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Research Article

Effect of Mn_3O_4 Nanoparticles on Lipopolysaccharide-Induced Inflammatory Factors in the Human Tendon Cells and Its Mechanism

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Objective. To investigate the effect of Mn_3O_4 nanoparticles (Mn_3O_4NPs) on inflammatory factors induced by lipopolysaccharide (LPS) in human tendon cells and its mechanism. *Methods*. The Mn_3O_4NPs were synthesized by a hydrothermal method. RT-qPCR was used to detect the expression levels of miRNAs related to inflammation in human tendon cells. The expression level of NLRP1 (NOD-like receptor containing pyrin domain 1) was measured by Western blotting. ELISA assay was used to measure the level of TNF-α, IL-1β, IL-4, and IL-10. The relationship between miR-181a-5p and NLRP1 was verified by dual-luciferase reporter assay. *Results*. Mn_3O_4NPs produced in this study were brown spherical particles with an average size of 7-10 nm. Mn_3O_4NP treatment significantly reduced the levels of TNF-α and IL-1β but increased the levels of IL-4 and IL-10 in the human tendon cells induced by LPS. In addition, Mn_3O_4NP treatment remarkably increased the expression level of miR-181a-5p. NLRP1 is one of the targets of miR-181a-5p, and miR-181a-5p downregulated its expression. Further study showed that Mn_3O_4NPs could alleviate the inflammatory response of human tendon cells induced by LPS by upregulating miR-181a-5p and thus downregulating the expression of NLRP1. *Conclusion*. Mn_3O_4NPs affect the expression of inflammatory cytokines in the human tendon cells induced by LPS by modulating the molecular axis of miR-181a-5p/NLRP1.

1. Introduction

Tendonitis is the inflammation of the tendon caused by strain. It often occurs in the hands, wrists, shoulders, and knees. With the increasing use of computers, the hand controlling the mouse has been the most common hand which affected by tendonitis and its incidence has been increasing in recent years [1]. Recently, nanomaterials with new structures, new properties, and new functions have been widely used in the treatment of a variety of diseases including tumors [2], autoimmune diseases, etc. [3]; studies also shown that nanoparticles have good efficacy in the treatment of inflammation-related diseases [4]. Among them, Mn_3O_4 nanoparticles (Mn_3O_4NPs) have been demonstrated to alleviate inflammation of mouse ears induced by ROS [5]. Lipopolysaccharide (LPS), as a cell wall component of Gram-negative bacteria, has been often used to stimulate cells to produce inflammatory mediators to build a model of cellular inflammation. However, there are no reports about the effects of Mn_3O_4NPs on LPS-induced tendon inflammatory factor levels and their regulatory mechanisms. Therefore, in this study, to provide a new strategy for the treatment of tendonitis, human tendon cells were induced by LPS to form a tendinitis model, and the effect of Mn_3O_4NPs on the expression level of inflammatory factors was explored.

2. Material and Methods

2.1. Cell Lines and Reagents. Human tendon cells HT (Cat. No. PR203) were purchased from Beijing Fubo Biotechnology Co., Ltd. Fetal bovine serum, DMEM medium, penicillin, and streptomycin were purchased from Guangzhou Ruite Biotechnology Co., Ltd. $Mn(OAc)_2{\cdot}4H_2O$ and TRIzol one-

step RNA extraction reagents were purchased from Shanghai Yanjin Biotechnology Co., Ltd. The reverse transcription kit, total protein extraction kit, and dual-luciferase reporter gene detection kit were purchased from Wuhan Purity Biotechnology Co., Ltd. Lipofectamine 2000 kit was purchased from Shanghai Mingming Biotechnology Co., Ltd. TNF-α, IL-1β, IL-4, and IL-10 ELISA kits were purchased from Shanghai Kemin Biotechnology Co., Ltd.

2.2. Preparation and Identification of Mn_3O_4 Nanoparticles. The low-temperature esterification method reported by Li et al. [6] was used to prepare Mn_3O_4 nanoparticles. Specifically, 1 g of Mn $(OAc)_2{\cdot}4H_2O$ was dissolved in 60 ml of absolute ethanol, stirring magnetically until completely dissolved, adding 100 ml of polytetrafluoroethylene, and placed in an autoclave at 120°C for processing. After 24 h, it was cooled to room temperature and washed with deionized water three times to obtain Mn_3O_4NPs. The transmission electron microscope (TEM) was used to observe the color, and a laser particle size analyzer was used for the analysis of the morphology and particle size of the prepared Mn_3O_4NPs. The prepared Mn_3O_4NPs were also analyzed with X-ray diffraction (XRD).

