas the maximum bearing capacity of a single integrin can reach 40 pN (Zhang et al., 2014). Without external force, the binding state of integrins and ligands is unstable and they easily dissociate, whereas the action of an external force prolongs the life of this combination, that is, it promotes bond behavior. However, this external force needs to be controlled within a suitable range because excessive external force can reduce the lifespan of the combination (Kolasangiani et al., 2022; Hertig et al., 2012; Banerjee et al., 2022). The FAs of mesenchymal cells (MSCs) are sensitive to ECM stiffness and can regulate the growth, differentiation and migration of MSCs (Jaumouillé et al.,

2019). For example, MSCs cultured on substrates with high stiffness ranging from 11–30 kPa tend to differentiate into osteoblasts, whereas those cultured on gels with lower stiffness tend to differentiate into adipocytes (Zhang et al., 2018). In addition, αMβ2 integrin, a complement receptor (CR) that is highly expressed in macrophages, mediates phagocytosis (Chen et al., 2017). Phagocytosis mediated by αMβ2 integrin prefers stiff targets (Jaumouillé et al., 2019). Thus, it is feasible to control the stiffness of the substrate to regulate macrophage phagocytic activity.

The connection between macrophages and the ECM does not take the form of FAs formed by integrins; instead, integrins aggregate to form podosomes (Murphy et al., 2011; Jain et al., 2019). As a cell membrane adhesion structure, the podosome is widely expressed in macrophages, dendritic cells, osteoclasts, and other cells and plays important roles in cell movement, immune monitoring, bone structure maintenance, and organogenesis. The podosome is composed of an F-actin-rich core and an adhesive ring surrounding it, which are connected by an actin network loaded with myosin II. Podosomes are more dynamic and unstable than FAs (Labernadie et al., 2014). Podosomes and FAs have similar protein compositions, including integrins, talin, vinculin, paxillin, and FAK. FA formation is initiated by integrin-ECM interactions and is determined by the integrin-ligand binding tension. However, the formation of podosomes is not regulated by the integrin-ligand binding tension but is completely mediated by actin and myosin. Scholars have speculated that the intracellular binding tension of integrin and actin can activate adaptor proteins and form podosomes (Pal et al., 2022) without integrin-ligand interactions. Podosomes participate in the adhesion, migration and phagocytosis of macrophages and degrade the pericellular matrix (Van Goethem et al., 2009; Zhou et al., 2020) via cell–matrix contact. In addition, owing to its contact with the intracellular cytoskeleton, the podosome can be used as a sensor of mechanical stimulation (Jain et al., 2019). Studies have suggested that the podosome can sense the physiological stiffness of most tissues of the human body in the range of approximately 1–100 kPa, and its formation is positively correlated with the stiffness of dynamics in this range (van den Dries et al., 2019). Hu et al. revealed that podosome clusters, rather than single podosomes, perform functions in macrophages, and microtubules act to stabilize podosome clusters and regulate their assembly (Hu et al., 2022). The aggregation of integrins, whether in the form of FAs or podosomes, can cause changes in the cytoskeleton dynamics, whereas the specific mechanism of their response to external forces needs further study.

2.2 Ion channels

Ion channels, such as transient receptor potential (TRP) channels, Piezo channels and epithelial sodium ion (ENaC) channels, are also known to be mechanical sensors that can change membrane tension by regulating the entry and discharge of ions, thus affecting cell function (Meli et al., 2019). Ca^{2+} is an important cation that participates in many signaling pathways involved in cell apoptosis and proliferation (Tu et al., 2023). Mechanically activated ion channels increase the permeability for Ca^{2+}, leading to an increase in the intracellular Ca^{2+} concentration. Intracellular Ca^{2+} then binds to Ca^{2+}-binding proteins such as calmodulin (CaM) to control the intracellular Ca^{2+} concentration by allowing ion channels to self-regulate Ca^{2+} entry (Hasan et al., 2018).

2.2.1 TRP

TRP is a nonspecific cation-selective access channel through which Ca^{2+}, Na^{+} and other cations can enter and exit the cell, causing changes in cell function. On the basis of amino acid arrangement, TRP is divided into seven subfamilies, namely, TRPC, TRPM, TRPV, TRPA, TRPML, TRPMN and TRPP (Clapham et al., 2005; Clapham et al., 2001), all of which have six transmembrane domains (S1--S6) in structure and a pore (Yu et al., 2005; Ramsey et al., 2006) between S5 and S6 and between the intracellular C-terminus and the N-terminus. TRP has weak voltage sensitivity and is regulated by temperature, mechanical factors, ligands, pH, etc. (Feske et al., 2015). Ca^{2+} influx induced by TRP is considered to contribute to the inflammatory response induced by inflammatory agonists (Santoni et al., 2018). Lv et al. revealed that TRPV1-evoked Ca^{2+} influx is involved in M1 macrophage polarization (Lv et al., 2021).

