

Arbutin-Modified Microspheres Prevent Osteoarthritis Progression by Mobilizing Local Anti-Inflammatory and Antioxidant Responses

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Abstract

Osteoarthritis (OA), a common chronic degenerative disease, is considered the leading cause of disability worldwide. However, the current clinical treatments for OA are still unsatisfactory. Arbutin (ARB) is a major active ingredient of the Chinese medicinal herb cowberry leaf, which exerts potential antioxidant and anti-inflammatory activity. Based on a microfluid platform, we fabricated ARB-loaded gelatine methacryloyl-Liposome (GM-Lipo@ARB) microspheres that showed long-term release and excellent cartilage-targeting effects. The ARB-loaded microspheres effectively reduced the inflammatory response in interleukin (IL)-1 β -treated arthritic chondrocytes. Moreover, GM-Lipo@ARB microspheres regulated cartilage extracellular matrix (ECM) homeostasis by upregulating nuclear factor erythroid 2-related factor 2 (Nrf2)-associated antioxidant properties. Intraarticular injection of GM-Lipo@ARB microspheres alleviated cartilage erosion in the destabilization of the medial meniscus (DMM)-mediated mouse OA model. Our results indicated that ARB-modified microspheres prevented cartilage degeneration by not only inhibiting the inflammatory reaction but also mitigating oxidative stress. This study proposes a novel ARB-laden functional microsphere for treating OA through the mobilization of local anti-inflammatory and antioxidant responses.

1. Introduction

Osteoarthritis (OA) has been reported to be a common chronic degenerative disease worldwide. This disease is characterized by extreme pain and disability, making it a major public health concern [1]. The major pathologic changes in OA include joint inflammation, progressive destruction of articular cartilage, osteophytes, subchondral bone remodelling, and joint effusion [2]. Many factors can contribute to the development of OA, including age, obesity, genetic changes, and mechanical shocks [3]. Nonoperative measures to treat OA involve physical therapy, pharmacological treatment with nonsteroidal drugs, and intra-articular injection of hyaluronic acid [4]. At present, nonsteroidal anti-inflammatory drugs (NSAIDs) are the major drugs for treating OA [5]. Unfortunately, NSAIDs are only palliative, rendering them ineffective in treatment regimens. These methods all have some disadvantages, such as frequent injections and gastrointestinal and cardiovascular side effects [6]. To overcome this drawback, the development of a novel treatment strategy for OA is needed.

Arbutin (ARB), a natural extract from the Chinese medicine herb cowberry leaf, has been shown to suppress disease-related inflammation in animal models of diabetes [6], nervous system disease [7], and acute lung injury by regulating cellular inflammation [8]. ARB exerted antioxidant effects and is a reliable component that is widely used in the organic synthesis and cosmetic industries. Recently, ARB has been shown to have great potential in different medical fields. Previous studies have shown that ARB has beneficial therapeutic effects, including antitumour effects [9], and certain protective effects against nerve cell oxidative stress, apoptosis, and irreversible kidney damage caused by diabetes. In addition, ARB has been reported to reduce acute alcohol-induced liver damage and prevent oxidative damage in mice [10]. Unlike conventional NSAIDs, ARB has been shown to exhibit potent anti-inflammatory effects

and minimal side effects [11]. However, the anti-inflammatory effect of ARB on chondrocytes and its effect on OA have not been examined.

Gelatine methacryloyl (GM) is a natural polymer originating from hydrolysed collagen that has a biological activity similar to that of collagen and has been widely used as a raw material for biological materials [12]. GM microspheres are porous, polymeric microspheres that are used topically and can achieve near uniform drug release [13]. However, GM alone cannot effectively slow-release the water-soluble ARB and target chondrocytes. Liposomes are self-assembling vesicles that can efficiently encapsulate both water-soluble and fat-soluble drugs [14]. Moreover, cationic Liposomes are the most prevalent synthetic vector and can target negatively charged cartilage [15]. Functionalized microspheres composed of Liposomes and GM microspheres can achieve sustained release and targeting at the same time [16]. Thus, the anti-inflammatory and antioxidant effects of ARB can be better administered to the OA environment. In theory, the ARB-coated hydrogel could effectively inhibit inflammation in cartilage cells and slow the progression of OA.

In this study, we fabricated an ARB-modified microsphere (GM-Lipo@ARB) by integrating cationic Liposomes and GM microspheres based on a microfluid platform. *In vitro* protection of cartilage extracellular matrix (ECM) homeostasis, *in vivo* therapeutic effects on posttraumatic OA, and the underlying mechanisms were investigated (Fig. 1). Our research aimed to introduce a dual functional microsphere to ameliorate OA by simultaneously regulating inflammation and redox balance.

2. Results

2.1. Synthesis and characterization of GM-Lipo@ARB microspheres

The mixture of GM, Liposomes and ARB was cut by Span 80 oil to form uniform droplets, which were cross-linked to form microspheres under UV light. Loading drugs into positive Liposomes and packaging them into GM microspheres increases the encapsulation efficiency of the drugs while effectively reducing the release rate of ARB (Fig. 2A). Scanning electron microscopy showed that the Liposomes were uniformly distributed on the surface of the GM microspheres (Fig. 2B). The average particle size of the Liposomes was less than 200 nm, which facilitated entry into chondrocytes. A PDI of 0.147 indicated that the microspheres showed good dispersion (Fig. 2C), and the zeta potential at +20 mV indicated that they could effectively adsorb negatively charged glycosaminoglycans in chondrocytes (Fig. 2D). FTIR indicated an unaffected composite endowed with Liposomes and ARB (Fig. 2E). When placed in a solution containing 0.2 mg/mL collagenase, the weight of GM-Lipo@ARB microspheres decreased by 64.8% compared with that of GM microspheres, which decreased by 72.0% at 28 days, showing a slower *in vitro* degradation rate (Fig. 2F). In drug release experiments, the cumulative release spectra of GM@ARB, Lipo@ARB, and GM-Lipo@ARB were all bipolar. After three days of rapid release, the release efficiencies of the GM@ARB and Lipo@ARB microspheres were 75.6% and 76.3%, respectively, while that of GM-Lipo@ARB microspheres was only 46.1%. In addition, the cumulative release of GM-Lipo@ARB

microspheres was 67.6% at 28 days, compared with 82.0% for GM@ARB and 81.6% for Lipo@ARB microspheres, indicating an excellent sustained-release system (Fig. 2G).

2.2. GM-Lipo@ARB was cell-friendly and showed cartilage-targeting effects

To verify the biocompatibility of the modified microspheres, micromorphology, cell proliferation, and cell viability assays were performed on the cell-microsphere complex. Microscopy images indicated that the cells grew steadily with the microspheres after five days of coculture (Fig. 3A). CCK8 analysis showed that the microspheres had no effect on chondrocyte proliferation at any time point (Fig. 3B). Moreover, when cells grew with the microspheres, quantitative analysis indicated no significant difference in the percentage of live cells between each group (Fig. 3C-D).

In this study, a red fluorescence signal was observed in chondrocytes after they were incubated with Deere-labelled cationic Liposomes (Fig. 3E), indicating that the cationic Liposomes had been absorbed by chondrocytes. As shown in Fig. 3F, Dil-labelled Liposomes were used to examine Liposome attachment to the cartilage surface, and red fluorescence was distributed on the cartilage surface, confirming the cartilage-targeting property of the Liposomes. Cationic Liposomes can interact with negatively charged cells and are easily internalized by them.

