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Crocin inhibits titanium particle-induced inflammation and promotes osteogenesis by regulating macrophage polarization

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ABSTRACT

Wear particle-induced periprosthetic inflammatory osteolysis and resultant aseptic loosening are major causes of orthopedic implant failure, for which there are no effective treatments other than revision surgery. Crocin, a carotenoid compound derived from crocus flowers, has anti-inflammatory properties, but its immunomodulatory function and role in particle-induced osteolysis are not well characterized. Here we report the effect of crocin on titanium (Ti) particle-induced macrophage polarization and osteogenic differentiation. We found that crocin induced anti-inflammatory (M2) macrophage polarization and attenuated Ti particle-induced inflammation by promoting the expression of anti-inflammatory cytokines *in vitro* as well as *in vivo* in a mouse air-pouch model. Additionally, crocin pre-treated macrophages promoted osteogenic differentiation of co-cultured mouse bone mesenchymal stem cells (BMSCs). These effects were mediated *via* inhibition of p38 and c-Jun N-terminal kinase signaling. Our results indicate that crocin suppresses Ti particle-induced inflammation and enhances osteogenic differentiation of BMSCs by inducing M2 macrophage polarization, highlighting its therapeutic potential for preventing wear particle-induced osteolysis.

1. Introduction

Total joint arthroplasty (TJA) is a highly beneficial treatment for end-stage joint diseases such as osteoarthritis and femoral head necrosis [1,2]. Excessive inflammation and enhanced bone resorption caused by periprosthetic particle-induced osteolysis (PIO) is a major cause of aseptic loosening, which is the most common reason for revisions in TJA [3,4]. Although the mechanism underlying PIO remains unclear, it is generally thought that increased osteoclastogenesis resulting from wear particle-induced inflammation plays a role. The initial host response to foreign bodies is mediated by the innate immune system in which macrophages are main effector cells [5,6]. Macrophages secrete immunomodulatory factors including cytokines and chemokines by adopting various phenotypes upon contact with wear particles: M1 macrophages are considered the pro-inflammatory subtype that stimulates the expression of pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α, while M2 macrophages exhibit anti-inflammatory activity and promote wound healing [7–10].

Although osteoclast formation is more relevant than osteogenesis to the dysregulation of bone homeostasis caused by osteolysis, bone regeneration through osteogenesis and the influence of osteoimmunomodulation are also important [11,12]. M2 macrophages contribute to osseointegration and angiogenesis by secreting cytokines such as vascular endothelial growth factor (VEGF) and bone morphogenetic protein (BMP)-2 [13,14]. Thus, regulating macrophage polarization is a potentially effective strategy for alleviating wear particle-induced osteolysis and maintaining bone homeostasis through enhanced bone formation.

Crocin is a chemical compound found in *Crocus sativus* L (saffron) that has traditionally been used as a colorant and seasoning in food but has demonstrated antioxidant and anti-inflammatory properties [15–17]. Crocin was shown to attenuate PIO by inhibiting receptor activator of nuclear factor κB ligand-induced osteoclast formation and bone resorption [18,19]. The recent report of an unexpected role for crocin in macrophage phenotype determination has provided novel insight into its function: crocin was found to alleviate coronary atherosclerosis by inducing M2 macrophage polarization [20]. However, it is unclear whether the immunomodulatory properties of crocin apply to wear particle-induced inflammation and bone dynamics.

We speculated that crocin attenuates titanium (Ti) particle-induced osteolysis by modulating macrophage polarization and enhancing osteogenic differentiation, and tested this hypothesis by *in vitro* and *in vivo* experiments. We found that crocin alleviated Ti particle-induced inflammation and stimulated osteogenesis by inducing M2 macrophage