TRPV4 is a widely studied TRP that participates in many physiological activities (Swain et al., 2020; Li et al., 2019), such as blood flow regulation, epithelial ciliary movement, and vascular dilation induced by shear force. It promotes the production of reactive oxygen species in macrophages and regulates the ability of macrophages to phagocytose pathogens. It is also known to promote M1 polarization, which is dependent mainly on Ca^{2+} signaling (Chen et al., 2023). TRPV4 can be activated directly or indirectly by mechanical forces. According to the theory of direct activation, membrane deformation under mechanical forces can cause changes in

lipid membrane tension and directly activate TRPV4 (Michalick et al., 2020). A number of studies have also shown that when subjected to osmotic pressure and mechanical forces, activated integrins, the mechanical messengers of Ca^{2+} released by ion channels, as well as lipid metabolites such as arachidonic acid and its derivatives, indirectly activate TRPV4 (Swain et al., 2021). Activated TRPV4 further triggers the downstream integrin and Ca^{2+} pathways, causing cytoskeleton remodeling. Some proteins that can produce or promote the production of mechanical messengers also play a role in activating TRPV4. For example, G protein-coupled receptors can promote the generation of arachidonic acid to activate the TRPV4 channel, and its downstream signaling proteins PKA, PKC and other tyrosine protein kinases can directly phosphorylate the TRPV4 protein to improve channel sensitivity (Veldhuis et al., 2015). The expression of nuclear factor kappa B (NF-κB) is further promoted by Ca^{2+} entering through TRPV4, to ultimately achieve a proinflammatory effect (Nguyen et al., 2022; Baratchi et al., 2016) Furthermore, mechanically sensitive TRPV4 channels can be recruited to the surface of the plasma membrane to increase mechanical sensitivity when subjected to external mechanical forces.

2.2.2 Piezo

Piezo channels are mechanically sensitive cation channels that can be divided into two types: Piezo1 and Piezo2. Piezo1, expressed mainly in nonsensory tissues such as the lung, bladder and skin (Fang et al., 2021), can mediate distinct Ca^{2+} signaling pathways. Piezo2 mainly exists in sensory tissues as an important medium for sensory neurons to perceive and transmit touch, proprioception and interoception (Shin et al., 2021). Piezo1 has been associated with macrophage polarization. He et al. reported that Piezo1 deletion suppressed macrophage inflammation in a Ca^{2+}-dependent manner (He et al., 2022). Geng et al. (2021) showed that LPS-activated TLR4 activates Piezo1, which participates in phagocytosis by macrophages and mediates the inflammatory response. Luo et al. reported that Piezo1 knockout leads to decreased M1 polarization (Luo et al., 2023). In addition, Piezo1 plays an important role in the recombination of cytoskeleton in macrophages and the formation of filamentous pseudopodia.

Mouse Piezo (mPiezo), which consists of three surrounding protomers forming a three-bladed, propeller-shaped trimeric architecture, has been proven to have a 38-transmembrane helix topology with a total of 114 TM helices. It contains a central ion-conducting pore module topped with an extracellular cap domain and three peripheral mechanosensing blades. Each blade consists of 36 transmembrane helices, while the remaining 2 transmembrane helices form the structure of the central cap. There are three beam-like structures in the intracellular part that connect the blades to the caps (Zhao et al., 2018), which are approximately 90 nm long and 30° to the membrane plane. Piezo1 and Piezo2 are similar in structure, but their sequence homology is only 42% (Coste et al., 2010; Szczot et al., 2021). On the basis of the above structure, Ge et al. (Ge et al., 2015) suggested that when highly curved blade-like structures are subjected to external forces, the beam-like structures in the cell are used as a fulcrum to form a lever-like structure, which promotes the opening of the inner pore and then leads to ion communication inside and outside the cell. Guo et al. (2017) reported that Piezo bears the ability to deform the plasma membrane into a dome-shaped structure. Piezo is similar to the nanobowl configuration in its side view, which results in the primary dome-shaped membrane having a certain energy. Under tension, the mechanosensing blades of Piezo flatten, and the surface area of the nanobowl expands to an in-plane membrane area, taking up up to 700 nm^2, providing enough energy to gate the ion channel. The energy difference caused by the change in membrane curvature leads to the opening of the Piezo channel (Li Wang et al., 2019; Lin et al., 2019).

Recent studies have confirmed that Piezo1, another ion channel different from TRP, is one of the most abundant ion channels in mechanosensitive tissues. Piezo1 is also a nonspecific cation-selective ion channel that is distributed in the plasma membrane, endoplasmic reticulum and nuclear membrane (Pathak et al., 2014; Chen et al., 2018). When stimulated by mechanical forces, the cell membrane will pick up these signals and then transmit them to various components in the body, including the double layer, cytoskeleton, and the ECM. The Piezo1 channel changes from a closed state to an open state, regulating Na^+, K^+ and Ca^{2+} to enter and leave the cell, which can regulate protein synthesis in the body and affect the functions of cell proliferation, apoptosis and

migration (H. Liu et al., 2022). Solis et al. (2019) applied circulating hydrostatic pressure from 45 mmHg to 60 mmHg per second to mouse BMDMs with and without the Piezo1 gene knockout (Piezo1ΔLysM and Piezo1fl/fl). They reported that the expression of proinflammatory genes in Piezo1fl/fl BMDMs was significantly greater than that in Piezo1ΔLysM BMDMs, demonstrating the important role of the Piezo1 ion channel in the perception and conduction of hydrostatic pressure in macrophages. By using HEK293 cell membrane blebs largely free of the cytoskeleton, Cox et al. (2016) also proved that Piezo1 can be directly activated by membrane stretching.