2.4. GM-Lipo@ARB maintained cartilage ECM homeostasis

A primary feature of OA is the degradation of cartilage, which results in an imbalance between the synthesis and degradation of chondrocyte ECM. An *in vitro* arthritic environment was established by treatment with the classical proinflammatory factor interleukin (IL)-1 β . Immunofluorescence staining showed that GM-Lipo@ARB microspheres could effectively promote the expression of the synthetic marker Collagen II (Col II) and suppress the activity of the matrix degrading enzyme MMP13 in IL-1 β -treated chondrocytes (Fig. 4A-B). Then, the results were analyzed (Fig. 4C-D). Intriguingly, the cells that grew with the microspheres exhibited a consistent trend, as evidenced by the enhanced ECM anabolism level in the GM-Lipo@ARB group (Fig. 4E-G). Western blot assays indicated that GM-Lipo@ARB upregulated the expression of Col II and Aggrecan but decreased the expression of MMP13 and ADAMTS5 (Fig. 4I). Western blot assays further corroborated the positive regulation of GM-Lipo@ARB on cartilage ECM equilibrium (Fig. 4J). Moreover, analysis of the WB results was performed (fig. S1A)

2.5. GM-Lipo@ARB inhibited the inflammatory response via the NF- κ B axis

To explore the effects of the modified microspheres on inflammation, we measured the changes in inflammatory cytokines following treatment with GM-Lipo@ARB microspheres. RT-PCR showed that GM-Lipo@ARB decreased the expression levels of iNOS, COX-2, tumour necrosis factor (TNF)- α , and IL-6, all of which participate in the generation and development of the inflammatory cascade (Fig. 5A).

Subsequent Western blot and ELISA experiments confirmed the inhibitory effect of the released ARB on the local inflammatory microenvironment (Fig. 5B-C). Meanwhile, analysis of the WB results was performed (fig. S2A). Immunofluorescence staining revealed that IL-1 β -mediated production of iNOS was alleviated by treatment with GM-Lipo@ARB (Fig. 5D). Immunofluorescence analysis was performed for iNOS (fig. S2B). Furthermore, we explored the potential mechanisms by which ARB-modified microspheres yielded anti-inflammatory effects. Western blot assays indicated that the protein level of p65 was reduced in GM-Lipo@ARB-treated cells; however, the I κ B α protein level was elevated, indicating inhibition of NF- κ B dimers in the cytoplasm (Fig. 5E). Furthermore, analysis of the WB results was performed (fig. S2C). In addition, immunofluorescence stain verified that the nuclear translocation of p65 was comprehensively blocked following GM-Lipo@ARB treatment (Fig. 5F). Immunofluorescence analysis was performed for p65 (fig. S2D). Similar results were confirmed by immunofluorescence for I κ B α (fig. S2E).

2.4. GM-Lipo@ARB alleviated oxidative stress injury through nuclear factor erythroid 2-related factor 2 (Nrf2) activation

We then evaluated the therapeutic effects of ARB-laden microspheres on IL-1 β -induced oxidative stress injury. Molecular docking experiments indicated that Nrf2 may act as a target protein of ARB (Fig. 6A), the remaining set of result is presented in the supplementary materials (fig. S3A-C). Nrf2 plays an indispensable role in modulating redox balance by binding the antioxidant response element (ARE) sequence in the nucleus. Western blot analysis indicated that the Nrf2-HO-1 axis was activated by the modified microspheres; however, inhibiting Nrf2 abolished these beneficial effects on redox balance (Fig. 6B). Results from WB were statistically analyzed in fig. S3E. Immunofluorescence staining also indicated that the nuclear translocation of Nrf2 was increased by GM-Lipo@ARB (Fig. 6C) and the results were statistically analyzed (fig. S3F). Consistently, immunofluorescence staining of HO-1 confirmed the antioxidant effect of GM-Lipo@ARB (fig. S3D).

Moreover, Nrf2-specific siRNA was transfected into GM-Lipo@ARB-treated cells. Furthermore, insufficiency of Nrf2 impaired the protective effect of GM-Lipo@ARB on cartilage ECM homeostasis (Fig. 6D). Supplementary fig. S3G presents the statistical results of wb. More importantly, Nrf2 inhibition activated the NF- κ B-mediated inflammatory pathway, as indicated by increased p65 protein levels (Fig. 6E and fig. S3H), reducing the anti-inflammatory effect of GM-Lipo@ARB (Fig. 6F). The above results indicated a regulatory effect of GM-Lipo@ARB on both inflammation and redox status.

2.6. Intra-articular injection of GM-Lipo@ARB delayed OA progression

We further investigated the *in vivo* effect of GM-Lipo@ARB on cartilage degeneration in experimental OA mice. Intra-articular injection of GM-Lipo@ARB preserved the expression of glycosaminoglycan in articular cartilage, as shown by H&E and safranin O staining (Fig. 7A-B). Similarly, the OARSI score was decreased by treatment with ARB-modified microspheres (Fig. 7F). Moreover, immunohistochemical

analysis showed that GM-Lipo@ARB increased the expression of Col II but decreased the expression of MMP13 in hyaline cartilage in destabilization of the medial meniscus (DMM)-impaired mice (Fig. 7C-D), indicating improved protective potency against ECM metabolism *in vivo*. More specifically, as shown in the immunofluorescence assay, the transcription factor Nrf2 was significantly increased following the injection of ARB-laden microspheres (Fig. 7E). The corresponding results are reported in Fig. 7F. These results demonstrated that GM-Lipo@ARB exerted dual regulatory effects on inflammation and oxidative stress balance and prevented OA progression *in vivo* by activating the Nrf2 pathway.

3. Discussion

The overactivated inflammatory response acts as an indispensable driver of the progression of OA. Patients with OA show increased serum levels of IL-1 β , IL-2, IL-6, and IL-8, which are associated with aggregated bone marrow lesions (BMLs) and impaired knee functional scores [17]. Moreover, treatment with proinflammatory factors, such as IL-1 β or TNF- α , undermines cartilage ECM balance in chondrocytes and accelerates OA development in a mouse model [18]. Clinical application of anti-inflammatory drugs, such as etoricoxib, diclofenac, and celecoxib, only alleviates pain but cannot delay the progression of endogenous inflammation [19]. According to our findings, the natural product ARB was a potential anti-arthritis agent that inhibited the inflammatory cascade. Consistently, the robust anti-inflammatory effect of ARB has been evidenced in multiple diseases, including epilepsy [20], lung injury [21], and cardiac hypertrophy [22]. More specifically, the underlying mechanisms by which ARB affects inflammatory reactions are not clear. Our results demonstrated that the anti-inflammatory effects of ARB depended on the inhibition of p65 nuclear translocation, indicating the involvement of the NF- κ B signalling pathway. The latest studies reported that ARB-induced expression of NAD-dependent protein deacetylase sirtuin 1 (SIRT1) not only recoupled apoptosis and autophagy but also reduced the secretion of inflammatory cytokines [23]. Our previous study revealed that the pineal-produced hormone melatonin rescued IL-1 β - or TNF- α -impaired chondrogenic potential in synovial mesenchymal stem cells (SMSCs) via the activation of SIRT1 [24]. Therefore, the crosstalk between ARB and SIRT1 will be investigated in our future work.