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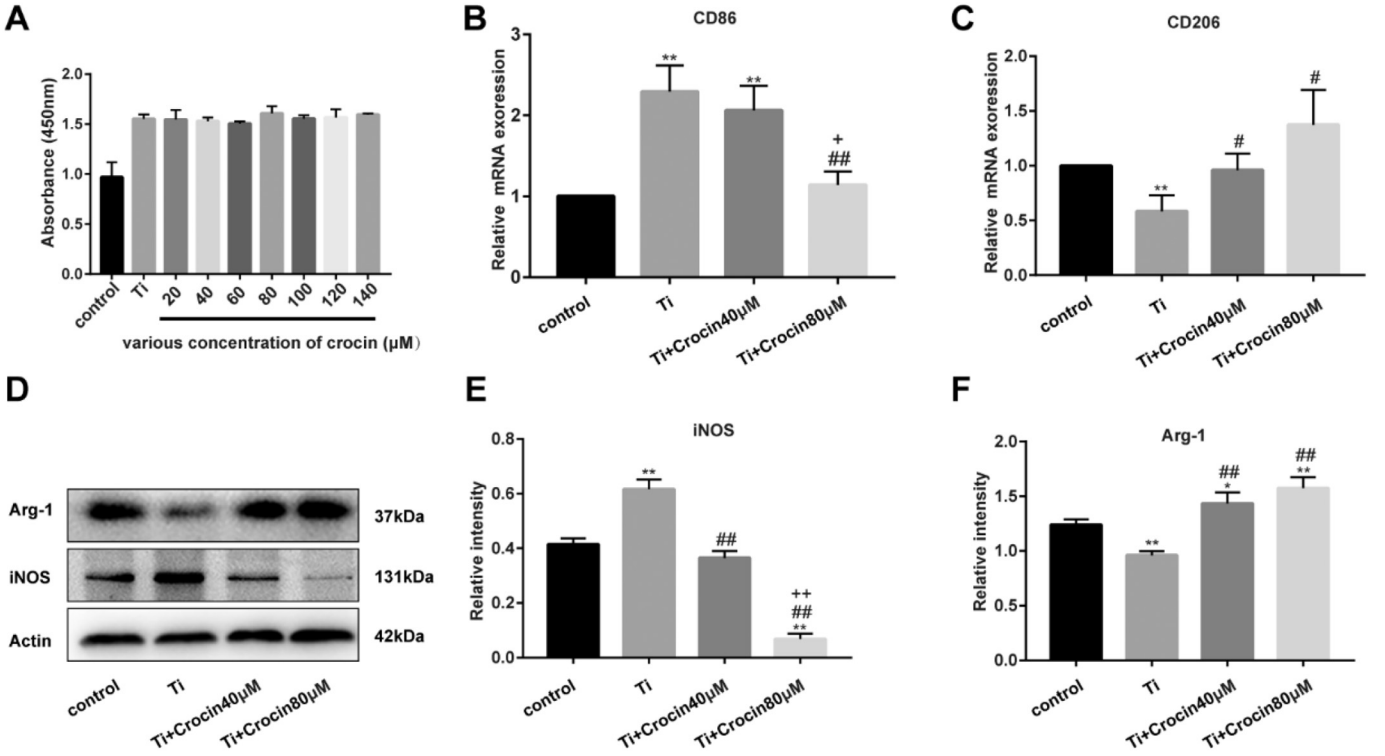

Fig. 1. Crocin enhances the mRNA and protein levels of macrophage polarization markers *in vitro* without cytotoxicity. (A) Cell viability was evaluated with the CCK-8 assay after 24 h of culture. (B, C) CD86 and CD206 mRNA levels were assessed by RT-PCR. GAPDH was used as an internal control. (D) Protein levels of iNOS and Arg-1 were measured by western blotting. (E, F) Quantitative analysis of signal intensity of protein bands shown in panel D. $^{*,\#,+}P < 0.05$, $^{**,\#\#,++}P < 0.01$ *vs.* Control, Ti, and Ti + Crocin40 μM, respectively.

