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# Low–Molecular Weight Fucoidan Polysaccharide Inhibits Pulmonary Fibrosis by Regulating M1/M2 Macrophages Polarization

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# Abstract

In this study, sulfated polysaccharides extracted from *Laminaria japonica* were degraded by free radicals to obtain low–molecular weight fucoidan (LMWF). The effects of LMWF on bleomycin-treated pulmonary fibrosis (PF) mice were evaluated in precious research. In this study, the expression of M2 macrophage polarization biomarker arginase was detected by immunohistochemistry, and the expression of M1/M2 markers was detected by Western blot and qPCR. It was found that the expression of M1/M2 macrophage markers were significantly increased in the lung tissue of pulmonary fibrosis mice, and LMWF could effectively reduce its expression. Subsequently, in order to verify the mechanism of LMWF inhibiting pulmonary fibrosis by regulating macrophage polarization, MH-S cells were induced by LPS combined with IFN-γ and IL-4 to construct the in vitro macrophage polarization models, and the expression of biomarkers were analyzed by Western blot and qPCR. The results showed that LMWF could regulate M1 macrophage polarization through early anti-inflammatory effect and inhibit the development of pulmonary fibrosis by inhibiting M2 macrophage polarization in the later stage.


# 1. Introduction

Pulmonary fibrosis (PF) is a disease characterized by chronic progressive fibrosing interstitial pneumonia with unknown etiology (Hutchinson et al., 2015; Richeldi et al., 2017). The pathogenesis of PF includes chronic disease, epithelial cell injury and activation, fibroblast differentiation, excessive angiogenesis, and oxidative stress. In recent years, the research on the pathogenesis of PF has no longer been limited to the pathogenesis of alveolitis. PF may be caused by endogenous and exogenous cell stress, resulting in fibroblast activation, alveolar structure destruction, extracellular matrix protein deposition, gas exchange disorder, and respiratory failure (Meyer 2017). Macrophages are involved through the whole process of the PF pathogenesis, and their different phenotypes have different effects on PF (Laskin et al., 2019). The polarization of macrophages is regulated by microbial signals, tissue-specific inducements, cytokines, and other factors (Yunna et al., 2020). When they polarize to different subtypes, macrophages are regulated by different signaling pathways (Zhou et al., 2014). At different stages of PF, the macrophages cells polarize to different subtypes. The phenotypic migration of macrophages may be one of the mechanisms of the occurrence and progression of PF (Kolahian et al., 2016).

It has been shown that the polarization subtype of macrophages corresponds to the early and late stage of the development of PF (Byrne et al., 2016). In the early stage of PF, that is, the stage of alveolitis, pulmonary macrophages shift to M1 subtype (Guo et al., 2018); in the late stage of PF, that is, the stage of fibrosis repair, macrophages shift to M2 subtype (Guo et al., 2020; Wang et al., 2021). Inflammatory macrophages usually show M1 activation type; they secrete nitric oxide (NO), Reactive Oxygen Species (ROS), interleukin, and tumor necrosis factor, which have strong antimicrobial activity (Shapouri et al., 2018). Moreover, M1 macrophages secrete matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9) so as to promote the degradation of extracellular matrix, which is also conducive to the infiltration of the damaged tissue by inflammatory cells. When inflammation continues, M1 macrophages change the way they respond to inflammation, and then recruit Th17 cells and

neutrophils, resulting in sustained tissue damage (Dong et al., 2017). If the inflammatory stimulus is eliminated or the pathogen is cleared, M1 macrophages gradually disappear. Recent studies have shown that the activation of M2 macrophages is closely related to the development of PF (Yao et al., 2016). It has been pointed out that M2 macrophages play an important role in a mouse fibrosis model and human PF (Su et al., 2015), as they can produce fibrogenic mediators. In addition to the fibrogenic effect, the change of macrophage phenotype in patients with PF can explain why patients have frequent lung infections, leading to lung failure and eventually death. Therefore, inhibiting M2 macrophage phenotype is a potential therapeutic strategy for PF (Pan et al., 2021). This phenotype expresses a large number of growth factors and profibrotic cytokines in vitro, which may contribute to the persistence of fibrotic microenvironment. Previous studies have revealed the molecular entities involved in the alternative activation of macrophages. However, the key signal transduction pathway controlling M1/M2 transition in PF remains to be determined (Pan et al., 2020). If these signaling pathways controlling cell polarization are determined, they can be used as a therapeutic target for M2 macrophage-dependent PF.

Low–molecular weight fucoidan (LMWF) is a sulfated polysaccharide mainly composed of fucose. It has shown an inhibitory effect in a PF mice model (Wang et al., 2019; Wu et al., 2021). LMWF can block the ROS to inhibit PF (Dong et al., 2022), but previous studies have not described the mechanism by which LMWF acts on the alveolar macrophage. The purpose of this study was to clarify whether LMWF could act on the alveolar macrophage polarization.

## 2. Materials And Methods

## 2.1 Reagents and Animals

Antibodies against arginase-1, Fizz-1, CD86, iNOS and TGF-β were produced by Affbiotech (Cincinnati, OH, USA); when used in western blot or immunohistochemistry, they were diluted one thousand or one hundred times, respectively, using a primary anti-diluent (Beyotime, Shanghai, China). Antibodies against YM-1 was produced by Abcam (Woburn, England), and those against TNF-α and CD163 (diluted 1:1000) were purchased from Bioswamp (Wuhan, China). Secondary antibodies, including biotin-conjugated affinipure goat anti-mouse IgG (H + L) and biotin-conjugated affinipure goat anti-rabbit IgG (H + L) were provided by Affbiotech. MH-S was purchased from Zhongchu Biotechnology (Jinan,China). SeqState-primer was purchased from Sangon Biotech (Shanghai) Co., Ltd. All of the other chemicals and reagents were obtained from general commercial sources and used without prior treatment, unless otherwise specified. Reverse transcription Kit was purchased from Toyobo (Shanghai) Biotech Co., Ltd.