Reactive oxygen species (ROS) are mainly produced through oxidative phosphorylation reactions in mitochondria and consist of superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (HO) [25]. The accumulation of ROS impairs cartilage redox equilibrium and activates matrix proteinases [26]. In addition to negatively regulating inflammation, ARB preserved antioxidant properties by enhancing the expression of the redox-sensitive element Nrf2 and its downstream antioxidant enzyme HO-1. Strengthening the Nrf2-HO-1 pathway not only alleviates OA-related oxidative stress injury but also benefits ECM anabolic metabolism to protect against OA. A possible upstream mediator of Nrf2 was revealed to be microRNA (miRNA)-146a-5p, which targets the 3' untranslated region (3'UTR) of Nrf2 to block its translational process [27]. Indeed, the other miRNAs are considered potential regulators of Nrf2, such as miR-132 [28], miR-27a [29], or miR-3175 [30], some of which may be targets of ARB but need further corroboration. Moreover, ARB ameliorated Lipopolysaccharide (LPS)-induced acute kidney injury (AKI) by upregulating the phosphatidylinositol 3-kinase (PI3K)-AKT-Nrf2 signalling pathway [31].

Polouliakh et al. indicated that α -ARB promoted wound healing by activating the insulin-like growth factor 1 receptor (IGF1R) pathway in human dermal fibroblasts [32], and alterations induced by ARB treatment should be explored. On the other hand, Nrf2-mediated redox regulation is not restricted to HO-1. Our previous research showed that enhancing Nrf2 improved the expression of catalase (CAT) and superoxide dismutase 1/2 (SOD1/2), which are responsible for abrogating the overproduction of ROS [33]. Cano et al. recently revealed that Nrf2 deficiency worsened the function of mitochondrial antioxidant enzymes, including isocitrate dehydrogenase 2 (IDH2) and peroxiredoxin 3 (PRDX3), which are essential for mitochondrial respiration [34]. Therefore, the specific impacts of ARB on mitochondrial functions need further exploration.

In vivo administration of anti-arthritic agents faces several limitations, such as rapid loss, nonspecific exposure, and off-target effects [35]. Generally, systemic injection of drugs, such as the intraperitoneal method, exerts broad-spectrum therapeutic effects on the whole body [36]; however, articular cartilage is free of blood and oxygen supply, and so systemic drug delivery shows little success [37]. To overcome this problem, we designed and synthesized an injectable microsphere drug delivery system (DDS), in which ARB was packaged in Liposomes and then integrated with GM microspheres. The ARB-modified microspheres showed long-term drug release and excellent biocompatibility. Notably, both *in vitro* and *in vivo* experiments revealed favourable repair functions. Bian et al. constructed a porous peptide-cell-hydrogel microsphere to reduce the inflammatory cytokine storms in intervertebral disc degeneration (IVDD) development [38]. Similarly, based on its strong ability to eliminate inflammatory cytokines *in vitro*, the GM-Lipo@ARB microspheres achieved powerful anti-inflammatory effects following intra-articular injection. Beyond that, the injury-inflammation-oxidative stress vicious cycle should be emphasized during the progression of OA [39]. Due to robust antioxidant properties, ARB-modified microspheres scavenge excessive ROS via the Nrf2-HO-1 signalling pathway. Li et al. also demonstrated that hollow manganese dioxide nanoparticles (hMNPs) promoted bone defect repair by decomposing H₂O₂ and generating oxygen to eliminate intracellular ROS [40]. Hence, we hypothesized that the dual regulation of inflammation and redox induced by GM-Lipo@ARB could boost cartilage regeneration in the microenvironment.

Another limitation of intra-articular injection-based OA treatment is the residual time and mode of action during long-term follow-up [41]. It is widely reported that regular injection, such as once or twice per week, of a drug is used to treat OA; however, repeated puncture is itself a form of injury. Based on this, we chose cationic Liposomes as carriers of ARB to achieve cartilage-targeting. Due to the negatively charged surface of articular cartilage [42], cationic Liposomes selectively accumulate on cartilage without diffusing into the synovial tissues. Furthermore, the positively charged Liposome penetrates deep into the cartilage matrix and controllably releases ARB to regulate the cartilaginous inflammation-redox balance [43]. Additionally, our *in vitro* assays indicated a superb cell-microsphere complex, in which cells proliferated stably and produced abundant levels of cartilage-related matrix components. Thus, we concluded that the injected microsphere complex not only releases ARB-laden Liposomes to attach to the cartilage surface but also recruits endogenous cells, especially stem cells, such as SMSCs [44] and

adipose mesenchymal stem cells (AMSCs) [45]. Coincidentally, Lei et al. fabricated a cell island microsphere to recruit endogenous stem cells and promote chondrogenic differentiation [46]. Given its strengthened association with cell survival and differentiation, future studies should focus on the niche effects [47] or cell factory [48] provided by microspheres.

4. Material And Methods

4.1. Preparation and characterization of Liposomes

Liposomes were prepared via a film dispersion method. In short, HPSC (Aladdin, China), cholesterol and octylamine (Aladdin, China) were dissolved in chloroform at a ratio of 40:10:2. The solution was completely dried by rotary evaporator at 60°C. The Liposomes then extruded through 0.45 µm and 0.22 µm membrane filters (Millex, Ireland). Finally, the size distribution, PDI and Zeta potential of Liposomes were measured by Malvin Zeta potential and nano particle size analyzer.

4.2. Fabrication and characterization of GM-Lipo@ARB

The mixture of GM 10 wt %, Liposome and photoinitiator was cut by 5 wt % Span 80 oil to form uniform droplets in microfluidic device, which were cross-linked by ultraviolet (UV) irradiation. The Liposomes were encapsulated in GM microspheres and the cross-linked microspheres were cleaned with 75% ethanol and stored in deionized water. The size and shape of the microspheres were observed under a light microscope. The structure of the freeze-dried microspheres was observed by scanning electron microscopy (SEM).

4.3. Encapsulation and drug release

The encapsulation rate of ARB was measured by ultraviolet spectrophotometer. Drug release was then measured. In simple terms, GM-Lipo@ARB was loaded into a dialysis bag (MWCO,1000 kDa) and immersed in a 37°C PBS solution at 60rpm for continuous stirring until complete drug release. The drug release at a specific time point was measured by UV spectrophotometer and PBS was replaced with the equivalent volume.

4.4. Degradation tests

A total of 100 mg GM-Lipo /GM/GM-Lipo@ARB lyophilized particles were soaked in 0.2 mg/mL collagenase solution. Microspheres were taken out every two days to measure the remaining solid weight and re-soaked into collagenase solution. Compare the remaining solid weight to the initial weight.

4.5. Cell biocompatibility

Mice chondrocytes from C57BL/6 mice will be used throughout the study. In order to test the biocompatibility of GM-Lipo@ARB, chondrocytes were inoculated on the surface of microspheres and the growth of the cells was observed under a light microscope at 1, 3 and 5 days. Cell proliferation was

detected by live/dead staining. Cells were incubated with calcein -AM/propidium iodide (Beyotime, China) for 30 min and examined using a fluorescence microscope.

4.6. Cell proliferation and transfection

Detailed information is provided in the Supplementary data.

4.7. Immunofluorescence staining

Col II, Aggrecan, MMP13, iNOS, p65, IκBα, Nrf2 and HO-1 protein levels were detected by immunofluorescence staining. In a nutshell, cells or tissues were immobilised with 4% paraformaldehyde for 15 minutes and treated with 2% bovine serum albumin for 1 hour to reduce background noise. Cells or tissues were labeled with antibody overnight at 4°C and then incubated with fluorescent secondary antibody (Servicebio, China) for 2 hours. Nuclear staining with DAPI (Servicebio, China) 15 min. The cytoskeleton was stained with phalloidin (Servicebio, China) 1 hour. The samples were viewed using Confocal Microscope.

4.8. RT-PCR

Detailed information is provided in the Supplementary data.