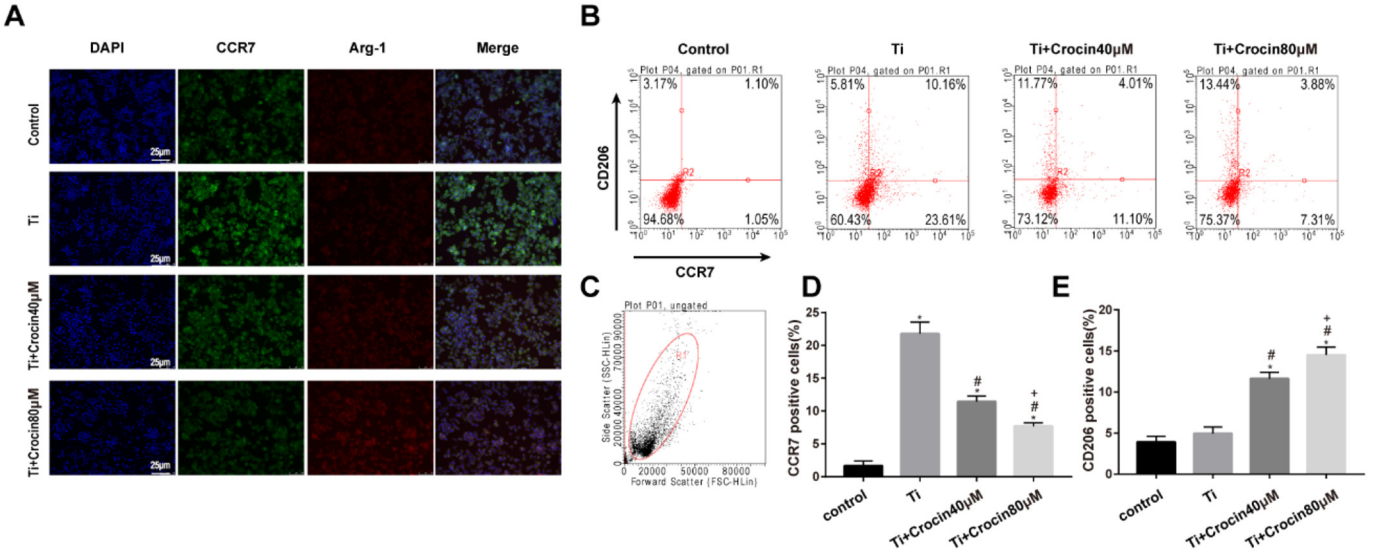

Fig. 2. Crocin modulates macrophage polarization *in vitro*. (A) Immunofluorescence labeling of RAW cells; M1 and M2 macrophages were positive for CCR7 (green) and Arg-1 (red), respectively. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; blue). (B) Flow cytometry analysis of cell surface makers on macrophages. (C) Representative gates of forward and side scatter. (D, E) Representative histograms of flow cytometry results shown in panel B. $^{*,\#,+}P < 0.05$, $^{**,\#\#,++}P < 0.01$ *vs.* Control, Ti, and Ti + Crocin40 μM, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

polarization *via* inhibition of p38 and c-Jun N-terminal kinase (JNK) signaling.

2. Material and methods

2.1. Particle preparation

Pure Ti particles were purchased from Johnson Matthey Pharma Services (Devens, MA, USA). Endotoxins were removed from the particles by baking at 180 °C for 6 h and then washing in 70% ethanol for