2.3. Cell Culture and Transfection. Human tendon cell HT was cultured in DMEM medium containing 10% fetal bovine serum, 100 U/l penicillin, and 100 mg/l streptomycin (containing 10% fetal bovine serum), at 37°C with 5% CO_2. Once the cell growth was stable, 1 mg/ml lipopolysaccharide (LPS) was added to build the human tendon cell inflammation model. After 24 h of treatment, miR-181a-5p mimics, miR-181a-5p inhibitor, and si-NLRP1 were transfected into cells according to the experimental design. The experimental groups included NC group (HT cells without transfection treatment), miR-181a-5p mimics group (overexpression of miR-181a-5p in HT cells), si-NLRP1 group (knockout NLRP1 in HT cells), and si-NLRP1+miR-181a-5p inhibitor group (simultaneously knockout NLRP1 and miR-181a-5p in HT cells). Lipofectamine 2000 kit was used for transfection according to the instructions. After 48 h, the transfected cells were collected for subsequent experiments.

2.4. Determination of Mn_3O_4NP Cytotoxicity and Treatment of Mn_3O_4NPs. MTT method was used to detect the cytotoxicity of Mn_3O_4NPs to HT cells. The HT cells were cultured in 96-well plates. After incubation overnight, the fresh medium was changed, and different concentrations (0, 2, 4, 6, 8, and 10 μg/ml) of Mn_3O_4NPs were added and incubated. After 48 hours of incubation, the fresh medium and MTT was added, and the absorbance of the cells at 570 nm was detected by a microplate reader to determine the cytotoxicity of Mn_3O_4NPs to HT cells. After cytotoxicity testing, HT cells in each experimental group were treated with 10 μg/ml Mn_3O_4NPs for 48 h.

2.5. RT-qPCR. Total RNA of cells in each experimental group was extracted by TRIzol one-step method. 2 μl of total RNA was reversely transcribed into cDNA according to the reverse transcription kit instructions. The reverse transcription product was placed in a PCR instrument for the reaction. The 20 μl reaction system contains 0.5 μl reverse transcription product, 1 μl upstream and downstream primers, 10 μl SYBR Premix Ex Taq, and 7.5 μl RNase-FREE water. The reaction conditions were 90°C for 10 min, 95°C for 30 s, and 55°C for 1 min. A total of 50 cycles were performed.

The primer sequences are as follows:

miR-181a-5p upstream primer sequence: 5′-CGGCAA CATTCAACGCTGT-3′;

miR-181a-5p downstream primer sequence: 5′-GTGC AGGGTCCGAGGTATTC-3′. U6 upstream primer sequence: 5′-CTTCGGCAGCACATATAC-3′; U6 downstream primer sequence: 5′-GAACGCTTCACGAATTTGC-3′.

Quantitative fluorescence detection results were calculated by the $2^{-\Delta\Delta Ct}$ method.

2.6. Western Blotting. The total protein of each experimental group was extracted using a total protein extraction kit. The equivalent amount of denatured protein sample was loaded, and SDS-PAGE electrophoresis was performed. After 2 h of electrophoresis, the membrane was transferred at 100 V. After the film is transferred, it was sealed in 5% skimmed milk powder. After 2 h, the membrane was washed, and a primary antibody (ani-NLRP1, 1 : 1000; ani-GAPDH, 1 : 1000) was added and incubated at 4°C overnight. The next day, after washing the membrane, a secondary antibody (1 : 1000) was added and incubated at room temperature for 2 h. After the reaction was complete, the film was washed, and a chemiluminescence developing solution was added for development and photographs. Grayscale analysis of protein bands was performed using ImageJ software.

2.7. ELISA. The cell supernatants of each experimental group were obtained by centrifugation, and the contents of TNF-α, IL-1β, IL-4, and IL-10 were measured according to the instructions of the ELISA kit. In specific, 100 μl of the standard sample and the test sample were added to a 96-well plate and incubated at 37°C for 1 hour and rinsed with a washing solution. 100 μl of the primary antibody working solution was added to each well, mixed, and incubated at 37°C. After 1 h, the plate was washed; 100 μl enzyme-labeled antibody working solution was added and incubated at 37°C. After 30 minutes, the plate was washed again; 100 μl of substrate working solution was added and reacted at 37°C in the dark. After 15 min, 100 μl of reaction stop solution was added and samples were further incubated at 37°C for 10 min. After the reaction was completed, the OD value was measured by a microplate reader, and a standard curve was drawn to determine the contents of TNF-α, IL-1β, IL-4, and IL-10 in the measured samples.

2.8. Double Luciferase Reporter Assay. The 3′-UTR fragments of the wild-type and mutant NLRP1 genes were amplified and inserted into a double luciferase reporter gene plasmid. The reporter gene plasmid and miR-181a-5p mimics were cotransfected into T293 cells and cultured. After 48 h, luciferase activity was measured.