4.9. Enzyme-linked immunosorbent assay

Chondrocyte supernatants were measured by ELISA kit (Becton, Dickinson and Company) following the manufacturer's instructions.

4.10. Western blot assay

Detailed information is provided in the Supplementary data.

4.11. Mice model and treatment

C57BL/6J male mice (6–8 weeks) were purchased from Suzhou Healthytech Bio-pharmaceutical Co., Ltd. (Suzhou, China). All mice were maintained in a SPF (specific-pathogen-free) environment with free access to water and diets under the standard of 12 h-light and 12 h-dark cycle at $22 \pm 1^\circ\text{C}$. OA model of knee joint was established by surgical destruction of medial meniscus (DMM). mice were first anesthetized with a mixture of 2.0% isoflurane and 30% oxygen (RWD Life Sciences, Shenzhen, China). The medial meniscus ligament (MML) of OA group was cut with microscissors. In the sham operation group, only the knee capsule was exposed and the integrity of MML was not affected. One week after surgery, the mice were randomly divided into 5 groups. PBS, ARB, Lipo@ARB and GM-Lipo@ARB microspheres were injected into the joint cavity of the mice.

4.12. Histology and immunohistochemistry staining

Eight weeks after the operation, the mice were euthanized and the isolated knee joint was fixed with paraformaldehyde, decalcified and paraffin embedded. Sagittal sections with a thickness of 5 mm were

histologically analyzed by HE and Saffron O-fast green staining and grading the degree of cartilage degeneration according to the Osteoarthritis Research Society International (OARSI) scoring system. For immunohistochemical staining, sections were incubated with Col II /MMP13 antibody at 4°C overnight and then incubated with secondary antibody for 1 hour. Then, the tissue sections were stained with 3, 3'-diaminobenzidine (DAB) substrates. The relative expression levels of Col II and MMP13 were quantified by ImageJ software.

4.13. Statistical analysis

Each work was carried out at least three times. The statistical analysis was performed by a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test via SPSS 26 (SPSS Inc., USA). All Data are presented as mean $\pm$ SEM. $P < 0.05$ was considered statistically significant.

5. Conclusions

Our study fabricated an ARB-modified injectable microsphere known as GM-Lipo@ARB as a potential candidate for treating OA. *In vitro* treatment with ARB-loaded microspheres prevented OA-induced ECM disruption. Mechanistically, ARB exerted anti-inflammatory effects to alleviate the inflammatory cytokine storm mediated by the NF- κ B signalling pathway; moreover, ARB alleviated ROS-mediated oxidative stress injury by enhancing Nrf2 functions. *In vivo* administration of GM-Lipo@ARB prevented posttraumatic cartilage degeneration. Collectively, our proposed modified microsphere acts as a dual regulator that maintains local inflammation and redox equilibrium to counteract OA progression.

Declarations

Ethics approval and consent to participate

All animal experiments and studys were guidedand approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University.

Availability of data and materials

All datasets used and analyzed in this article can be obtained from the corresponding author on reasonable grounds.

Consent for publication

Not applicable.

Conflicts of interest

No potential conflicts of interest were identified.

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Authors' contributions

Yue Wang, Chi Zhang, Yijian Zhang designed the research; Jiale Jin, Yang Liu, and Chao Jiang conducted the experiments in vitro and in vivo; Guanrui Huang, Yifan Shen and Guangyu Chu prepared the figures. Liu Can, Lejian Jiang and Yifang Qin analyzed the data; Jiale Jin and Chi Zhang wrote the paper. All authors contributed to the article and approved the submitted version.

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References

1. Long H, Liu Q, Yin H, Wang K, Diao N, Zhang Y, Lin J and Guo A. Prevalence trends of site-specific osteoarthritis from 1990 to 2019: findings from the Global Burden of Disease Study 2019. *Arthritis Rheumatol.* 2022.
2. Angelini F, Widera P, Mobasheri A, Blair J, Struglics A, Uebelhoer M, Henrotin Y, Marijnissen AC, Kloppenburg M, Blanco FJ, Haugen IK, Berenbaum F, Ladel C, et al. Osteoarthritis endotype discovery via clustering of biochemical marker data. *Ann Rheum Dis.* 2022.
3. Zhang Y, Zhu T, He F, Chen AC, Yang H and Zhu X. Identification of Key Genes and Pathways in Osteoarthritis via Bioinformatic Tools: An Updated Analysis. *Cartilage.* 2021; 13(1_suppl):1457s-1464s.
4. Cherian JJ, Jauregui JJ, Leichliter AK, Elmallah RK, Bhave A and Mont MA. The effects of various physical non-operative modalities on the pain in osteoarthritis of the knee. *Bone Joint J.* 2016; 98-b(1 Suppl A):89-94.
5. Schattner A. Review: Some NSAIDs, notably diclofenac, improved knee or hip pain and function in osteoarthritis vs other NSAIDs. *Ann Intern Med.* 2016; 165(2):Jc9.
6. Lv L, Zhang J, Tian F, Li X, Li D and Yu X. Arbutin protects HK-2 cells against high glucose-induced apoptosis and autophagy by up-regulating microRNA-27a. *Artif Cells Nanomed Biotechnol.* 2019; 47(1):2940-2947.
7. Saeedi M, Khezri K, SeyedZakaryaei A and Mohammadamini H. A comprehensive review of the therapeutic potential of α -arbutin. *Phytother Res.* 2021; 35(8):4136-4154.
8. Kumar M, Kumar A, Sindhu RK and Kushwah AS. Arbutin attenuates monosodium L-glutamate induced neurotoxicity and cognitive dysfunction in rats. *Neurochem Int.* 2021; 151:105217.
9. Jiang L, Wang D, Zhang Y, Li J, Wu Z, Wang Z and Wang D. Investigation of the pro-apoptotic effects of arbutin and its acetylated derivative on murine melanoma cells. *Int J Mol Med.* 2018; 41(2):1048-

1054.