## 2.2 Chemical Composition of LMWF

Crude polysaccharides were extracted from *Laminaria japonica* with water (100℃) as extraction solvent and precipitated with ethanol. Then, low molecular weight fucoidan was obtained by free radical degradation ($H_2O_2$ : Vc = 1:1, 30mmol/L). Elute with CL-6B column and use NaCl as eluent to obtain 1.0 mol NaCl eluting component (previous studies found that this component has better inhibitory activity

against pulmonary fibrosis (Wu et al., 2021)). Based on High performance liquid chromatography (HPLC) analysis after 3-Methyl-1-phenyl-2-pyrazolin-5-one precolumn derivatization, to evaluate the main monosaccharide component of LMWF (Honda et al., 1989). Using the appropriate chemical methods to test the total sugar, sulfate, and fucose contents, respectively (Dische and Shettles, 1948; Kawai et al., 1969).

## 2.3 Animal experimental samples

Animal experimental samples were from previous research: male C57BL/6 (8–12 weeks) mice were randomly divided into six groups, with 12 mice in each group: sham (Sham); bleomycin-induced PF; bleomycin-induced PF + low-dose LMWF (25 mg/kg, L-LMWF); bleomycin-induced PF + medium-dose (50 mg/kg, M-LMWF); bleomycin-induced + high-dose LMWF (100 mg/kg, H-LMWF); bleomycin-induced + Nintedanib (NiN) (Dong et al., 2022).

## 2.4 M2 macrophage biomarkers expression

Paraffin sections of mouse lung tissues were taken for immunohistochemistry detection to observe the expression and localization of arginase-1. The mouse lung tissue was grinded in liquid nitrogen to extract protein and quantified content with BCA kit. The expression of M2 biomarker was detected by Western blot. Image J software was used to analyze the differences (Anders et al., 2021; Wculek et al., 2022).

## 2.5 qPCR test the expression of M1/M2 macrophage biomarkers

qPCR method was used for the determination of the differential gene expressions of arginase-1, Fizz-1, IL-10, YM-1, TGF-β, CD86, TNF-a, IL-6, TLR4 and NOS2 in lung tissues (Li et al., 2017). Total cellular RNA was isolated via TRIzol reagent (Thermo Fisher Scientic), and cDNA was synthesized using the PrimeScript RT Reagent Kit (Toyobo). The qPCR reactions were performed on the Roche Light Cycler 96 in a 20 μL reaction system by using SYBR Green One Step qPCR Kit (Toyobo, Shanghai, China). The primer sequences were as follows: arginase-1: F: CTCCAAGCCAAAGTCCTTAGAG, R: GGAGCTGTCATTAGGGACATCA; Fizz-1: F: CCAATCCAGCTAACTATCCCTCC, R: CCAGTCAACGAGTAAGCACAG; IL-10: F: GCTCTTACTGACTGGCATGAG, R: CGCAGCTCTAGGAGCATGTG; YM-1: F: GCAAGACTTGCGTGACTATGAA, R: AACGGGGCAGGTCCAAA; TGF-β: F: ACGTCACTGGAGTTGTACGG, R: GGGGCTGATCCCGTTGATT; CD86: F: GAGCTGGTAGTATTTTGGCAGG, R: GGCCCAGGTACTTGGCATT; TNF-a: F: CCTGTAGCCCACGTCGTAG, R: GGGAGTAGACAAGGTACAACCC; IL-6: F: TAGTCCTTCCTACCCCAATTTCC, R: TTGGTCCTTAGCCACTCCTTC; TLR4: F: TTTGACACCCTCCATAGACTTCA, R: GAAACTGCAATCAAGAGTGCTG; NOS2: F: GGAGTGACGGCAAACATGACT, R: TCGATGCACAACTGGGTGAAC;

## 2.6 Cell Viability by CCK-8 Assay

The mouse alveolar macrophage cell lines (MH-S cells) were purchased from Zhongchu Biological Technology Co. Ltd. (Jinan, China), an agent of ATCC cells. The MH-S cells were grown in RPMI-1640 medium (Hyclone) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 2 mM L-

glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells incubated in the incubator under 37°C and 5% CO2 conditions. Cell viability was tested with the CCK-8 kit (Solarbio, Beijing, China) according to the instructions. In brief, cells were seeded into 96-well plates at a density of 2000 cells/well. Absorbance was read in a spectrophotometer at a wavelength of 450 nm.

## 2.7 Cell Culture and Treatment

MH-S cells seeded at a density of 2000 cells/well were exposed to IL-4 (20 ng/mL) for 48 h to induce M2-like differentiation (Peng et al., 2021; Huang et al., 2022), the cells were also treated with LMWF (100, 200, 400, 800, 1000 μg/mL). M1-like differentiation was induced with IFN-γ (20 ng/mL) and LPS (10 ng/mL) for 24h (Lin et al., 2020), the cells were also treated with LMWF (25, 50, 100, 200, 400, 800 μg/mL). Cell viability was tested with the CCK-8 kit to screen the effective concentration.

## 2.8 LMWF act on M1/M2 macrophage polarization

MH-S cells seeded at a density of 1 x $10^5$ cells/mL were exposed to IL-4 (20 ng/mL) for 48 h and the cells were also treated with LMWF (800, 900, 1000 μg/mL). M1-like differentiation was induced with IFN-γ (20 ng/mL) and LPS (100 ng/mL) for 24h, the cells were also treated with LMWF (200, 400, 800 μg/mL). Total cellular RNA and protein were collected. The total RNA was taken to qPCR test, the primer sequences were as follows: E-cad: F: CAGGTCTCCTCATGGCTTTGC, R: CTTCCGAAAAGAAGGCTGTCC; α-SMA: F: GTGACTCACAACGTGCCTATC, R: CTCGGCAGTAGTCACGAAGC; Collagen: F: ATGGATTCCCGTTCGAGTACG, R: TCAGCTGGATAGCGACATCC; Fibronectin: F: AAGACCATACCTGCCGAATG, R: GAACATGACCGATTTGGACC; β-actin: F: CCTCTATGCCAACACAGT, R: AGCCACCAATCCACACAG.

## 2.9 Western blot Analysis

To quantify the proteins, western blot analyses were conducted. First, each sample of cells was treated with 200 μL of a lysis buffer. Subsequently, the resulting mixture was centrifuged at 4°C and 12,000 *g* for 10 min. To determine the total protein concentration, the supernatant was collected and analyzed using a BCA Protein Assay kit, with the measurement process conducted on ice. After denaturation, western blot analyses were conducted to visualize proteins. The results were then analyzed by chemiluminescence (Tanon, Shanghai, China), using β-actin as a loading control.