2.9. Statistical Analysis. All experimental data in this study were expressed as $\bar{x} \pm s$. Analysis of data was performed using

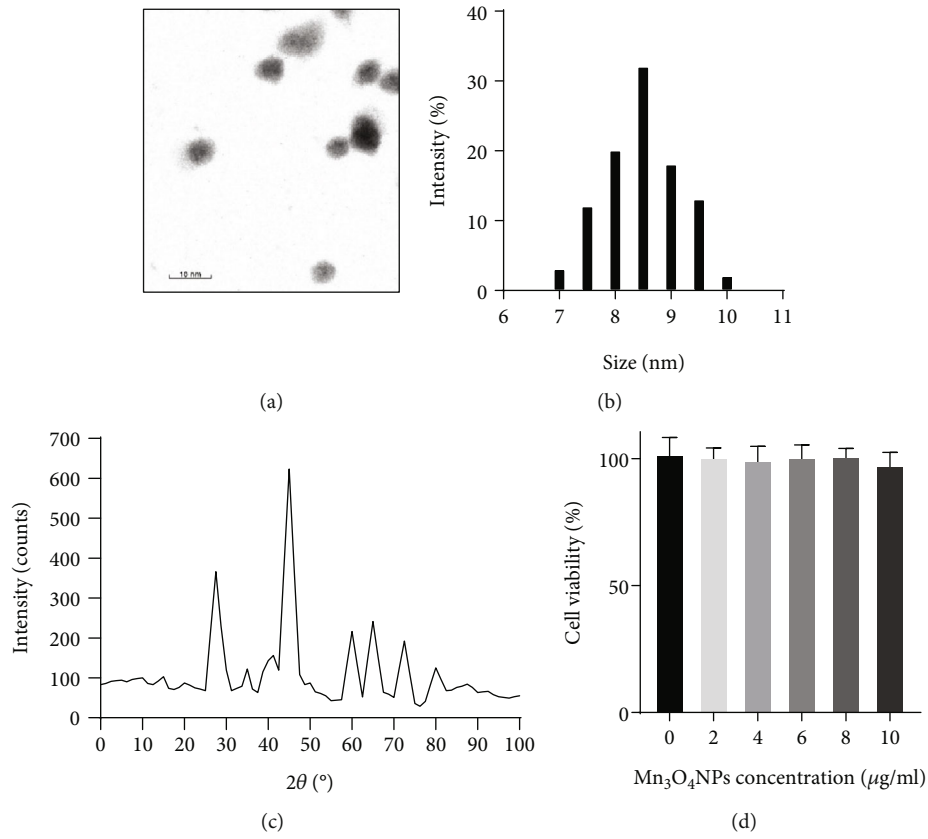

FIGURE 1: Identification of Mn_3O_4NPs and their cytotoxicity detection. (a) The morphology of Mn_3O_4NPs by transmission electron microscope. (b) The particle size distribution range of Mn_3O_4NPs analyzed by laser particle size analyzer. (c) T he XRD pattern of Mn_3O_4NPs detected by X-ray diffraction. (d) Detection of the cytotoxicity of Mn_3O_4NPs with MTT method.

SPSS 22.0. The *t*-test was used to compare the two groups, and the one-way analysis of variance was used to compare the multiple groups. $P < 0.05$ or $P < 0.01$ indicates that the difference is statistically significant.

3. Results

3.1. Identification of Mn_3O_4 Nanoparticles and Detection of Their Cytotoxicity. Observed with a transmission electron microscope, the prepared Mn_3O_4NPs were spherical particles, as shown in Figure 1(a). The particle size distribution of Mn_3O_4NPs analyzed by laser particle size analyzer was about 7-10 nm, as shown in Figure 1(b). X-ray diffraction analysis results showed that the prepared Mn_3O_4NPs diffraction peaks are in good concordance with the Mn_3O_4 standard chart (JCPDS card No. 24-0734) and do not contain impurity peaks as shown in Figure 1(c), which was used in subsequent experiments. The results of the MTT assay showed that Mn_3O_4NPs had no significant toxicity to HT cells in the experimental range, as shown in Figure 1(d).

3.2. Effects of Mn_3O_4 Nanoparticles on Inflammatory Factors in Human Tendon Cells Induced by LPS. ELISA results showed that the expression levels of TNF-α and IL-1β in HT cells were significantly increased after LPS induction ($P < 0.01$), and the expression levels of IL-4 and IL-10 were significantly decreased ($P < 0.01$). Compared with the LPS group, Mn_3O_4NP treatment significantly reduced the expression levels of TNF-α and IL-1β in cells ($P < 0.05$), and significantly increased the expression levels of IL-4 and IL-10 ($P < 0.05$) (Figure 2). Therefore, Mn_3O_4NP treatment significantly inhibited LPS-induced human tendon cell inflammation.