10. Wang R and Mu J. Arbutin attenuates ethanol-induced acute hepatic injury by the modulation of oxidative stress and Nrf-2/HO-1 signaling pathway. *Journal of biochemical and molecular toxicology*. 2021; 35(10):e22872.
11. Zhao J, Kumar M, Sharma J and Yuan Z. Arbutin effectively ameliorates the symptoms of Parkinson's disease: the role of adenosine receptors and cyclic adenosine monophosphate. *Neural regeneration research*. 2021; 16(10):2030-2040.
12. Kong B, Chen Y, Liu R, Liu X, Liu C, Shao Z, Xiong L, Liu X, Sun W and Mi S. Fiber reinforced GM hydrogel to induce the regeneration of corneal stroma. *Nature communications*. 2020; 11(1):1435.
13. Yuan Z, Yuan X, Zhao Y, Cai Q, Wang Y, Luo R, Yu S, Wang Y, Han J, Ge L, Huang J and Xiong C. Injectable GMCryogel Microspheres for Modularized Cell Delivery and Potential Vascularized Bone Regeneration. *Small*. 2021; 17(11):e2006596.
14. Chen CH, Kuo SM, Tien YC, Shen PC, Kuo YW and Huang HH. Steady Augmentation of Anti-Osteoarthritic Actions of Rapamycin by Liposome-Encapsulation in Collaboration with Low-Intensity Pulsed Ultrasound. *International journal of nanomedicine*. 2020; 15:3771-3790.
15. Kim YB, Zhao KT, Thompson DB and Liu DR. An anionic human protein mediates cationic Liposome delivery of genome editing proteins into mammalian cells. *Nature communications*. 2019; 10(1):2905.
16. Mehrotra S, Singh RD, Bandyopadhyay A, Janani G, Dey S and Mandal BB. Engineering Microsphere-Loaded Non-mulberry Silk-Based 3D Bioprinted Vascularized Cardiac Patches with Oxygen-Releasing and Immunomodulatory Potential. *ACS Appl Mater Interfaces*. 2021; 13(43):50744-50759.
17. Zhu J, Ruan G, Cen H, Meng T, Zheng S, Wang Y, Li B, Zhu Z, Han W, Winzenberg T, Wluka AE, Cicuttini F, Wang B, et al. Association of serum levels of inflammatory markers and adipokines with joint symptoms and structures in participants with knee osteoarthritis. *Rheumatology (Oxford)*. 2022; 61(3):1044-1052.
18. Zhang Y, Lin J, Zhou X, Chen X, Chen AC, Pi B, Pan G, Pei M, Yang H, Liu T and He F. Melatonin Prevents Osteoarthritis-Induced Cartilage Degradation via Targeting MicroRNA-140. *Oxidative medicine and cellular longevity*. 2019; 2019:9705929.
19. da Costa BR, Pereira TV, Saadat P, Rudnicki M, Iskander SM, Bodmer NS, Bobos P, Gao L, Kiyomoto HD, Montezuma T, Almeida MO, Cheng PS, Hincapié CA, et al. Effectiveness and safety of non-steroidal anti-inflammatory drugs and opioid treatment for knee and hip osteoarthritis: network meta-analysis. *Bmj*. 2021; 375:n2321.
20. Ahmadian SR, Ghasemi-Kasman M, Pouramir M and Sadeghi F. Arbutin attenuates cognitive impairment and inflammatory response in pentylenetetrazol-induced kindling model of epilepsy. *Neuropharmacology*. 2019; 146:117-127.
21. Ye J, Guan M, Lu Y, Zhang D, Li C and Zhou C. Arbutin attenuates LPS-induced lung injury via Sirt1/Nrf2/ NF- κ Bp65 pathway. *PulmPharmacolTher*. 2019; 54:53-59.

22. Nalban N, Sangaraju R, Alavala S, Mir SM, Jerald MK and Sistla R. Arbutin Attenuates Isoproterenol-Induced Cardiac Hypertrophy by Inhibiting TLR-4/NF- κ B Pathway in Mice. *Cardiovasc Toxicol.* 2020; 20(3):235-248.
23. Ma C, Zhang D, Ma Q, Liu Y and Yang Y. Arbutin inhibits inflammation and apoptosis by enhancing autophagy via SIRT1. *Adv Clin Exp Med.* 2021; 30(5):535-544.
24. Yan J, Chen X, Pu C, Zhao Y, Liu X, Liu T, Pan G, Lin J, Pei M, Yang H and He F. Synovium stem cell-derived matrix enhances anti-inflammatory properties of rabbit articular chondrocytes via the SIRT1 pathway. *Materials science & engineering C, Materials for biological applications.* 2020; 106:110286.
25. Yang Y, Shen P, Yao T, Ma J, Chen Z, Zhu J, Gong Z, Shen S and Fang X. Novel role of circRSU1 in the progression of osteoarthritis by adjusting oxidative stress. *Theranostics.* 2021; 11(4):1877-1900.
26. Coryell PR, Diekman BO and Loeser RF. Mechanisms and therapeutic implications of cellular senescence in osteoarthritis. *Nat Rev Rheumatol.* 2021; 17(1):47-57.
27. Hou M, Zhang Y, Zhou X, Liu T, Yang H, Chen X, He F and Zhu X. Kartogenin prevents cartilage degradation and alleviates osteoarthritis progression in mice via the miR-146a/NRF2 axis. *Cell Death Dis.* 2021; 12(5):483.
28. Mao M, Yang L, Hu J, Liu B, Zhang X, Liu Y, Wang P and Li H. Oncogenic E3 ubiquitin ligase NEDD4 binds to KLF8 and regulates the microRNA-132/NRF2 axis in bladder cancer. *Exp Mol Med.* 2022; 54(1):47-60.
29. Wang L, Bayanbold K, Zhao L, Wang Y, Adamcakova-Dodd A, Thorne PS, Yang H, Jiang BH and Liu LZ. Redox sensitive miR-27a/b/Nrf2 signaling in Cr(VI)-induced carcinogenesis. *Sci Total Environ.* 2022; 809:151118.
30. Chen J, Liang JQ, Zhen YF, Chang L, Zhou ZT and Shen XJ. DCAF1-targeting microRNA-3175 activates Nrf2 signaling and inhibits dexamethasone-induced oxidative injury in human osteoblasts. *Cell Death Dis.* 2021; 12(11):1024.
31. Zhang B, Zeng M, Li B, Kan Y, Wang S, Cao B, Huang Y, Zheng X and Feng W. Arbutin attenuates LPS-induced acute kidney injury by inhibiting inflammation and apoptosis via the PI3K/Akt/Nrf2 pathway. *Phytomedicine.* 2021; 82:153466.
32. Polouliakh N, Ludwig V, Meguro A, Kawagoe T, Heeb O and Mizuki N. Alpha-Arbutin Promotes Wound Healing by Lowering ROS and Upregulating Insulin/IGF-1 Pathway in Human Dermal Fibroblast. *Frontiers in physiology.* 2020; 11:586843.
33. Zhou X, Zhang Y, Hou M, Liu H, Yang H, Chen X, Liu T, He F and Zhu X. Melatonin Prevents Cartilage Degradation in Early-Stage Osteoarthritis Through Activation of miR-146a/NRF2/HO-1 Axis. *J Bone Miner Res.* 2022.
34. Cano M, Datta S, Wang L, Liu T, Flores-Bellver M, Sachdeva M, Sinha D and Handa JT. Nrf2 deficiency decreases NADPH from impaired IDH shuttle and pentose phosphate pathway in retinal pigmented epithelial cells to magnify oxidative stress-induced mitochondrial dysfunction. *Aging cell.* 2021; 20(8):e13444.