## 2.10 Statistical Analysis

The results of western blot and immunohistochemistry staining were analyzed using Image-Pro software, and statistical analyses were carried out using GraphPad Prism 8.0 software. The variations among groups were assessed by one-way analysis of variance (ANOVA), followed by Dunnett's test. All of the quantitative data are expressed as the mean ± standard deviation (SD), and differences were considered to be statistically significant and very significant at $p < 0.05$ (*/#) and $p < 0.01$ (**/##), respectively.

# 3. Results

# 3.1 Chemical Composition of LMWF

The degraded polysaccharides were classified by CL-6B chromatographic column. 0–2 mol NaCl were gradient eluted to obtain 2 fractionated components (F0.5 and F1.0), and F 1.0 component was collected (Fig. 1a). Although the chemical structure of F1.0 has been clarified (Wang et al., 2010), because its basic chemical composition is slightly different with the source of *Laminaria japonica*, we still retested the basic chemical composition of F1.0. Molecular weight test showed that it was 9801Da (Fig. 1c). Monosaccharide composition analysis found that fucose was the main component, followed by galactose and glucuronic acid (Fig. 1b). The chemical composition test results showed that the total sugar was 52.53%, fucose was 38.75%, uronic acid content was 2.98%, and sulfate content was 34.33% (Fig. 1d).

# 3.2 M2 Macrophage Biomarkers Expression

Immunohistochemical staining revealed that the expression levels of arginase-1 increased significantly in the lung tissue of bleomycin-induced mice (Figs. 2a, b), whereas the expression levels of the M2 macrophage polarization marker in the lung tissue of LMWF-treated mice decreased significantly. Then, M2 macrophage polarization biomarkers were detected by western blot (Fig. 2c). The results showed good stability of LMWF in inhibiting the expression of the biomarkers, revealing significant reduction of the expression levels of Fizz-1, arginase-1, YM-1 and TGF-β (Figs. 2d, e, f and j). Nintedanib had a good inhibitory effect on the expression of M2 macrophage polarization biomarkers, but it did not show a significant inhibitory effect on the expression of YM-1.

# 2.3 Expression of M1 and M2 Macrophages' Biomarkers in Lung

To investigated whether LMWF can regulate macrophage polarization, we examined the mRNA levels of M1 and M2 polarization biomarkers in lung tissues. As shown in Fig. 3a-d, the M2 markers Fizz-1, IL-10, Arginase-1 and YM-1 were increased, while LMWF was capable of reducing all four M2 markers. We tested whether LMWF could reduce the pro-fibrotic phenotype of macrophage, and result showed that the pro-fibrotic cytokines TGF-β was elevated by LMWF treatment (Fig. 3e). We also measured changes the M1 biomarker factors, and the results showed the same trend as shown in Fig. 3f-j. We detected CD86, TNF-α, IL-6, TLR4 and NOS2, the result shows that LMWF could decrease the inflammation cytokines.

# 2.4 LMWF Regulate M2 Macrophages Polarization

In order to explore the regulation effect of LMWF on macrophage polarization, we detected the effect of LMWF on IL-4 induced MH-S cells. The CCK-8 Assay was used to detect the cytotoxicity. The results showed that LMWF (0-1mg/mL) was nontoxic on MH-S cells (Fig. 4b). After IL-4 induction, cell morphology changed, and LMWF could effectively regulate cell morphology and reduce the damage of IL-4 to cells (Fig. 4a). After concentration screening, 800, 900, 1000mg/mL (L-, M-, H-LMWF group) was

selected as the experimental concentration (Fig. 4c). Similarly, we also detected the protein and mRNA expression of M2 macrophage polarization biomarkers, and the results showed that LMWF could effectively reduce the expression of M2 type biomarkers (Fig. 4d-e; Fig. 5a-e). At the same time, the mRNA expression of fibrosis markers was also detected. The results also showed that after IL-4 treated, fibrosis was aggravated. However, LMWF could reduce the expression of fibrosis markers (Fig. 5f-h).

## 2.5 LMWF Regulate M1 Macrophages Polarization

LMWF has been proved to regulate the polarization of M2 macrophages and delay the process of pulmonary fibrosis. However, LMWF has obvious anti-inflammatory effect. Does it have the effect on regulating M1 macrophages in the early stage? In order to clarify this problem, MH-S cells was induced by LPS + IFN-γ to construct M1 macrophage polarization model (Fig. 6a). The morphological changes of cells were not obvious when treated with LPS, we screened the effective concentration through the CCK-8 test. The result showed LMWF is effective on inhibiting inflammatory, and 200, 400, 800 μg/mL were selected (Fig. 6b). The expression of M1 macrophage biomarkers were detected through western blot and qPCR. LPS reduced the CD163 expression, enhanced the expression of iNOS, TNF-α, CD86. However, LMWF regulated the biomarkers expression (Fig. 6c **and d**). Then, qPCR test also showed the similar result (Fig. 6e).

## Discussion

Through previous experiments, we found that LMWF effectively alleviated the symptoms of PF and significantly reduced collagen deposition in mice. However, the specific mechanism of LMWF on PF is not clear. Although it has been confirmed that LMWF could block the TGF-β/Smad signaling pathway and Nrf-2 to inhibit PF (Wu et al., 2021; Dong et al., 2022), the exact mechanism is still to explore.

In the early stage of PF, a variety of injuries and stimuli can recruit circulating monocytes to differentiate into macrophages after entering the diseased tissue (Cheng et al., 2021; Rao et al., 2021). Macrophage differentiation can form two subpopulations with different inflammatory states. Classically activated M1 macrophages and alternatively activated M2 macrophages are the two subgroups of polarization. They have different gene expression profiles and protein markers. In the early stage of tissue injury, i.e., in acute inflammatory stage, necrotic cells recruit proinflammatory monocytes to aggregate, differentiate into classically activated M1 macrophages, clear necrotic tissue, and express a large number of proinflammatory cytokines, such as TNF-α and IL-6. Subsequently, the proinflammatory signal is inhibited and the cells are more inclined to differentiate into alternatively activated M2 macrophages, expressing high levels of anti-inflammatory cytokines and growth factors, such as IL-10 and TGF-β. M2 macrophages promote the differentiation and proliferation of epithelial and endothelial cells, restore the tissue morphological structure, promote the phenotypic transformation of fibroblasts into myofibroblasts, synthesize and secrete extracellular matrix, and promote tissue repair (Wang et al., 2021); however, if M2 macrophages are continuously activated, they promote myofibroblasts to further secrete extracellular matrix and lead to fibrosis (Zhu et al., 2017).