3.3. Effects of Mn_3O_4 Nanoparticles on the Expression Level of Inflammation-Related miRNA in Human Tendon Cell-Induced LPS. RT-PCR results showed that Mn_3O_4NPs affected the expression level of inflammation-related miRNAs in HT cells induced by LPS, and the expression level of miR-181a-5p was significantly higher than that of the untreated group, as shown in Figure 3. The mechanism of miR-181a-5p affecting LPS-induced inflammation of human tendon cells was further explored in subsequent experiments.

3.4. Targeting Relationship between miR-181a-5p and NLRP1. The StarBase database was used to predict the binding sites of NLRP1 and miR-181a-5p as shown in Figure 4(a). The results of the double luciferase experiment showed that miR-181a-5p mimics significantly reduced the luciferase activity of the NLRP1 wild-type vector ($P < 0.05$), but had no effect on the luciferase activity of the NLRP1 mutant vector (Figure 4(b)). In addition, Western blotting results showed that overexpression of miR-181a-5p significantly reduced the expression level of NLRP1 protein in LPS-

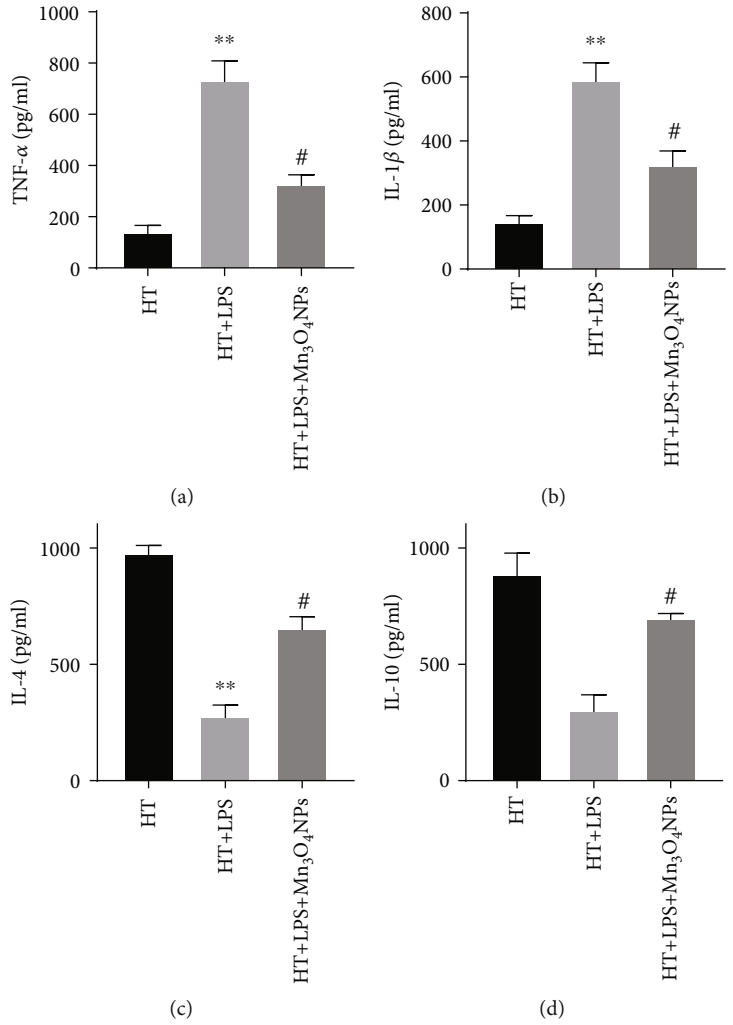

Figure 2: Effects of Mn_3O_4 nanoparticles on the inflammatory factors levels in human tendon cells induced by LPS. (a) TNF-α level measured by ELISA. (b) IL-1β level measured by ELISA. (c) IL-4 level measured by ELISA. (d) IL-10 level measured by ELISA. $^{**}P < 0.01$ vs. HT group; $^{\#}P < 0.05$ vs. HT+LPS group.

induced HT cells ($P < 0.05$) (Figure 4(c)). From these results, it seemed that there is a targeting relationship between miR-181a-5p and NLRP1 in LPS-induced human tendon cells and miR-181a-5p downregulated the NLRP1 expression.