2.3 Interactions between Mechanical Receptors

Both ion channels and integrins sense and conduct mechanical stimuli, and they also interconnect with each other. After being subjected to external forces, β1 integrin on endothelial cells can recruit CD98hc to form FAs. CD 98hc can further cause conformational changes in TRPV4, thus causing the rapid (<4 ms) activation of TRPV4 (Garcia-Elias et al., 2013; Potla et al., 2020). Du et al. applied cyclic stretching force to chondrocytes and reported that the calcium signal mediated by the TRPV4 channel exhibited a stronger response to low cyclic stretching of 3% and 8%. While Piezo1 and Pizeo2 showed a stronger response to a moderate stretching force of 13%, only Piezo2 exhibited a stronger response under an 18% stretching force (Du et al., 2020); that is, different ion channels can sense external forces of different sizes. In addition, the activation of TRPV4 channels in LPS-activated macrophages promotes the expression of anti-inflammatory factors such as IL-10 and inhibits the expression of proinflammatory factors (Orsini et al., 2021), indicating that TRPV4 has anti-inflammatory properties. However, in diseases such as arthritis and pancreatitis, TRPV4 can have synergistic proinflammatory effects with Piezo1. Phospholipase A2 phospholipase A2 (PLA2) produced after the activation of Piezo1 triggers TRPV4, and the two ion channels jointly promote an increase in the intracellular Ca^{2+} concentration, resulting in cell apoptosis (Du et al., 2020). Meanwhile, the specific mechanisms of integrins and ion channels in the development of diseases and how to apply these findings to the treatment of diseases require further discussion.

3 Signaling is Involved in Mechanotransduction in Macrophages

Many studies have suggested that mechanical stimulation is first recognized by adhesion molecules and ion channels on the surface of macrophages, then converted into biological signals, which can activate downstream signaling pathways to activate transcription factors to regulate gene expression and protein translation or directly change cytoskeleton dynamics to regulate cell phagocytosis, migration and polarization (Fig. 2).

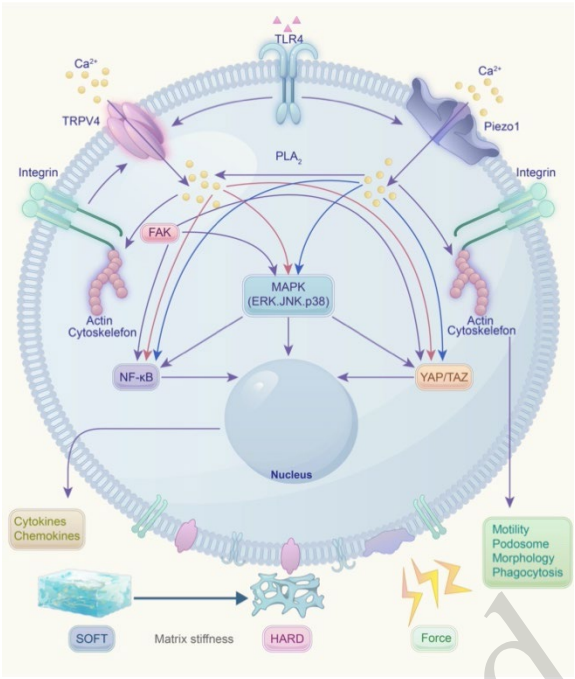

Fig. 2 Illustration of the signaling pathways activated by mechanoreceptors. Integrin, TRPV4 and Piezo1 are important mechanoreceptors that can be activated by diverse mechanical stimuli, such as forces, exogenous ligands and biomaterials with different matrix stiffnesses. Activated mechanoreceptors regulate multiple pathways to impact the transcription factor NF-κB and the transcription assistant activator YAP, leading to downstream effects on the function of macrophages. TRPV4, transient receptor potential vanilloid 4; PLA2, phospholipase A2; TLR4, toll-like receptor 4; NF-κB, nuclear transcription factor kappa B; YAP, Yes-associated protein; MAPK, mitogen-activated protein kinase; FAK, focal adhesion kinase. TAZ，Tafazzin; ERK, extracellular regulated protein kinases; JNK, c-Jun N-terminal kinase. The red lines refer to the Ca^{2+} signaling pathway related to TRPV4. The blue lines refer to the Ca^{2+} signaling pathway related to Piezo1.

3.1 NF-κB

Nuclear transcription factor kappa B (NF-κB) is related to inflammation, cell cycle regulation and protection against apoptosis (Ergun et al., 2023). In macrophages, NF-κB has been demonstrated to participate in polarization and mechanotransduction. After applying 5%, 10% and 15% tensile forces to mouse RAW264.7 cells for 5–120 min, an increase in intracellular phosphorylated FAK protein levels and M1 macrophage polarization was found (Shan et al., 2019). In addition, after the detection of molecules downstream of FAK, only the phosphorylation of NF-κB p65 was found to be specifically regulated by FAK. The nuclear translocation level of NF-κB p65 was significantly increased in macrophages subjected to tensile force, which revealed an inflammation-related FAK/NF-κB mechanical conduction pathway (Meli et al., 2020). Tu et al. (2022) applied tensile force to mouse RAW264.7 cells and reported that the expression of RhoA and ROCK 1/2 in the cells increased significantly, suggesting the mechanical activation of the RhoA–ROCK pathway. The activation of RhoA-ROCK was accompanied by the upregulation of NF-κB signaling and a significant increase in IκB phosphorylation levels and proinflammatory cytokines. These findings indicate that external mechanical force can promote the M1-type polarization of macrophages through the RhoA-ROCK-NF-κB pathway. The above results collectively prove that NF-κB participates in the mechanical signal transduction of macrophages and that NF-κB usually causes a proinflammatory reaction in macrophages.

The NF-κB signaling pathway consists of 15 dimers composed of five NF-κB monomers and five IκB inhibitory proteins (Dorrington et al., 2019). The NF-κB signaling pathway activates innate immunity and participates in the development and activation of adaptive immune cells, as well as the development of secondary lymphoid organs (Liu et al., 2017; Cildir et al., 2016). The binding of IκB inhibitory protein and NF-κB dimer results in the inactivation of transcription factor NF-κB. Under normal circumstances, inactive NF-κB is localized in the cytoplasm (Zambrano et al., 2014). Once the NF-κB signaling pathway is activated, the phosphorylated IKK2 complex phosphorylates the IκB protein, which promotes the degradation of IκB by the proteasome. The activated NF-κB dimer then enters the nucleus, binds to the corresponding DNA site and ulti-

mately initiates the transcription of inflammatory genes (Schröfelbauer et al., 2012; Kanarek et al., 2012).