To clarify the mechanism by which LMWF inhibits PF through macrophage polarization, we detected the expression levels of M2 macrophage biomarkers, such as arginase-1, Fizz-1, YM-1, and TGF-β, in lung by western blot, qPCR and Immunohistochemistry. We found that LMWF effectively regulated the expression levels of these biomarkers. This shows that LMWF could inhibit the development of pulmonary fibrosis by regulating the polarization of M2 macrophages. In the late stage of pulmonary fibrosis, M2 macrophages is dominant, the most critical factor is TGF-β. The LMWF could effectively block its secretion, it is also important for inhibiting the process of pulmonary fibrosis. In addition, inflammation is the early clinical manifestation of pulmonary fibrosis, and the main clinical manifestation in the later stage is collagen deposition, which is also why we detected the expression of inflammatory factors in this experiment. We found that inflammatory factors and M1 macrophage biomarkers were also highly expressed in qPCR results, and LMWF could effectively reduce their expression. This suggested whether LMWF inhibits pulmonary fibrosis through the early anti-inflammatory effect and regulating the M2 macrophages polarization in the late stage.

Then we verified the mechanism by in vitro experiments. First, we induced MH-S cells with IL-4 to construct M2 macrophage polarization model. In the experiment, we found that the morphological changes of cells were obvious after IL-4 treatment. Subsequently, the Western blot and qPCR results showed that the expression of M2 macrophage biomarkers increased, while LMWF could effectively reduce its expression, especially TGF-β. This also showed that LMWF could regulate the polarization of M2 macrophages and the results of in vivo experiments were verified. In addition, we found collagen, fibronectin and α-SMA expression increased after IL-4 induction and decreased after LMWF treatment.

Secondly, we induced MH-S cells by LPS combined with IFN-γ to construct M1 macrophage polarization model. Through experiments, we found that LMWF could reduce the secretion of inflammatory factors (iNOS and TNF-α,) and reduce the expression of inflammation. This shows that LMWF could effectively block the M1 macrophages polarization and inhibit the development of inflammation through its anti-inflammatory effect, which is also consistent with our previous experimental research (Dong et al., 2022). Combined with in vitro experimental studies, we confirmed that LMWF could regulate the polarization of M1/M2 macrophages, inhibited the development of inflammation, and blocked the process of fibrosis. However, we also found that LMWF did not act macrophage at the same time, but could regulate the inflammatory response in the early stage, inhibit the secretion of inflammatory factors, and block the polarization of M2 macrophages and inhibit TGF-β in the late stage to inhibit TGF- β Induce fibrosis symptoms.

Finally, we summarized the results of this experiment: previous in vivo experimental studies found that LMWF could regulate M2 macrophage polarization and reduce inflammatory response, and then in vitro experiments found that LMWF could inhibit pulmonary fibrosis by regulating M1/M2 macrophage polarization.

# Declarations

**Author Contributions:** Conceptualization, Bo Zhang, analysis, Yanli Yuan, resources, Yanjuan Liu and Xinpeng Li, investigation, Jie Xin, data curation, Fengli Shao, writing—original draft preparation, Bo Zhang, writing—review and editing, Zhen Wang, visualization, Tao Xue, supervision, Meiying Sun. All of the authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** There are no conflicts of interest to declare.

**Availability of data and material:** All of the experimental data have been included in the manuscript.

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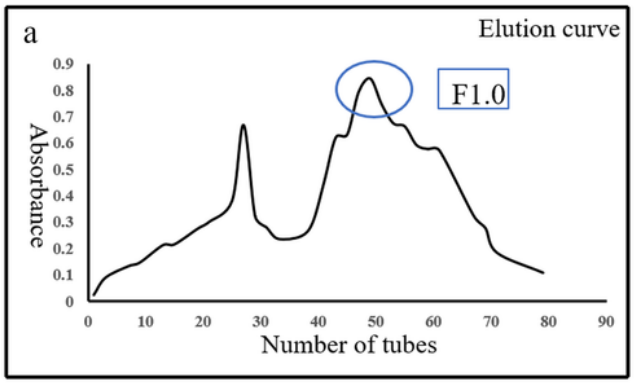
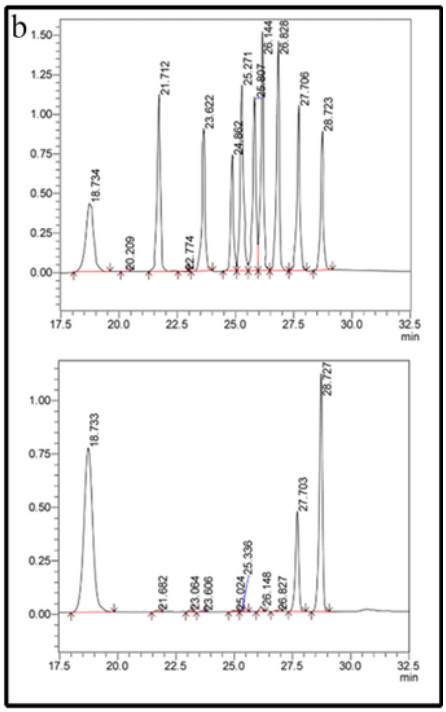
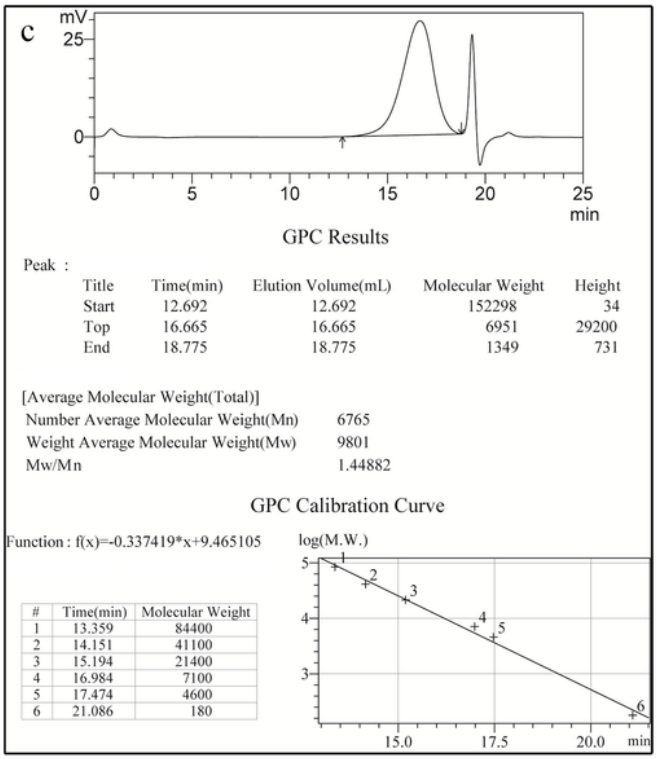
# Figures