3.5. Mechanism of Mn_3O_4 Nanoparticles Affecting LPS-Induced Inflammatory Factors in Human Tendon Cells. ELISA results showed that knockout of NLRP1 significantly reduced the expression levels of TNF-α and IL-1β in HT cells induced by LPS ($P < 0.01$) and significantly increased the expression levels of IL-4 and IL-10 ($P < 0.01$). Compared with the NLRP1 knockout group, the treatment of NLRP1 knockout with Mn_3O_4NPs significantly reduced the expression levels of TNF-α and IL-1β ($P < 0.05$) and at the time significantly increased the expression levels of IL-4 and IL-10 ($P < 0.05$). The expression levels of TNF-α and IL-1β in the si-NLRP1+Mn_3O_4NPs+miR-181a-5p inhibitor group were higher than those in the si-NLRP1+Mn_3O_4NPs group ($P < 0.05$), and the expression levels of IL-4 and IL-10 were lower than si-NLRP1+Mn_3O_4NPs group ($P < 0.05$). Compared with the si-NLRP1+Mn_3O_4NPs+miR-181a-5p inhibitor group, the expression levels of TNF-α and IL-1β were increased in the Mn_3O_4NPs+miR-181a-5p inhibitor group ($P < 0.05$), whereas IL-4 and IL expression levels were decreased ($P < 0.05$) (Figure 5). From the above experimental results, it seems that Mn_3O_4NPs upregulated miR-181a-5p in HT cells induced by LPS to downregulate the expression of NLRP1, thereby inhibiting LPS-induced HT cell inflammation.

4. Discussion

Tendonitis can cause abnormalities in the normal biological properties of tendons, such as thickening of the tendon and damage of the synovium, and the pain caused by it severely affects the daily activities of patients [7]. As the hands are the main organ for daily labor, the tendon of the hands is extremely susceptible to cumulative strain; therefore, hand tendinitis is also very common in clinical practice [8]. Local hormone injection is currently the most commonly used method of treating tendinitis, but studies have shown that local hormone injection has the risk of causing necrosis of

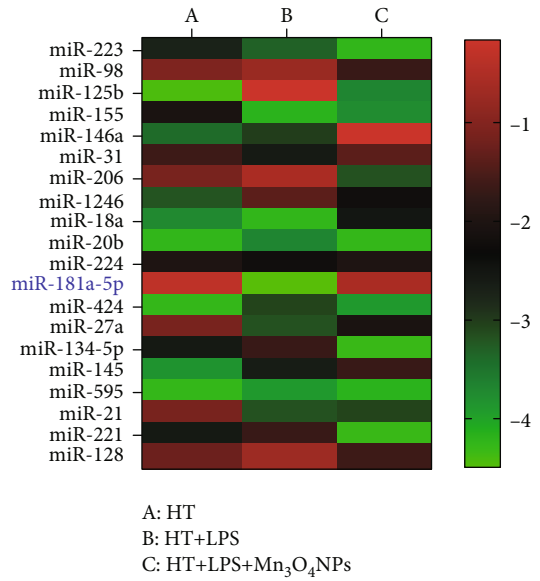

Figure 3: Mn_3O_4 nanoparticles effect on the expression of inflammatory-related miRNA in human tendon cells induced by LPS.

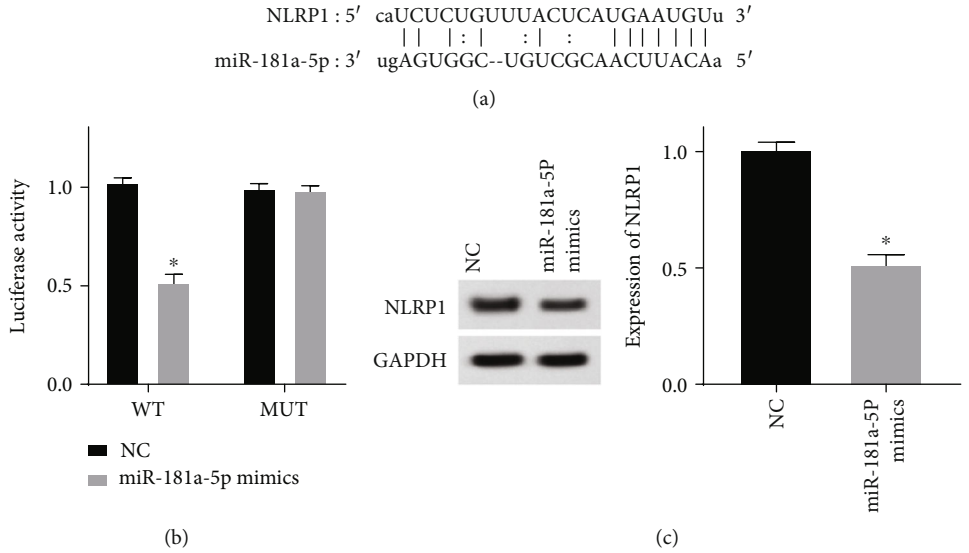

Figure 4: Relationship between miR-181a-5p and NLRP1. (a) Binding sites between miR-181a-5p and NLRP1 predicted by StarBase. (b) The interaction of miR-181a-5p and NLRP1 determined by dual-luciferase reporter assay. (c) NLRP1 expression level measured by western blotting. $^*P < 0.01$ vs. NC group.

tendon collagen [9]. In recent years, with the in-depth study of nanoparticles, its important role in the treatment of inflammation-related diseases has also been confirmed [10].