The mechanically sensitive ion channels TRP and Piezo promote the expression of proinflammatory transcription factor NF-κB and inhibit the expression of anti-inflammatory transcription factor STAT6 through transient Ca^{2+} influx. The important role of TRPC1 in promoting the inflammatory response was demonstrated experimentally and the related molecular mechanism has been revealed. Specifically, the pathogen binds to the TLR4 receptor to activate TRPC1, causing the release of Ca^{2+} in the endoplasmic reticulum. Ca^{2+} enters the cytoplasm and activates PLC-γ, subsequently inducing TRPC1-mediated Ca^{2+} influx, which further activates PKCα, leading to the migration of the transcription factor NF-κB from the cytoplasm to the nucleus and the transcription of inflammation-related genes (Zhou et al., 2015). In contrast, an increase in the intracellular Ca^{2+} concentration inhibits the phosphorylation of the transcription factor STAT6 and its migration toward the nucleus, thereby inhibiting the expression of anti-inflammatory genes (Nascimento et al., 2021).

In addition to being a downstream signaling molecule of mechanoreceptors, NF-κB also plays an important role in the response to activating biochemical signals. Different pathogens and cytokines stimulate different NF-κB monomers to dimerize in macrophages, leading to the expression of different genes. Even the same NF-κB dimer can lead to the expression of different genes in different macrophage types (Grigoriadis et al., 1996). However, the intersection of different signals, such as mechanical and chemical stimuli, and the interference between activated downstream signals, may eventually affect the types and activities of the NF-κB dimer. Relatively speaking, the current level of research on NF-κB in innate immunity and macrophages is insufficient, and further studies are needed to clarify the regulatory factors of the NF-κB signaling pathway in macrophages. Through in-depth research, the aim of reducing the inflammatory response can be ultimately achieved.

3.2 MAPK

Mitogen-activated protein kinase (MAPK) is involved in the downstream signal transduction of TRPV4 and Piezo1. The MAPK signaling pathway includes three signaling pathways mediated by ERK, JNK and p38. TLR4 receptors on the surface of macrophages recognize LPS and then initiate the NF-κB pathway, interferon regulatory factor 3 (IRF3) pathway or MAPK pathway to mediate cell phagocytosis (Takeda et al., 2004). JNK and p38 are activated by external mechanical forces involved in the release of inflammatory mediators from macrophages (Xiao et al., 2015; Yanagisawa et al., 2007; Tang et al., 2014; Zhang et al., 2002). The MAPK pathway also regulates the phenotypic transformation of macrophages. When magnetomechanical stimulation was applied to mouse BMDMs, a decrease in the level of phosphorylated JNK and M2 macrophage polarization was detected in BMDMs, but no obvious changes in the expression of phosphorylated ERK or phosphorylated p38 occurred (Shao et al., 2023). Therefore, magnetomechanical stimulation may inhibit the M2-type polarization of macrophages by reducing the level of phosphorylated JNK.

Some scholars such as Shen et al. (2020) have shown that the ERK signaling pathway is involved in mechanical conduction mediated by Piezo1, whereas p38 is associated with signal conduction mediated by the actin cytoskeleton. In normal lung tissue, JNK is the dominant molecule of the MAPK signaling pathway in TRPV4. However, when macrophages perceive a pathological increase in lung matrix hardness, DUSP1 of the bispecific serine–threonine phosphatase family (DUSPs/MKPs) regulates MAPK conversion by inactivating JNK molecules, and signal transduction changes from the JNK-dominated pathway to the p38-dominated pathway. The P38 pathway further enhances the phagocytosis of macrophages and inhibits the secretion of proinflammatory factors, thus protecting against lung injury (Scheraga et al., 2020). The activation of Piezo1 also causes the phosphorylation of MAPK molecules and thus activates the MAPK signaling pathway. Liu et al. (2021) studied the mechanical conduction mechanism of Piezo1 in HepG2 human hepatocellular carcinoma cells. They reported that the activation of Piezo1 can induce the phosphorylation of MAPK family signaling molecules and then lead to the phosphorylation of the transcription-activating factor YAP, which ultimately determines cell fate. In conclusion, Piezo1 signaling can be transmitted through the Ca^{2+}/MAPK/YAP axis.

Once the MAPK signaling pathway has been activated by mechanical stimuli, biochemical stimuli and so on, it will sequentially promote one MAPK kinase kinase, one MAPK kinase and one MAPK (ERK, JNK and P38) (Zhang et al., 2002). MAPKs continue to activate downstream signaling molecules to regulate cell proliferation, differentiation, apoptosis, and other physiological functions. The network of the MAPK signaling pathway constitutes a variety of proteins. Moreover, various signals in the body are interrelated and jointly regulate cell functions. Further studies on the molecular mechanism of the MAPK signaling pathway in mac-

rophages may provide new ideas for future clinical treatments.

3.3 YAP/TAZ

Tang et al. (Tang et al., 2021) reported that macrophages cultured on hard substrates exhibit polarization toward the M1 type via the Piezo1/YAP axis, suggesting that downstream YAP can respond to mechanical stimuli recognized by ion channels. Song et al. (2020) and Zhou et al. (2019) reported that YAP can bind to the promoters of inflammation-related genes in human liver macrophages and human THP-1 cells to initiate the expression of inflammation-related genes. Feng et al. (2018) reported that YAP/TAZ are related to the TGFβ1-induced M2 polarization of macrophages. Huang et al. (2017) revealed that YAP/TAZ participate in the M2 polarization of macrophages in tumor tissues. All these studies indicate that YAP can play a role in the transmission of mechanical signals and regulate the polarization direction of macrophages.