GPC Results

Peak :

| Title | Time(min) | Elution Volume(mL) | Molecular Weight | Height |
|---|---|---|---|---|
| Start | 12.692 | 12.692 | 152298 | 34 |
| Top | 16.665 | 16.665 | 6951 | 29200 |
| End | 18.775 | 18.775 | 1349 | 731 |

[Average Molecular Weight(Total)]

| | |
|---|---|
| Number Average Molecular Weight(Mn) | 6765 |
| Weight Average Molecular Weight(Mw) | 9801 |
| Mw/Mn | 1.44882 |

GPC Calibration Curve

Function : f(x)=-0.337419*x+9.465105

log(M.W.)

| # | Time(min) | Molecular Weight |
|---|---|---|
| 1 | 13.359 | 84400 |
| 2 | 14.151 | 41100 |
| 3 | 15.194 | 21400 |
| 4 | 16.984 | 7100 |
| 5 | 17.474 | 4600 |
| 6 | 21.086 | 180 |

Gla:Galactose

Man:Mannose

Glc:Glucose

Rha:Rhamnose

Xyl:Xylose

Fuc:Fucose

GlcA:Glucuronic acid

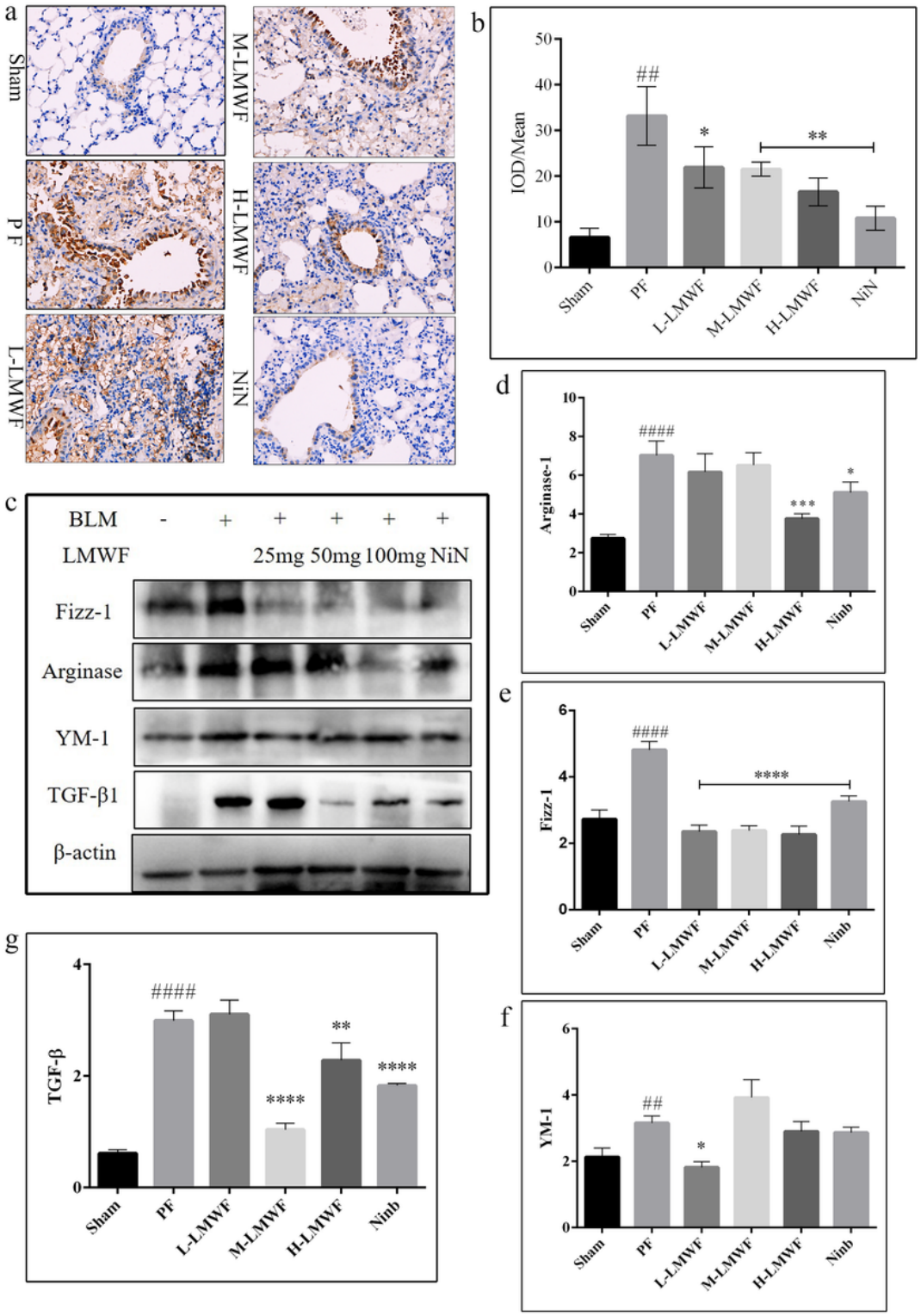
d

Chemical composition and neutral sugar of LMWF-F1.0

| Sample | Molecular weight(Da | Total sugar | Sulfate group | Fucose | Uronic acid | Neutral sugar (mole ration) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Gal | Man | Glc | Rha | Xyl | Fuc | GlcA |
| F1.0 | 9801 | 52.53% | 34.33% | 38.75% | 2.98% | 0.03 | 0.01 | 0 | 0.01 | 0.01 | 1 | 0.02 |