35. Zhang M, Hu W, Cai C, Wu Y, Li J and Dong S. Advanced application of stimuli-responsive drug delivery system for inflammatory arthritis treatment. *Mater Today Bio.* 2022; 14:100223.
36. Burton LH, Afzali MF, Radakovich LB, Campbell MA, Culver LA, Olver CS and Santangelo KS. Systemic administration of a pharmacologic iron chelator reduces cartilage lesion development in the Dunkin-Hartley model of primary osteoarthritis. *Free radical biology & medicine.* 2022; 179:47-58.
37. Brucker PU, Izzo NJ and Chu CR. Tonic activation of hypoxia-inducible factor 1alpha in avascular articular cartilage and implications for metabolic homeostasis. *Arthritis and rheumatism.* 2005; 52(10):3181-3191.
38. Bian J, Cai F, Chen H, Tang Z, Xi K, Tang J, Wu L, Xu Y, Deng L, Gu Y, Cui W and Chen L. Modulation of Local Overactive Inflammation via Injectable Hydrogel Microspheres. *Nano Lett.* 2021; 21(6):2690-2698.
39. Chen D, Lu D, Liu H, Xue E, Zhang Y, Shang P and Pan X. Pharmacological blockade of PCAF ameliorates osteoarthritis development via dual inhibition of TNF- α -driven inflammation and ER stress. *EBioMedicine.* 2019; 50:395-407.
40. Li J, Han F, Ma J, Wang H, Pan J, Yang G, Zhao H, Zhao J, Liu J, Liu Z and Li B. Targeting Endogenous Hydrogen Peroxide at Bone Defects Promotes Bone Repair. *Advanced Functional Materials.* 2022; 32(10):2111208.
41. Lu Y, Chen J, Li L, Cao Y, Zhao Y, Nie X and Ding C. Hierarchical functional nanoparticles boost osteoarthritis therapy by utilizing joint-resident mesenchymal stem cells. *J Nanobiotechnology.* 2022; 20(1):89.
42. Evans CH. Drug delivery to chondrocytes. *Osteoarthritis and cartilage.* 2016; 24(1):1-3.
43. Lin F, Wang Z, Xiang L, Deng L and Cui W. Charge-Guided Micro/Nano-Hydrogel Microsphere for Penetrating Cartilage Matrix. *Advanced Functional Materials.* 2021; 31(49):2107678.
44. Wang K, Li F, Yuan Y, Shan L, Cui Y, Qu J and Lian F. Synovial Mesenchymal Stem Cell-Derived EV-Packaged miR-31 Downregulates Histone Demethylase KDM2A to Prevent Knee Osteoarthritis. *Molecular therapy Nucleic acids.* 2020; 22:1078-1091.
45. Wu H, Peng Z, Xu Y, Sheng Z, Liu Y, Liao Y, Wang Y, Wen Y, Yi J, Xie C, Chen X, Hu J, Yan B, et al. Engineered adipose-derived stem cells with IGF-1-modified mRNA ameliorates osteoarthritis development. *Stem cell research & therapy.* 2022; 13(1):19.
46. Lei Y, Wang Y, Shen J, Cai Z, Zeng Y, Zhao P, Liao J, Lian C, Hu N, Luo X, Cui W and Huang W. Stem Cell-Recruiting Injectable Microgels for Repairing Osteoarthritis. *Advanced Functional Materials.* 2021; 31(48):2105084.
47. Matta C, Khademhosseini A and Mobasher A. Mesenchymal Stem Cells and their Potential for Microengineering the Chondrocyte Niche. *EBioMedicine.* 2015; 2(11):1560-1561.
48. Orsi E, Claassens NJ, Nikel PI and Lindner SN. Growth-coupled selection of synthetic modules to accelerate cell factory development. *Nature communications.* 2021; 12(1):5295.

Figures

Figure 1

Composite hydrogel (GM-Lipo@ARB) for the treatment of osteoarthritis.

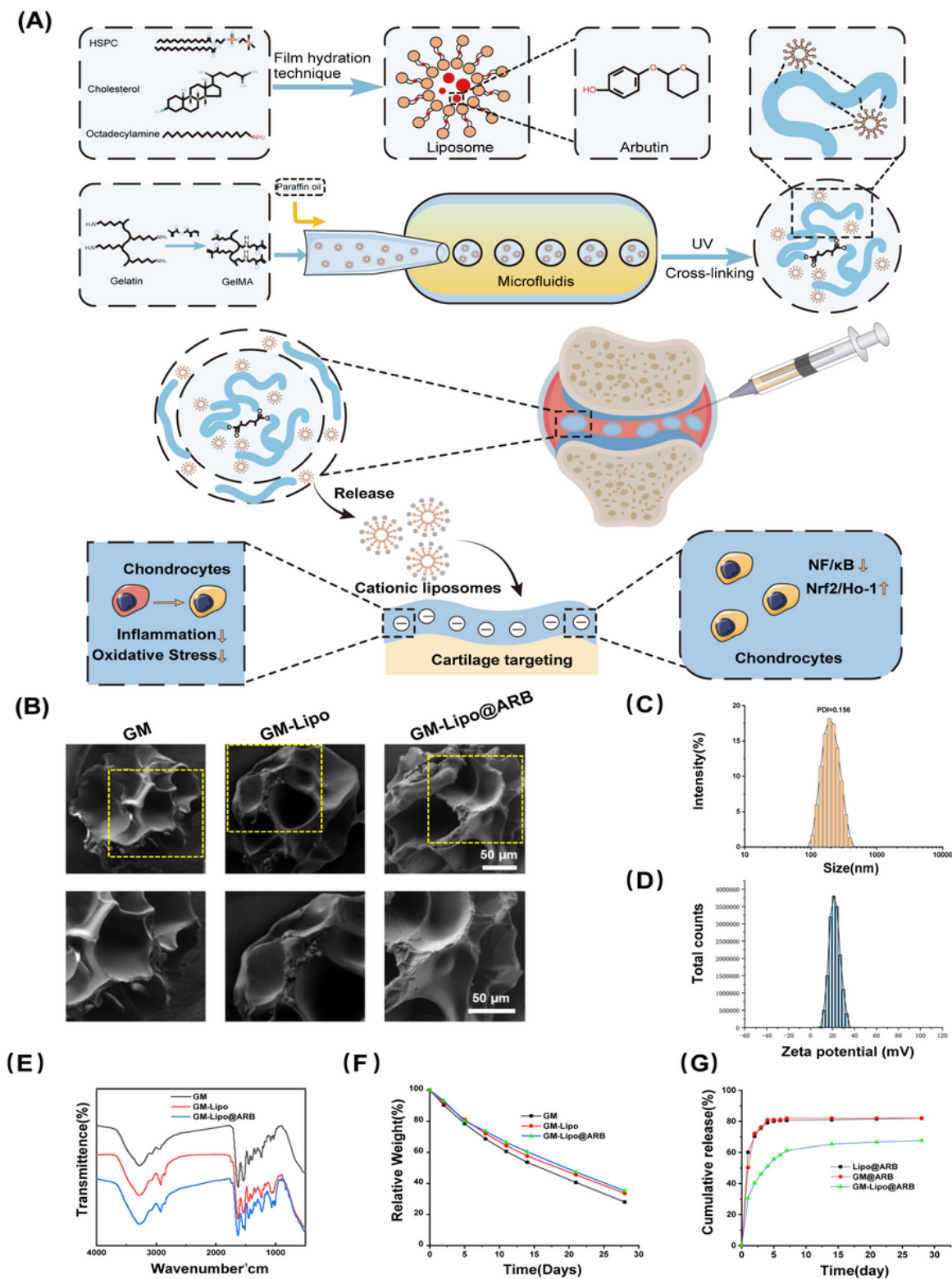


Figure 2

(A) The principle and fabrication of GM-Lipo@ARB. (B) SEM images of GM, GM+Lipo and GM-Lipo@ARB from the overall view and the local view. (C) Size distribution of liposomes. (D) Zeta potential of liposomes. (E) FTIR of GM, GM+Lipo and GM-Lipo@ARB. (F) The degradation curve of GM, GM+Lipo and GM-Lipo@ARB. (G) Release curves of ARB releasing from GM, GM+Lipo and GM-Lipo@ARB.