Mn_3O_4NPs, as nanomaterials with enzyme-like activity, display good therapeutic potential in the treatment of inflammatory diseases. Studies have confirmed that Mn_3O_4NPs can functionally mimic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), and can significantly remove superoxide anion radicals and peroxidation hydrogen and hydroxyl radicals to protect cells from oxidative damage [11]. ROS imbalances including superoxide anion free radicals, hydrogen peroxide, and hydroxyl free radicals often occurred in inflammation. Therefore, Mn_3O_4NPs with high active oxygen scavenging activity and high stability have been shown to significantly relieve ROS-induced mouse ear inflammatory response [12]. However, there are few reports about Mn_3O_4NPs in the treatment of inflammation, and the research on its mechanism of action is lacking. The results of this study indicate that Mn_3O_4NPs significantly reduced the levels of proinflammatory-related proteins in human tendon cells induced by LPS and increased the levels of anti-inflammatory-related proteins to alleviate the inflammatory response.

MicroRNAs (miRNAs), as a class of noncoding single-stranded small RNAs composed of 19 to 22 nucleotides, can target mRNAs to degrade them or inhibit the translation

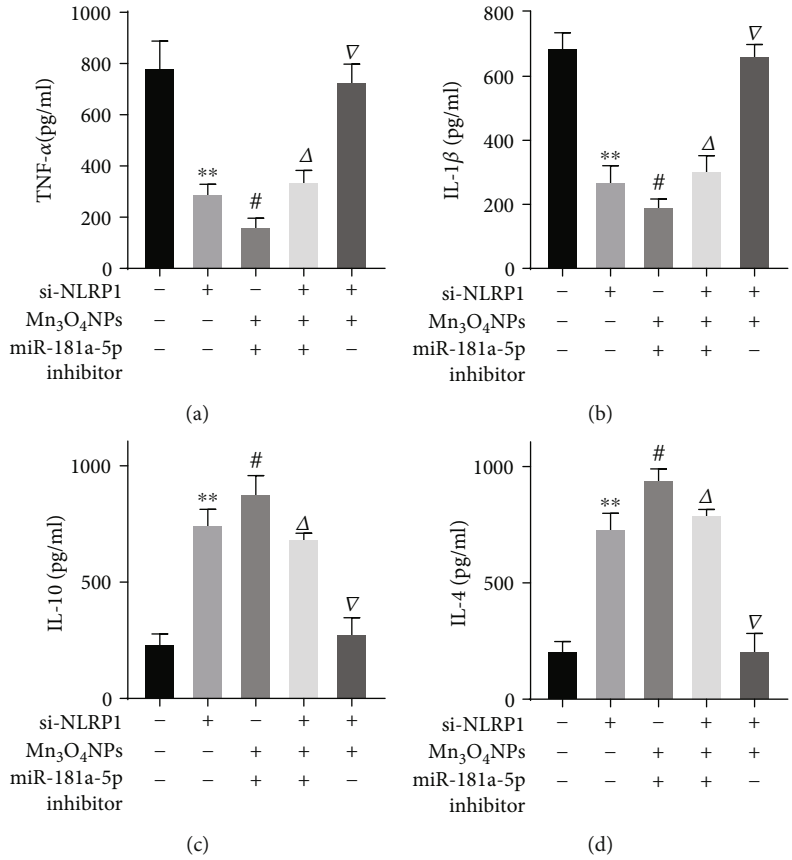

Figure 5: Mechanism of Mn_3O_4 nanoparticles effect on the inflammatory factors levels in human tendon cells induced by LPS. (a) TNF-α level measured by ELISA. (b) IL-1β level measured by ELISA. (c) IL-4 level measured by ELISA. (d) IL-10 level measured by ELISA. $^{**}P < 0.01$ vs. NC group; $^{\#}P < 0.05$ vs. si-NLRP1 group; $^{\Delta}P < 0.05$ vs. si-NLRP1+Mn_3O_4NPs group; $^{\nabla}P < 0.05$ vs. si-NLRP1 +Mn_3O_4NPs+miR-181a-5p inhibitor group.

process, thereby regulating the physiological processes of cells [13]. Many studies have confirmed that the occurrence and development of inflammatory-related diseases are closely associated with miRNAs [14]. For instance, studies have shown that the expression level of miRNA-146a is positively correlated with the severity of the inflammatory response and can be used as an indicator of the inflammatory response disease activity [15]. Studies by Marques-Rocha et al. [13] showed that continuously upregulated miR-155 can also lead to sustained inflammatory responses. Among them, miR-181a has also been shown to be associated with inflammation. Studies have shown that miR-181a can significantly inhibit the expression of inflammatory factors IL-1β, IL-6, and TNF-α in macrophages induced by LPS [16]. In addition, miR-181a can also affect the myometrial inflammatory response by regulating the expression levels of ER-α and c-Fos [17]. Studies have also illustrated that miR-181a participates in the homeostatic response to inflammatory stimuli by regulating the TLR-4 signaling pathway [18]. The experimental results of this study also confirmed that miR-181a-5p is involved in regulating the expression of inflammatory factors in human tendon cells induced by LPS.