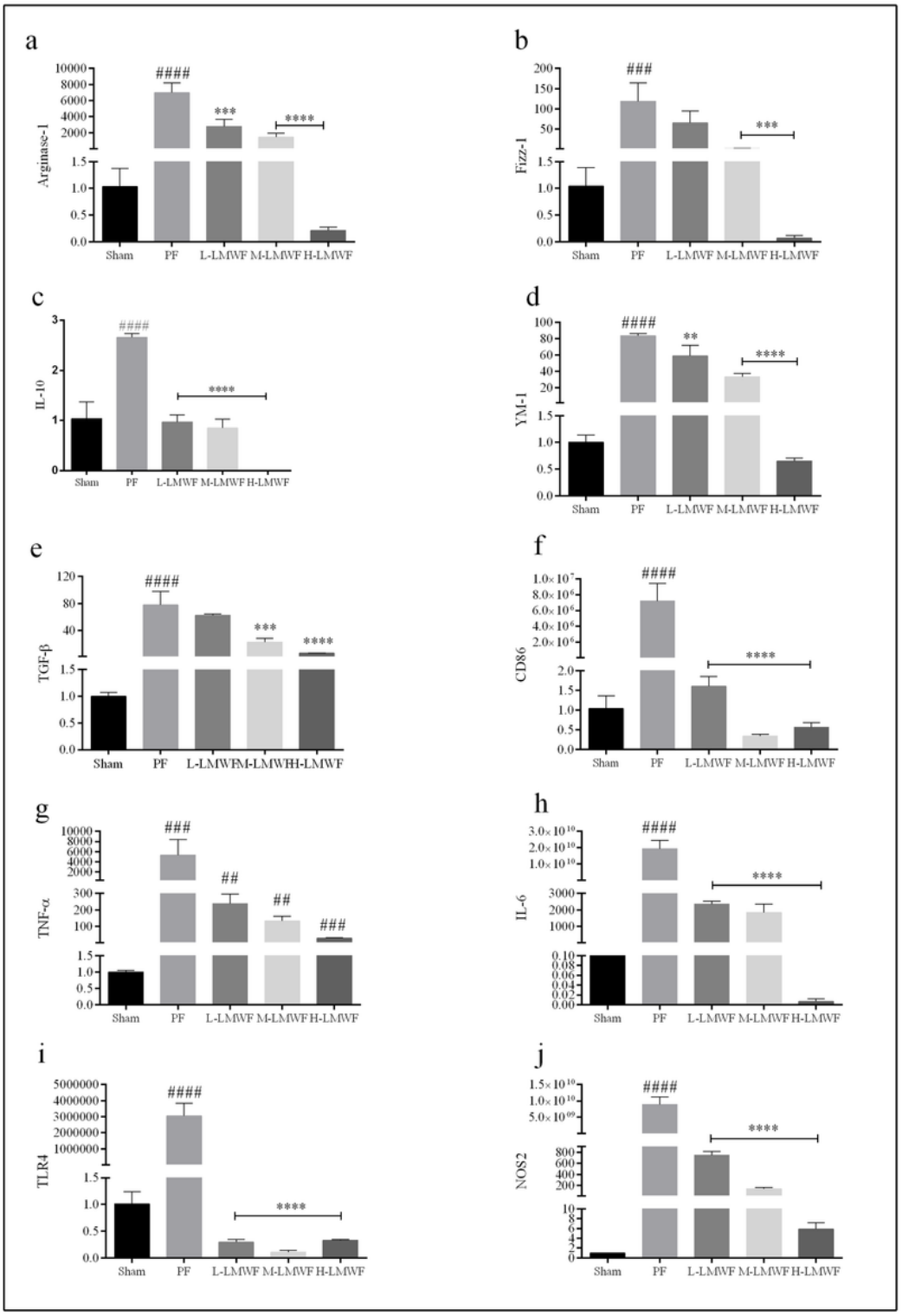
## Figure 1

(a) Elution curve of low molecular weight fucoidan, (b) Determination of the peak time of a neutral sugar standard by HPLC, (c) Determination of LMWF molecular weight by high-performance liquid chromatography with gel permeation chromatography, (d) Chemical composition and neutral sugar composition of LMWF, (Abbreviation: Fuc: Fucose, Gal: Galactose, Man: Mannose, Glc: Glucose, Rha: Rhamnose, Xyl: Xylose, and Rib: Ribose).