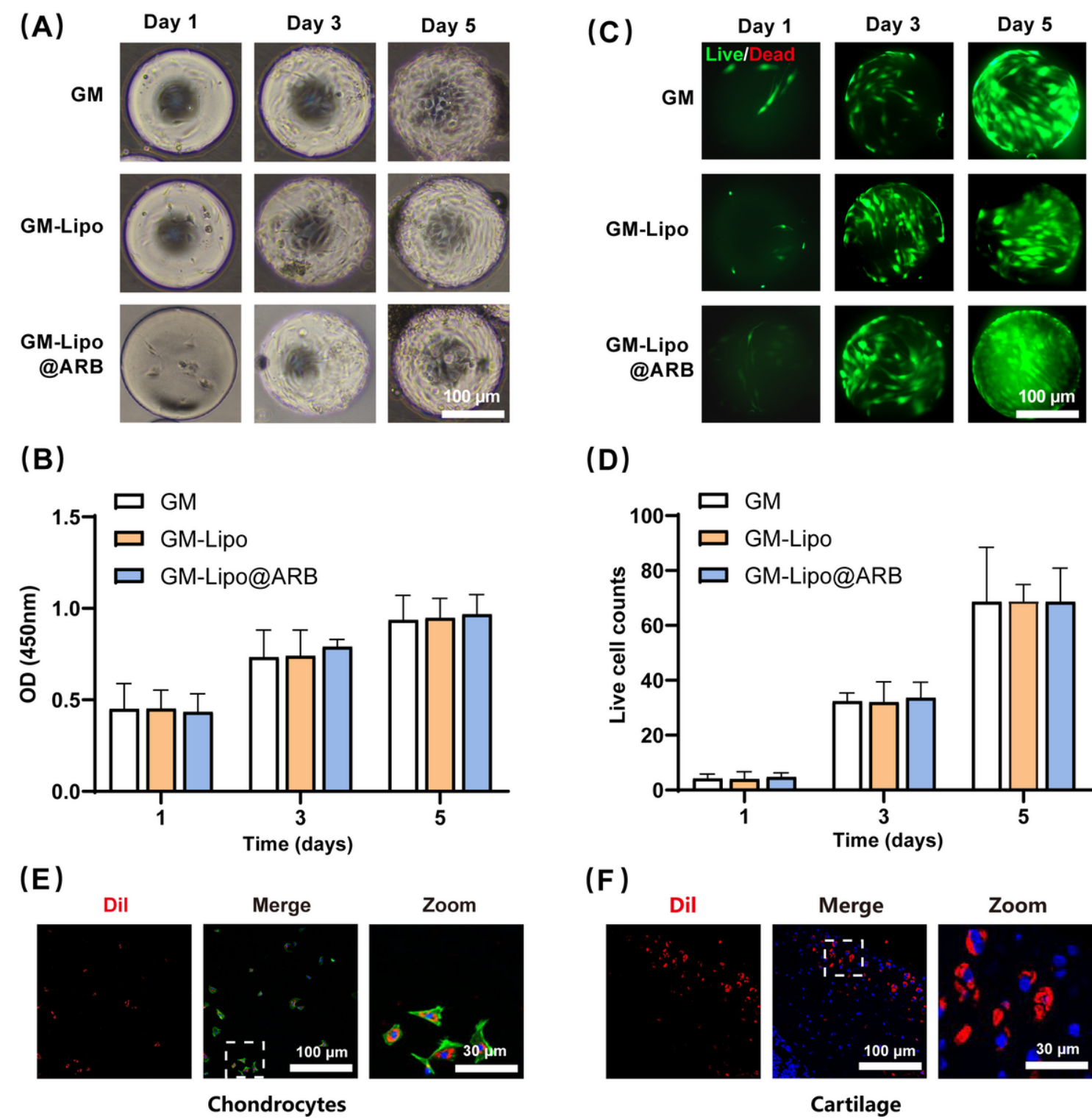


Figure 3

(A) Microscopy images of cells on micro spheres after 1, 3, and 5 days of incubation. (B) The CCK-8 results on 1, 3, and 5 days. OD, optical density. (C) Live/dead staining of cells on micro spheres after 1, 3, and 5 days of incubation. (D) Viable cell counts from the Live/Dead staining assay. (E) Immuno fluorescence of the chondrocytes incubated with Dil-labelled cationic liposomes. (F) Immuno fluorescence of the cartilage section incubated with Dil-labelled cationic liposomes.