Yes-associated protein (YAP) is a transcription assistant activator that can sense and be controlled by matrix hardness, cell shape, as well as stretching and shear forces. Along with another mechanically induced transcription assistant activator, TAZ, YAP participates in nuclear gene expression (Elosegui-Artola et al., 2017; Halder et al., 2012; Panciera et al., 2017). Research has shown that YAP/TAZ play important roles in cell proliferation, controlling organ size (Totaro et al., 2018), stem cell self-renewal, and cell differentiation (Piccolo et al., 2014). YAP/TAZ can be transported between the cytoplasm and the nucleus. Phosphorylated YAP/TAZ are inactive and located in the cytoplasm, whereas dephosphorylated YAP/TAZ enter the nucleus and bind with transcription factor TEADs, regulating the expression of related genes (Zanconato et al., 2015). The mechanical conduction of YAP can be regulated by mechanical conduction-related signaling pathways, such as the Hippo, RHO and MAPK pathways (Dupont, 2016). Meli et al. (2020) cultured human MDMs on glass and stained them with YAP one hour later. YAP was highly expressed both inside and outside the nucleus. As cell adhesion continued, YAP was gradually transferred to the nucleus. With increasing matrix hardness (20–280 kPa), the nuclear translocation of YAP increased. In addition, after the lentivirus expressing YAP-5SA was transduced to inhibit the phosphorylation and degradation of YAP, the expression of the YAP gene in macrophages increased significantly, accompanied by a significant increase in the TNFα level induced by LPS. These findings collectively indicate that increased YAP activity is related to increased inflammatory intensity.

The Hippo signaling pathway is mainly composed of MST1/2, LATS1/2 and YAP/TAZ (Y. Wang et al., 2022). After the activation of this pathway, MST1/2 is phosphorylated and downstream LATS1/2 is triggered, which leads to the phosphorylation of YAP and TAZ. Phosphorylated YAP and TAZ remain in the cytoplasm and cannot be transferred to the nucleus; thus, these proteins cannot further regulate the expression of related genes. When macrophages are cultured on hard substrates, dephosphorylated YAP/TAZ enters the nucleus from the cytoplasm, thereby promoting p53 transcription. Transcription of the p53 gene results in the production of many proinflammatory factors. On the other hand, macrophages cultured on soft substrates activate the Hippo pathway and confine phosphorylated YAP/TAZ to the cytoplasm.

Codelia et al. (2014) applied 20% uniaxial cyclic stretching force to the human epithelial cell line MCF10A and reported a decrease in LATS activity and a decrease in phosphorylated YAP expression caused by LATS, suggesting that stretching could promote YAP activation by inhibiting the Hippo pathway. Similarly, the expression level of JNK was upregulated in MCF10A cells under stretching, suggesting that the MAPK and Hippo pathways jointly regulate the expression level of YAP. However, after further use of the JNK-specific inhibitor SP600125, LATS activity was blocked and the proportion of YAP in the nucleus decreased, suggesting that JNK plays an important role in the nuclear localization of YAP. Consequently, the MAPK pathway may regulate the Hippo pathway to affect the activity of YAP/TAZ.

The RHO pathway is a component of the intracellular mechanical conduction network. Rho A is a key protein regulating the cytoskeleton that can activate downstream ROCK proteins and cause myosin contraction and actin rearrangement (Wei et al., 2021). The RHO pathway also plays an important role in the maturation of FAK. Notably, RHO regulates the phosphorylation of YAP/TAZ in a LATS-independent manner (Feng et al., 2014).

In endothelial cells, epithelial cells, fibroblasts, and mesenchymal stem cells, the intracellular YAP level is reduced because of the soft matrix, limited culture space, inhibition of cytoskeleton polymerization, and cell contraction. In macrophages, a soft matrix and the inhibition of cytoskeleton polymerization can also reduce the expression of YAP, while the restriction of cell culture space and the inhibition of cell contraction have no significant effect on the expression of YAP (Meli et al., 2020). This suggests that macrophages have a unique

regulatory mechanism involving YAP, which needs further clarification. In summary, deciphering which signaling pathways regulate YAP/TAZ and how different signaling pathways interact with each other require further study and discussion.

4 Mechanical Forces Directly Regulate Macrophage Polarization—The Results of Some In Vitro Studies

The functions of tissue repair and M2 macrophage remodeling are essential in the process of wound healing. However, in the early stages of wound repair, M1 macrophages are also indispensable for the phagocytosis of pathogens and damaged tissues. Thus, the timely transformation of the macrophage phenotype is necessary for tissue repair and regeneration. Researchers need to apply appropriate mechanical stimulation to obtain specific macrophage subtypes. On this basis, different types of external mechanical forces, cell types and force application devices have been adopted to obtain the target phenotype of macrophages under ideal conditions in vitro.