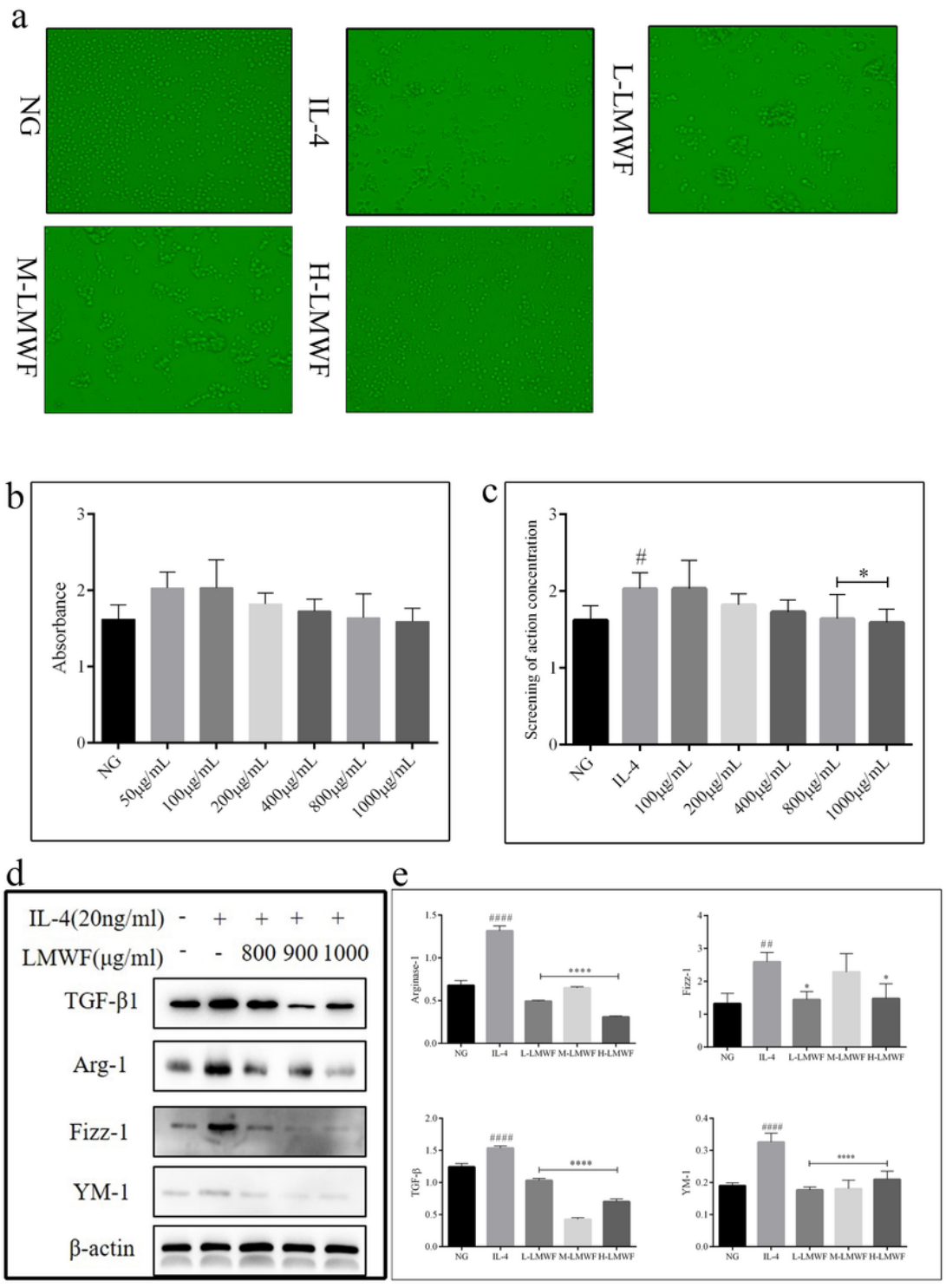

## Figure 2

Assessment of M2 macrophage polarization. **(a)** Immunohistochemical detection of arginase-1, **(b)** Statistical expressions of arginase-1 by an immunohistochemical assay (*$p$<0.05, **$p$<0.01 vs. the PF group, ##$p$<0.01 vs. the Sham group), **(c)** Expression of M2 macrophage polarization biomarkers by western blot, (**d–g**) Statistical expressions of M2 macrophage polarization biomarkers by western blot (*$p$<0.05, ****$p$<0.01 vs. the PF group, ####$p$<0.01 vs. the Sham group).



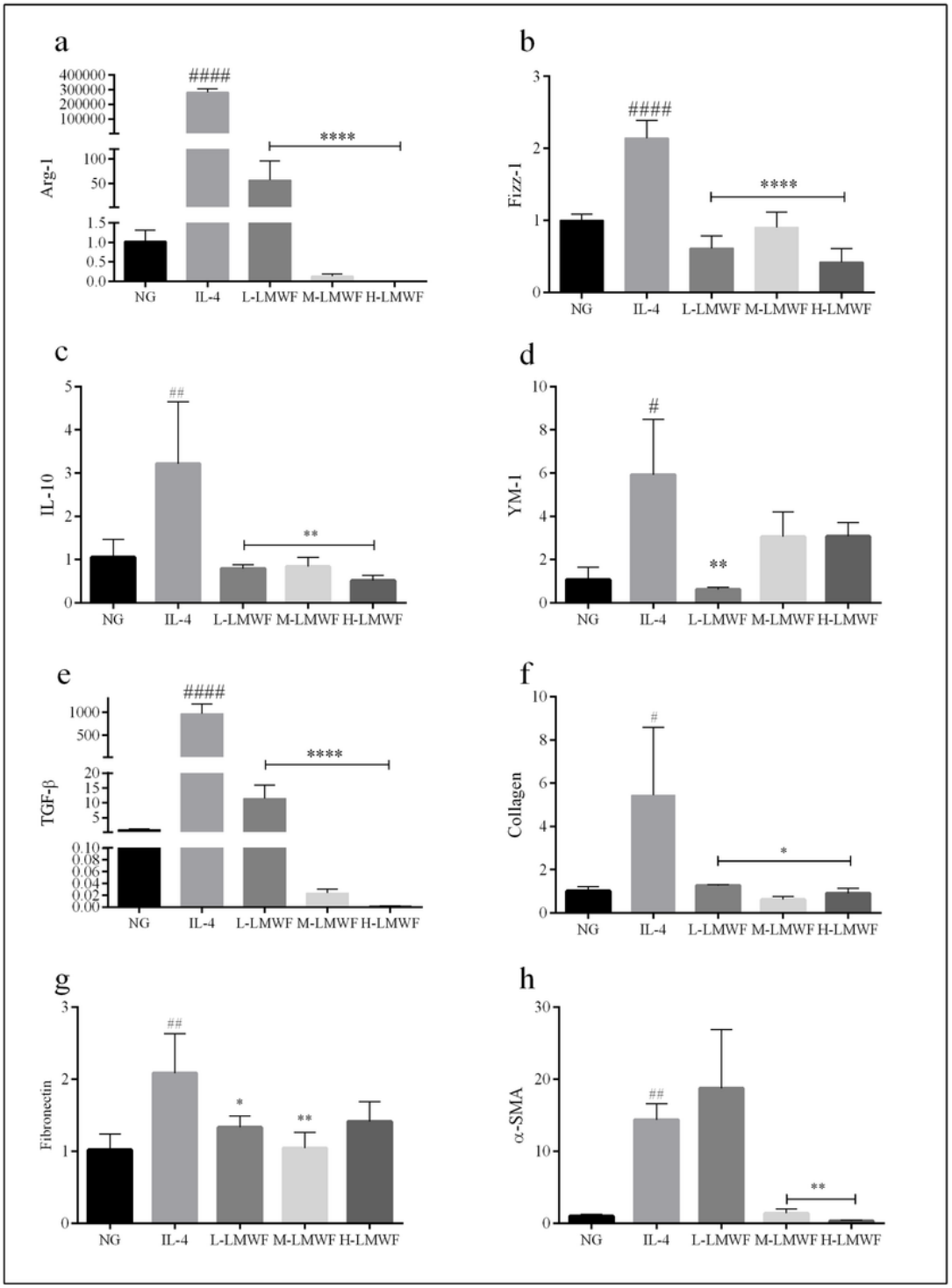

## Figure 3

Assessment of M1 and M2 macrophages biomarkers in lung by qPCR: **(a)** arginase, **(b)** Fizz-1, **(c)** IL-10, **(d)** YM-1, **(e)** TGF-β, **(f)** CD86, **(g)** TNF-α, **(h)** IL-6, **(i)** TLR4 and **(j)** NOS2 (****$p$<0.01 vs. the PF group, ####$p$<0.01 vs. the Sham group).