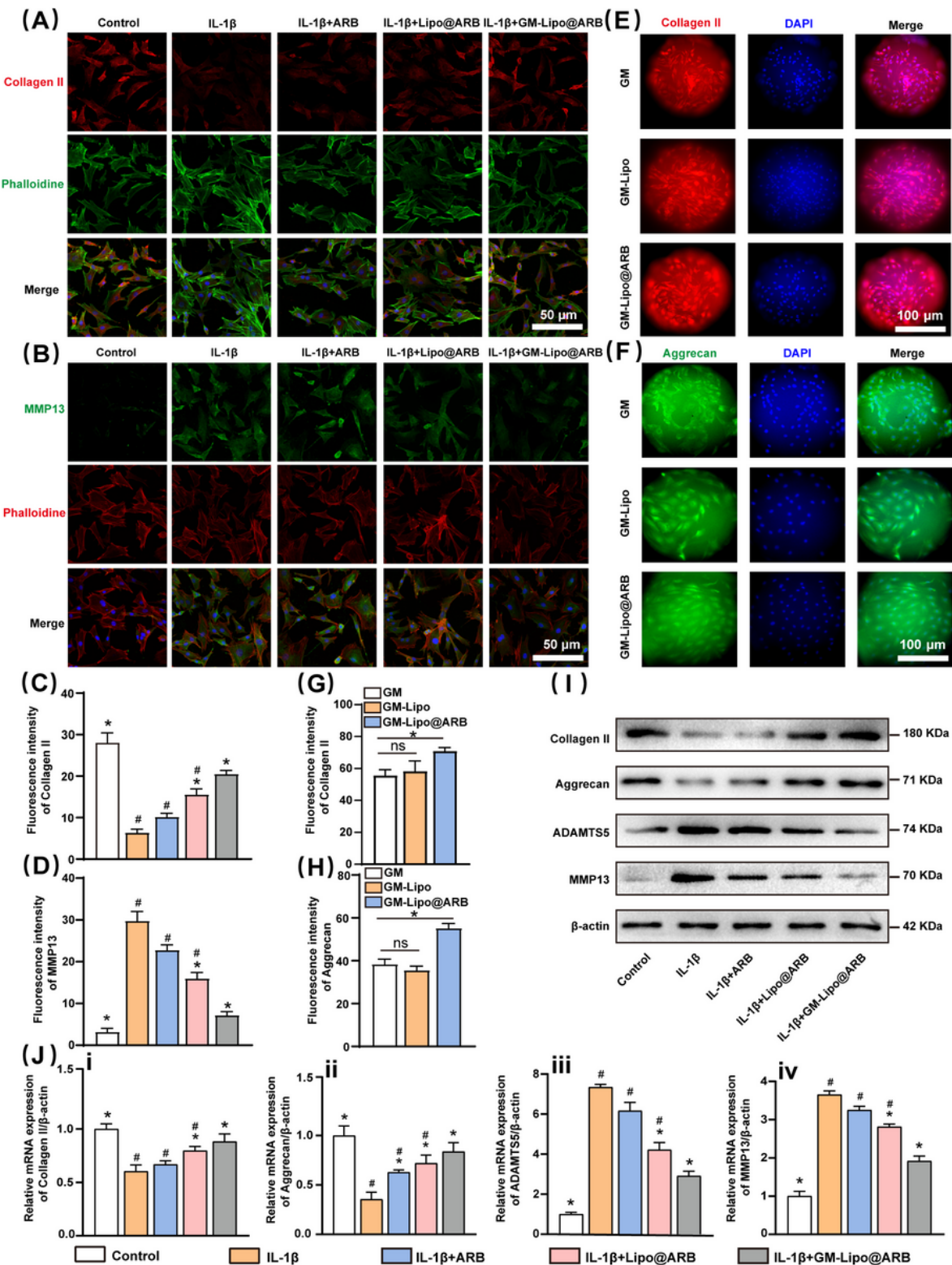


Figure 4

Figure 5

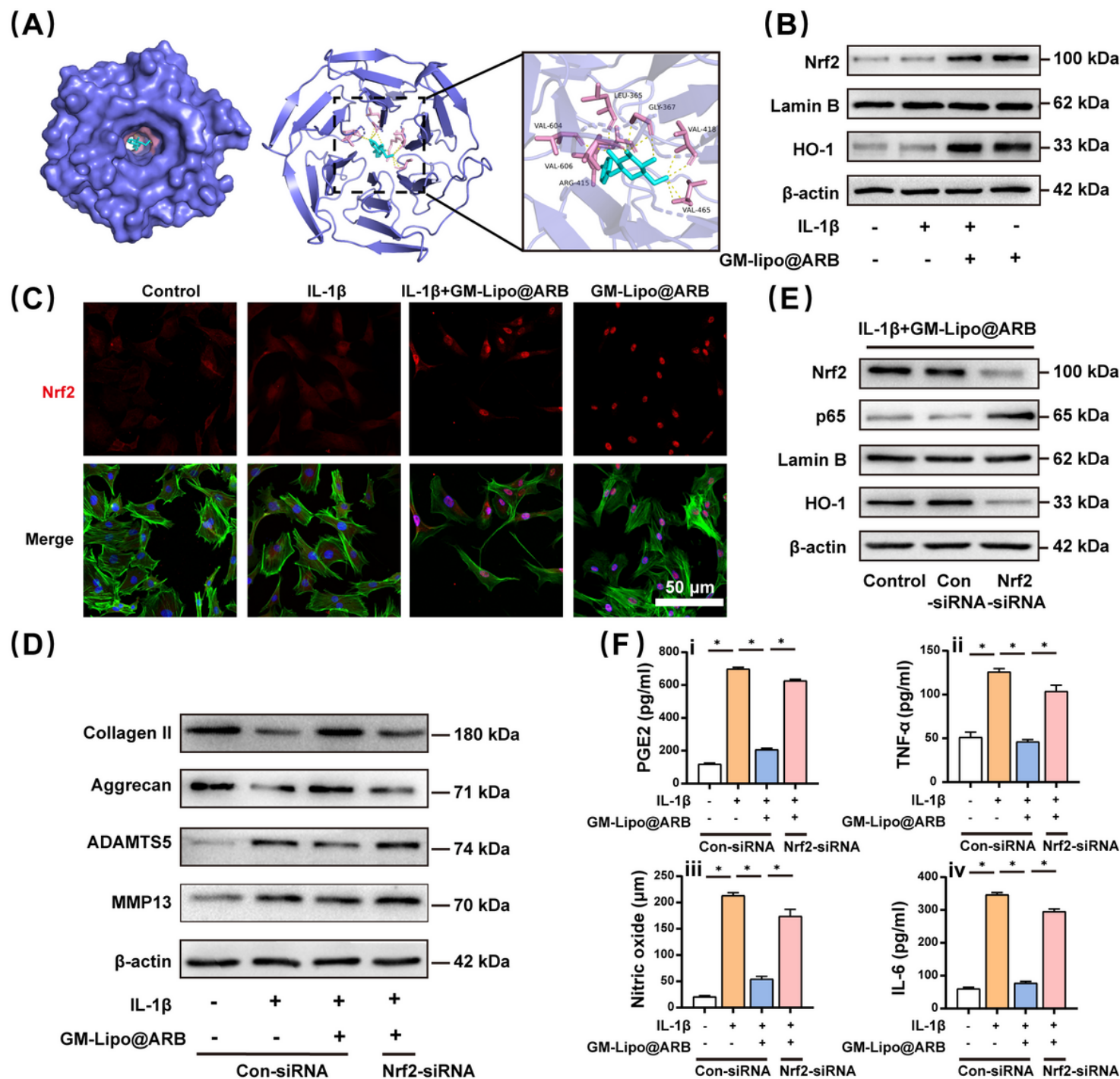


Figure 6

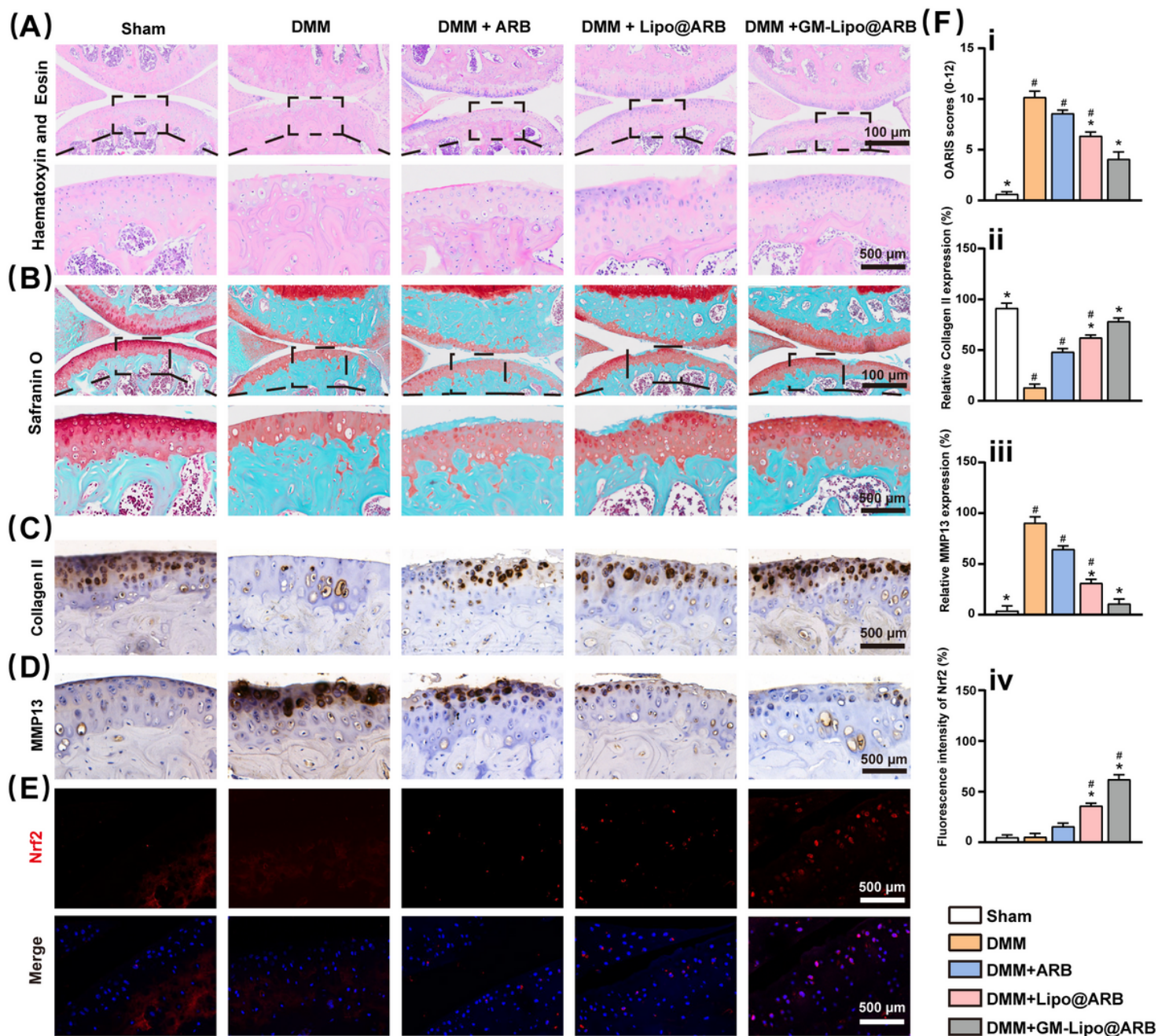


Figure 7

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