Table 1 lists some in vitro studies on the direct regulation of macrophage polarization by mechanical stress. The current in vitro studies on mechanical stress focus on three aspects: stretching, shear and pressure. Because stretching can be easily obtained through Flexcell cell-stretching bioreactors, which is most commonly used for in vitro research (Shan et al., 2019; Tu et al., 2022; Dong et al., 2021; Yang et al., 2022; Liang et al., 2021). The cells are often directly inoculated onto a BioFlex culture plate coated with collagen I, and a specific tensile force is applied to the culture plate through the Flexcell Tension Plus device. Some scholars (Schoenenberger et al., 2020) have chosen customized force application devices to study the influence of stretching. Specifically, the cells inoculated onto the fiber scaffold are endowed with a certain tensile force by the device, and the shearing force is usually obtained by passing fluids through pipelines. Wissing et al. (2019) used different pipeline radii and fluid flow rates to control the magnitude of shear stress in pipelines. Pfenniger et al. (2015) made a narrow segment on a model designed in vitro to mimic low laminar shear stress (LLSS), high laminar shear stress (HLSS) and oscillatory shear stress (OSS) in vivo, which are generated when blood flows through atherosclerotic plaques. In vitro studies of pressure effects typically focus on hydrostatic pressure. Wang et al. (2020) designed a customized pressure chamber system that uses an external manometer to regulate external pressure. Wang et al. (2023) later developed noncontact equipment to make heavy objects float on the medium to exert hydrostatic pressure on cells (Fig. 3). However, owing to the inconsistency in the size, frequency and duration of the selected external mechanical forces, the resulting polarization directions of macrophages vary, not to mention the different force application devices and cell types used by researchers.

Table 1 In vitro experiments on the regulation of macrophage polarization by mechanical forces

Mechanical microenvironment	Force characteristic	Cell types	Device	Cell behaviors/functions	Reference
Stretch	A sinusoidal pattern at 0.5 Hz with different strains (5, 10, and 15%) for the indicated durations (5 min, 10 min, 30 min, 1 h, 2 h, and 4 h)	Murine RAW264.7 cells	The FX-5000T Flexcell Tension Plus device	An increased expression of M1-type genes and an increased section of M1-type cytokine	(Shan et al., 2019)
Stretch	0.5 Hz, rounds of pull 6 s-stop 6 s for 8 h at 5, 10, and 15% elongation	Murine RAW264.7 cells	The Flexcell-5000T Tension system	1.Macrophage presented a polygonal shape, with elongated cell and irregular barbs 2.Proinflammatory cytokine secretion increased	(Tu et al., 2022)
Stretch	5% strain at frequency of 1.0 Hz for up to 12 h	Murine RAW264.7 cells	The FX-4000 Flexcell Tension System	The anti-inflammatory phenotype markers and cytokine secretion increased	(Dong et al., 2021)

Stretch	20% elongation, 0.5 Hz, Half Sinus Curve Shape, 24 h	Mice bone marrow-derived macrophage (BMDM)	The Flexcell cell stretching bioreactors	A significant increase in the proportion of M1-type macrophages	(Yang et al., 2022)
Stretch	Different longitudinal (control, 5, 10, and 15%) sinusoidal stretching (0.5 Hz) for 48 h	Mice bone marrow-derived macrophage (BMDM)	The Flexcell cell stretching bioreactors	1. An increased expression in M2-related genes under 10% stretching 2.15% stretching slightly induced BMDM apoptosis	(Liang et al., 2021)
Strain	7% cyclic strain at 1 Hz	M0 macrophages derived from human monocyte THP-1 cell line	A custom-made bioreactor	Macrophage polarized toward a pro-inflammatory phenotype	(Schoenenberger et al., 2020)
Stretch	1.04 at 0.5 Hz for 8 days	M0 macrophages derived from human monocyte THP-1 cell line	A custom-made bioreactor	1.An increase in cell number 2.An increase in M1/M2 ratios	(Wissing et al., 2019)
Shear	1 Pa for 8 days	M0 macrophages derived from human monocyte THP-1 cell line	A custom-made bioreactor	1.A reduction in the overall cell number 2.An increase in M1/M2 ratios	(Wissing et al., 2019)
Hydrostatic Pressure	60 mmHg for 7 days	Mice bone marrow-derived macrophage (BMDM)	A custom-made bioreactor	A pronounced anti-inflammatory phenotype of macrophages	(Wang et al., 2020)
Compression	1 g/cm2	Murine RAW264.7 cells	A custom-made bioreactor	1.No effect on cellular morphology and migration 2.No obvious increasion in polarization markers	(Wang et al., 2023)

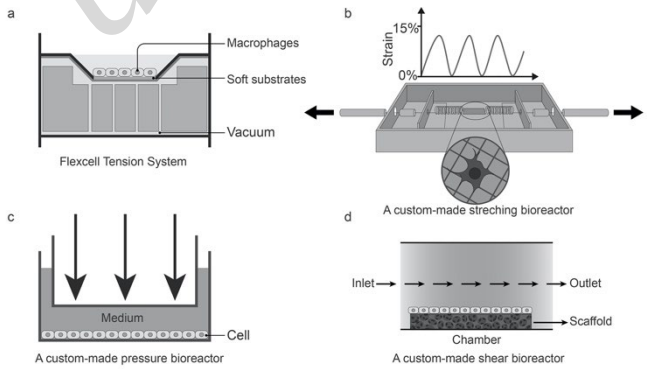

Fig. 3 Bioreactors often used in in vitro studies of macrophages. The bioreactors used in studies on stretching mainly include (a) the Flexcell Tension System and custom-made bioreactors such as (b). Typical structures of bioreactors in pressure and shear studies are listed in (c) and (d).

Mechanical forces can also indirectly regulate macrophage polarization by modulating the functions of other cells. Dziki et al. (2018) reported that stretching enhances the ability of myoblasts to promote M2 polarization. Wu et al. (2023) also reported that cyclic stretch stimulated human periodontal ligament cells to secrete exosomes, which further promoted M1 polarization. However, the mechanisms underlying the indirect regulation of macrophage polarization are more complex because the results may be influenced by more than one type of cell.