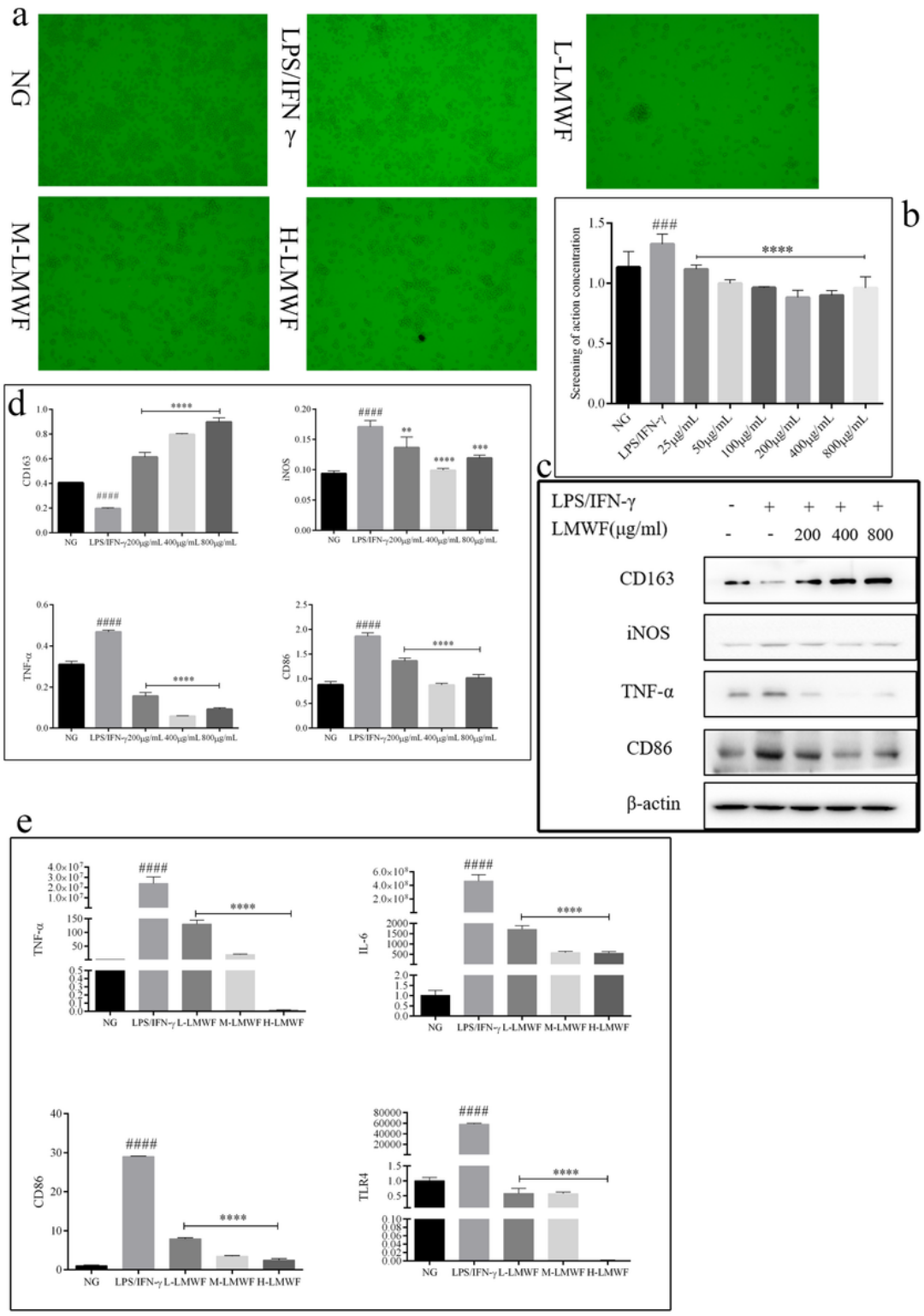

## Figure 4

**(a)** Observation of cell morphology, **(b)** Cytotoxicity assay, **(c)** Screening the effective concentration (*$p$<0.05, vs. the IL-4 group, #$p$ <0.05 vs. the NG group), **(d)** Expression of M2 macrophage polarization biomarkers by western blot, **(e)** Statistical expressions of western blot ($^{*}p$<0.05, $^{****}p$<0.01 vs. the IL-4 group, $^{\#}p$<0.05, $^{\#\#\#\#}p$<0.01 vs. the NG group).




## Figure 5

mRNA expression of M2 macrophage biomarkers: **(a)** arginase-1, **(b)** Fizz-1, **(c)** IL-10, **(d)** YM-1, **(e)**TGF-β, Fibrosis biomarkers expression: **(f)** Collagen, **(g)** Fibronectin, **(h)** α-SMA, ($^{*}p$<0.05, $^{****}p$<0.01 vs. the IL-4 group, $^{\#}p$ <0.05, $^{\#\#\#\#}p$<0.01 vs. the NG group).




## Figure 6

**(a)** Observation of cell morphology, **(b)** Screening the effective concentration ($^{****}p$<0.05, vs. the LPS/IFN-γ group, $^{\#\#\#\#}p$ <0.05 vs. the NG group), **(c)** Expression of M1 macrophage polarization biomarkers by western blot, **(d)** Statistical expressions of western blot ($^{****}p$<0.01 vs. the LPS/IFN-γ group, $^{\#\#\#\#}p$<0.01 vs.

the NG group), (**e**) mRNA expression of M2 macrophage biomarkers ($^{****}p<0.01$ vs. the LPS/IFN-γ group, $^{####}p<0.01$ vs. the NG group)

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- abstract.tif