miRNAs play an important role in the occurrence and development of inflammatory diseases by binding to target genes and regulating the expression of target proteins related to inflammation. The association of NLRP1 with inflammation has been demonstrated [19]. NLRP1 is widely present in T cells, B cells, macrophages, and dendritic cells. When not stimulated, NLRP1 leucine-rich repeat-rich domains bind to the central nucleotide-binding oligomerization regions (NACHT), self-oligomerization is inhibited and in an inactive state. Upon stimulation, its domain changes, and it binds to proteins such as apoptosis-related speckle-like protein (ASC) and semi-aspartase (caspase-1) to form a protein complex called an inflammasome, which can regulate the interleukin expression level, activates the NF-κB and MAPK signaling pathways, and participates in the body's inflammatory response [20]. The proven proinflammatory factors including tumor necrosis factor-α (TNF-α) produced by monocyte macrophages function in immune regulation, participate in fever and inflammation, and can further induce the production of other cytokines [21]. Interleukin-1β (IL-1β), as a pleiotropic factor, is the main mediator of the host's response to infection or tissue damage [22]. And the immune factors, especially the imbalance of Th1/Th2 immune response, often occur in the inflammatory response, among which interleukin-4 (IL-4) and interleukin-10 (IL-4) produced by the Th2 subset of CD4+ T cells. IL-10 has been shown to increase expression levels in the inflammatory response [23]. Studies have demonstrated that in rat

tendon cell inflammation models, stimulating factors can promote the activation of NLRP inflammasomes by disaggregating cytoskeleton F-actin, thereby increasing the expression and release of inflammatory factors TNF-α, IL-6, and IL-1β to aggravate the development of inflammation [24]. The results of this study indicate that miR-181a-5p downregulated NLRP1 expression in human tendon cells induced by LPS, and knocking out NLRP1 significantly alleviated the inflammatory response of human tendon cells induced by LPS.

The results of this study indicate that Mn_3O_4NP treatment can significantly reduce the levels of proinflammatory factors TNF-α and IL-1β in human tendon cells induced by LPS and increase the levels of IL-4 and IL-10. Further research confirmed that Mn_3O_4NPs upregulated miR-181a-5p in human tendon cells induced by LPS, and by doing that downregulated the expression of NLRP1 thus alleviating the LPS-induced human tendon cell inflammation.

Data Availability

All the data are available with the handwritten notebook documented in our lab.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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References

[1] L. Padua, D. Coraci, C. Erra et al., "Carpal tunnel syndrome: clinical features, diagnosis, and management," *Lancet Neurology*, vol. 15, no. 12, pp. 1273–1284, 2016.

[2] J. Ding, J. Chen, L. Gao et al., "Engineered nanomedicines with enhanced tumor penetration," *Nano Today*, vol. 29, article 100800, 2019.

[3] X. Feng, W. Xu, Z. Li, W. Song, J. Ding, and X. Chen, "Immunomodulatory nanosystems," *Advanced Science*, vol. 6, no. 17, article 1900101, 2019.

[4] Q. Zhang, D. Dehaini, Y. Zhang et al., "Neutrophil membrane-coated nanoparticles inhibit synovial inflammation and alleviate joint damage in inflammatory arthritis," *Nature Nanotechnology*, vol. 13, no. 12, pp. 1182–1190, 2018.

[5] J. Yao, Y. Cheng, M. Zhou et al., "ROS scavenging Mn_3O_4 nanozymes for *in vivo* anti-inflammation," *Chemical Science*, vol. 9, no. 11, pp. 2927–2933, 2018.

[6] X. Q. Li, L. P. Zhou, J. Gao, H. Miao, H. Zhang, and J. Xu, "Synthesis of Mn_3O_4 nanoparticles and their catalytic applications in hydrocarbon oxidation," *Powder Technology*, vol. 190, no. 3, pp. 324–326, 2009.

[7] L. Gaut and D. Duprez, "Tendon development and diseases," *Wiley Interdisciplinary Reviews: Developmental Biology*, vol. 5, no. 1, pp. 5–23, 2016.

[8] E. R. Wagner and M. B. Gottschalk, "Tendinopathies of the forearm, wrist, and hand," *Clinics in Plastic Surgery*, vol. 46, no. 3, pp. 317–327, 2019.