Existing studies mostly use murine RAW264.7 cells, mouse BMDMs and the human monocyte THP-1 cell line, whereas the most ideal cell type for the study of macrophages in vitro is macrophages from tissues or organs, such as Kupffer cells from the liver, alveolar macrophages from the lung, and microglia from nerve tissue. Owing to the difficulty of obtaining specific tissue-resident macrophages from tissues or organs, researchers often tend to adopt readily available immortalized cell models of human THP-1 and mouse RAW264.7 cells (Shiratori et al., 2017). THP-1 and RAW264.7 cells are human myeloid leukemia mononuclear cell lines and mouse leukemia mononuclear cell lines, respectively (Chanput et al., 2014). Compared with the primary macrophage line, the two cell lines grow faster, are easier to obtain and safer to apply. Moreover, the characteristic homogeneous genetic background of these two kinds of cells can help avoid genetic variation in primary cells and make the research results more repeatable (Cousins et al., 2003). However, these immortalized cells often have chromosomal abnormalities and cannot fully represent the phenotypic and functional characteristics of primary macrophages (Schildberger et al., 2013). Mononuclear-derived macrophages, as supplementary cells of resident macrophages, are relatively easy-to-extract primary macrophages and constitute the current gold standard for macrophage research (Mukherjee et al., 2018). However, considering the genetic heterogeneity of donors and ethical problems in practical applications, in vitro studies often use mouse BMDMs. Yet, the difference in gene expression between mice and humans can lead to difficulty in applying the results of macrophage studies to clinical practice. Raes et al. (2005) reported that the specific markers of mouse M2 macrophages include Arginase-1 and Ym1, whereas the detection of markers of human M2 macrophages did not support the above conclusion. Li et al. (2021) reported that the gene expression and protein secretion of human and mouse macrophages are not exactly the same after the same stimulation; that is, the genetic differences between mice and humans can affect the results of in vitro studies. Therefore, in vitro studies of mouse macrophages may have reference significance but no clinical application value.

In addition, if researchers aim to explore the prevention or treatment of a specific disease in the human body, the ideal macrophage type should involve cells of certain tissue residence. Macrophages residing in tissues play important roles in maintaining homeostasis and disease development, making them ideal research targets. For example, the ideal cell type for studying renal fibrosis is tissue-resident macrophages in the kidney, whereas microglia should be selected for studying nervous system diseases. However, the difficulty of obtaining resident macrophages and the relevant ethical considerations make practical application difficult. To solve this problem, researchers have proposed a scheme in which human induced pluripotent stem cells (hiPSCs) can be selectively induced to differentiate into target macrophages (Luque-Martin et al., 2021). Maintaining their original genetic background, macrophages induced by hiPSCs can serve as one of the sources of resident macrophages (Hale et al., 2015). Many in vitro studies are needed to verify the representativeness of macrophages induced from hiPSCs, which have a human genetic background and can represent the functional characteristics of primary macrophages in a cell model.

Another consideration of in vitro experiments is the controllability and restrictions of the research environment. The effect of a single factor acting on a macrophage in vitro cannot be compared with that in vivo. The interaction between macrophages, the ECM and neighboring cells in vivo often leads to chain reactions. As a typical example, Fresta et al. (2020) reported that when mouse RAW264.7 cells differentiated into the M1 type, the expression of SOD2 could not be detected in vitro, but in the presence of carnosine, the expression of SOD2 in mouse RAW264.7 cells increased. Carnosine is widely expressed in mammalian tissues. Many researchers have chosen to simulate the in vivo environment by coculturing macrophages with somatic cells, but the results obtained still require further verification.

In addition, some scholars have directly applied mechanical stress to animals to explore the phenotypic and functional changes in macrophages in these animals. Researchers have focused mostly on C57BL/6 mice and rats, whereas an external force was usually applied by running on a treadmill. For example, animal experiments performed by Shen et al. (2022) and Y. Liu et al. (2022) confirmed the positive effect of external mechanical forces on tissue healing. However, the force exerted in this way is a mixture of various forces, which are thus not comparable. Researchers such as Zheng et al. (2022) have also directly applied specific frequencies and sizes of external forces to specific parts of mice and obtained positive results, indicating that external mechanical forces promote healing. Xu et al. (2022) applied more specific orthodontic force to mice and concluded that orthodontic force promoted the proliferation of macrophages. Compared with in vitro settings, in vivo experiments in which external mechanical forces are applied to the wound often yield a unified conclusion: the appropriate kind and level of mechanical stress promotes tissue healing.

Furthermore, as a vital mechanical stimulus, matrix stiffness can also be recognized by macrophages. In the field of tissue regeneration, researchers often use scaffolds with different stiffnesses to specifically regulate the phenotype and function of macrophages. Some scholars believe that the mechanisms involved in the mechanosensing and mechanotransduction of macrophages under conditions of matrix stiffness may be the same as those under mechanical forces. However, the selected matrix stiffness range in studies is too broad, from <1 kPa to ~ GPa (Meli et al., 2020). Because of this inconsistency in matrix stiffness, there is no unified conclusion on the regulatory effect of matrix stiffness on macrophage polarization. Indeed, there is no clear definition of a soft matrix or a stiff matrix because different tissues and organs of the human body are characterized by their own ranges of stiffness (Butcher et al., 2009; Levental et al., 2007) (Table 2), even in different diseases. Table 3 lists some studies on the regulation of macrophage polarization by substrate stiffness. It seems that stiffer substrates are related to an increase in M1 polarization, while a soft substrate in one study may be a stiff one in another study, making the results confusing. Clearly, the different types of substrates and cell sources, as well as substrate deposition, may lead to different outcomes. For example, Vijaykumar S. Meli et al. (2024) reported that BMDMs cultured on Col-HEP-derived polyacrylamide hydrogel surfaces exhibited stiffness-dependent inflammatory activation, whereas BMDMs cultured on Col-AA-derived surfaces showed stiffness-independent inflammatory activation. This broad range of substrate stiffnesses in a single study may not make any sense. Instead, scholars should select specific types of macrophages and substrates corresponding to certain tissues or organs when exploring a certain disease or phenomenon. In addition, the mechanisms by which macrophages respond to matrix stiffness still need further study.

Table 2 Elastic moduli of different tissues/organs of humans

Tissues/ Organs	Fat	Brain	Lymph node	Mammary gland	Liver	Endothelial tissue	Smooth muscle	Thyroid	Skeletal muscle	Lung	Cartilage	Bone
Elastic modulus	17 Pa	100 Pa	120 Pa	160 Pa	640 Pa	1.2 kPa	5 kPa	9 kPa	12 kPa	5-30 kPa	100 kPa	2-4 GPa

Table 3 In vitro experiments on the regulation of macrophage polarization by matrix stiffness

Types of substrates	Elastic modulus			Cell types	Application	Related signaling pathways	Results	Reference
	soft	medium	stiff					
Polydimethylsiloxane (PDMS)	0.1 MPa	/	4.05 MPa	Mice bone marrow-derived macrophage (BMDM) and Murine RAW264.7 cells	Mimic physiological nature of bone tissue	Integrin– extracellular matrix signaling pathways	Stiffer PDMS substrates accelerated osteoclast differentiation.	(Q. Wang et al., 2022)
Polyacrylamide (PA) gels	11 kPa	88 kPa	323 kPa	Mice bone marrow-derived macrophage (BMDM)	Mimic implant-bone	Piezo1-YAP signaling axis	Stiffer substrates accelerated M1 polarization	(Mei et al., 2024)
3D-printed Ti6Al4V and Ti2448 scaffolds	1.92GPa (Ti2448 alloy)	/	16.1GPa (Ti6Al4V alloy)	Murine RAW264.7 cells	Titanium (Ti) and Ti-alloy implants	Piezo1-YAP signaling axis	A significant increase in the number of M2 macrophages was found around Ti2448 implants.	(Tang et al., 2021)
Polyacrylamide (PA)	1kPa	/	280kPa	Mice bone marrow-derived	Scaffolds for tissue engineer-	YAP-TAZ	Cells exhibited higher inflamma-	(Meli et al., 2024)

hydrogel functionalized with collagen I in HEPES buffer				macrophage (BMDM)	ing		tory activity on stiffer PA gels.	
Collagen-coated polyacrylamide (PA) gel	1kPa	/	50kPa	Mice bone mar-row-derived macrophage (BMDM)	Mimic normal skin and breast tissue (~1 kPa) and fibrotic skin (~8 to 50 kPa)	TRPV4	A significant increase in foreign body giant cells (FBGC) formation was found on high-stiffness PA gels.	(Goswami et al., 2021)
Soft ECM hydrogels and polystyrene	<1 kPa (fibrin hydrogels)	/	~GPa (polystyrene)	Human monocyte-derived macrophages	Scaffolds for tissue engineering	YAP/TAZ	Adhesion to soft hydrogels reduces inflammation.	(Meli et al., 2020)

5 Conclusions

Macrophages participate in many physiological processes and can cause pathological diseases under the action of external mechanical forces. Researchers need to continue to explore the in vivo mechanism of macrophage mechanical conduction, especially the interaction between mechanoreceptors and the corresponding downstream signaling pathways. The NF-κB pathway, MAPK pathway and YAP/TAZ pathway have been widely studied in fibroblasts, endothelial cells and other somatic cells, while the mechanisms by which they participate in the mechanical signal transduction of macrophages are unclear. To provide a new direction for the treatment of macrophage-related diseases, further studies are needed on the mechanisms of mechanosensing and mechanotransduction.

The mechanosensing and mechanotransduction abilities of macrophages have been proven by in vitro studies, but the variation in force application devices, as well as the inconsistency in the size, amplitude and duration of external mechanical forces, can lead to inconsistency in the research conclusions. To date, Flexcell cell-stretching bioreactors are the most popular devices for in vitro stretch research, and murine RAW264.7 cells, mouse BMDMs and the human monocyte THP-1 cell line are the most widely used cell types in studies. However, researchers should design reasonable devices and cell types for distinct research purposes. Moreover, given that hiPSCs have been found to achieve accurate acquisition of target cells, focusing on macrophages in in vitro experiments with hiPSCs has become a general trend. Finally, researchers need to design appropriate in vivo experiments to verify the conclusions of in vitro experiments.

Acknowledgments

This work is supported by the National Natural Science Foundation of China (No. 82202450).

Author contributions

Dan YU designed and led the project, and was responsible for the manuscript revision. Chenlu XU was responsible for reference searching, manuscript writing and revision and prepared the manuscript. Jinpeng JIANG and Wenyi SHEN assisted with revision of the article. Huiyong ZHU gave constructive comments on the revision of the scales, and was responsible for the supervision.All authors read and approved the final manuscript.

Compliance with ethics guidelines

This review does not contain any studies with human or animal subjects performed by any of the authors.Dan YU, Chenlu XU, Jinpeng JIANG, Wenyi SHEN and Huiyong ZHU declare that they have no conflict of interest.

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