[9] J. C. Kennedy and R. B. Willis, "The effects of local steroid injections on tendons: a biomechanical and microscopic correlative study," *The American Journal of Sports Medicine*, vol. 4, no. 1, pp. 11–21, 1976.

[10] C. Dianzani, F. Foglietta, B. Ferrara et al., "Solid lipid nanoparticles delivering anti-inflammatory drugs to treat inflammatory bowel disease: effects in an in model," *World Journal of Gastroenterology*, vol. 23, no. 23, pp. 4200–4210, 2017.

[11] N. Singh, M. A. Savanur, S. Srivastava, P. D'Silva, and G. Mugesh, "A redox modulatory Mn_3O_4 nanozyme with multi-enzyme activity provides efficient cytoprotection to human cells in a Parkinson's disease model," *Angewandte Chemie*, vol. 56, no. 45, pp. 14267–14271, 2017.

[12] M. Zendjabil, S. Favard, C. Tse, O. Abbou, and B. Hainque, "The microRNAs as biomarkers: what prospects?," *Comptes Rendus Biologies*, vol. 340, no. 2, pp. 114–131, 2017.

[13] J. L. Marques-Rocha, M. Samblas, F. I. Milagro, J. Bressan, J. A. Martínez, and A. Marti, "Noncoding RNAs, cytokines, and inflammation-related diseases," *The FASEB Journal*, vol. 29, no. 9, pp. 3595–3611, 2015.

[14] Y. Tan, L. Yu, C. Zhang, K. Chen, J. Lu, and L. Tan, "miRNA-146a attenuates inflammation in an *in vitro* spinal cord injury model via inhibition of TLR4 signaling," *Experimental and Therapeutic Medicine*, vol. 16, no. 4, pp. 3703–3709, 2018.

[15] S. Bala, T. Csak, B. Saha et al., "The pro-inflammatory effects of miR-155 promote liver fibrosis and alcohol-induced steatohepatitis," *Journal of Hepatology*, vol. 64, no. 6, pp. 1378–1387, 2016.

[16] X. J. Du, J. M. Lu, and Y. Sha, "MiR-181a inhibits vascular inflammation induced by ox-LDL via targeting TLR4 in human macrophages," *Journal of Cellular Physiology*, vol. 233, no. 10, pp. 6996–7003, 2018.

[17] L. Gao, G. Wang, W. N. Liu, H. Kinser, H. L. Franco, and C. R. Mendelson, "Reciprocal feedback between miR-181a and E2/ERα in myometrium enhances inflammation leading to labor," *The Journal of Clinical Endocrinology and Metabolism*, vol. 101, no. 10, pp. 3646–3656, 2016.

[18] H. Li, G. di, Y. Zhang, R. Xue, J. Zhang, and J. Liang, "MicroRNA-155 and microRNA-181a, via HO-1, participate in regulating the immunotoxicity of cadmium in the kidneys of exposed *Cyprinus carpio*," *Fish & Shellfish Immunology*, vol. 95, pp. 473–480, 2019.

[19] C. H. Yu, J. Moecking, M. Geyer, and S. L. Masters, "Mechanisms of NLRP1-mediated autoinflammatory disease in humans and mice," *Journal of Molecular Biology*, vol. 430, no. 2, pp. 142–152, 2018.

[20] R. Liu, A. D. Truax, L. Chen et al., "Expression profile of innate immune receptors, NLRs and AIM2, in human colorectal cancer: correlation with cancer stages and inflammasome components," *Oncotarget*, vol. 6, no. 32, pp. 33456–33469, 2015.

[21] D. Li, D. Chen, X. Zhang et al., "c-Jun N-terminal kinase and Akt signalling pathways regulating tumour necrosis factor-α-induced interleukin-32 expression in human lung fibroblasts: implications in airway inflammation," *Immunology*, vol. 144, no. 2, pp. 282–290, 2015.

[22] D. Gleiznys, A. Gleiznys, L. Abraškevičiūtė, A. Vitkauskienė, V. Šaferis, and J. Sakalauskienė, "Interleukin-10 and interleukin-1β cytokines expression in leukocytes of patients with chronic peri-mucositis," *Medical Science Monitor*, vol. 25, pp. 7471–7479, 2019.

[23] G. R. Gandhi, M. T. S. L. Neta, R. G. Sathiyabama et al., "Flavonoids as Th1/Th2 cytokines immunomodulators: a systematic review of studies on animal models," *Phytomedicine*, vol. 44, pp. 74–84, 2018.

[24] Q. Chen, J. Zhou, B. Zhang, Z. Chen, Q. Luo, and G. Song, "Cyclic stretching exacerbates tendinitis by enhancing NLRP3 inflammasome activity via F-actin depolymerization," *Inflammation*, vol. 41, no. 5, pp. 1731–1743, 2018.