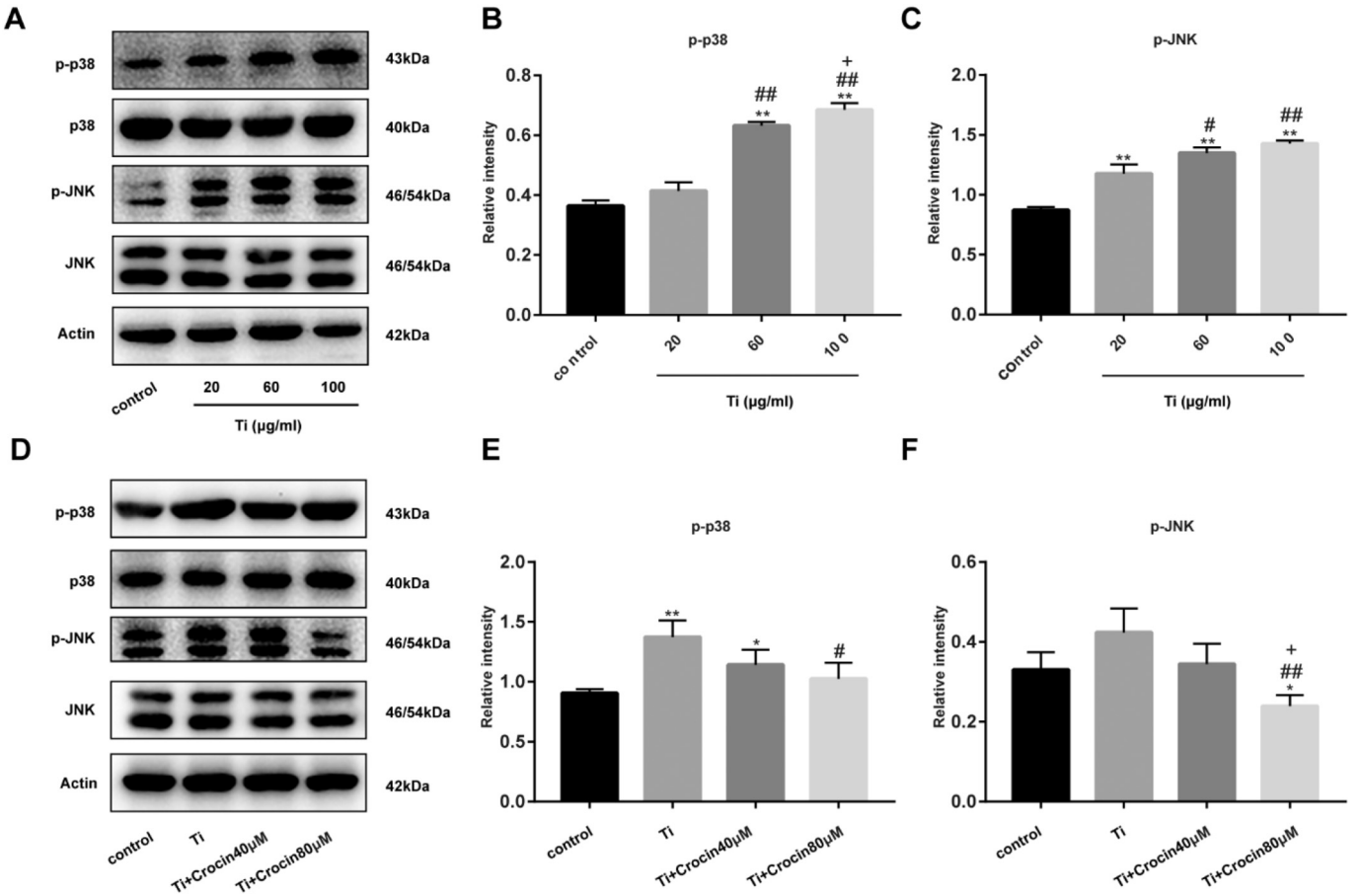

Fig. 3. Crocin induces M2 macrophage polarization by inhibiting p38 and JNK phosphorylation. (A) Western blot analysis of macrophages stimulated with different concentrations (0, 20, 60, and 100 μg/ml) of Ti particles for indicated times. (B, C) Quantification of signal intensity of protein bands shown in panel A. (D) Western blot analysis of RAW cells pretreated with crocin followed by Ti particles. (E, F) Quantification of signal intensity of protein bands shown in panel D. $^{*,\#,+}P < 0.05$, $^{**,\#\#,++}P < 0.01$ *vs.* Control, Ti, and Ti + Crocin40 μM, respectively.

48 h at room temperature [21]. The Limulus Amoebocyte Lysate assay (Biowhittaker, Walkersville, MD, USA) was used to confirm that the particles were endotoxin-free.

2.2. RAW 264.7 cell culture

Murine macrophage RAW 264.7 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, UT, USA) with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin/streptomycin (Hyclone) in a humidified atmosphere of 5% CO_2 at 37 °C. The cells were seeded in cell culture plates with or without Ti particles (0.1 mg/ml) or stimulated with a combination of Ti particles and various concentrations of crocin (0, 40, or 80 μM). The medium was changed every 2 days.

2.3. Cell viability assay

The Cell Counting Kit (CCK)-8 assay (Dojindo Laboratories, Kumamoto, Japan) was used to evaluate the viability of RAW264.7 cells. Cells were seeded in 96-well plates at a density of 1.0×10^5 cells/well. After 24 h of culture, the cells were incubated for 2 h at 37 °C with fresh medium and CCK-8 reagent at a 10:1 (*v*/v) ratio, and the absorbance at 450 nm was assessed on a microplate reader.

2.4. Immunofluorescence analysis

The expression of the M1 marker C—C chemokine receptor type (CCR)7 and the M2 marker arginase (Arg)-1 was detected by immunofluorescence analysis. After 24 h of culture, RAW264.7 cells were fixed in 4% paraformaldehyde for 30 min, washed three times with phosphate-buffered saline (PBS), and blocked with 1% bovine serum albumin (BSA) for 30 min. They were then incubated overnight at 4 °C with primary antibodies against CCR7 and Arg-1 (both at 1:100 and from Abcam, Cambridge, MA, USA), followed by Alexa Fluor 594-conjugated donkey anti-mouse and Alexa Fluor 488-conjugated goat anti-rabbit IgG (both at 1:200 and from Abcam) for 1 h in the dark. Cell nuclei were stained with 4′,6-diamidino-2-phenylindole for 5 min, and images were acquired with a fluorescence microscope (Leica, Wetzlar, Germany).

2.5. Flow cytometry

RAW264.7 cells expressing CCR7 and the M2 marker cluster of differentiation (CD)206 were detected by flow cytometry. After 24 h of culture, the cells were collected, resuspended in 1% BSA for 30 min, and labeled with phycoerythrin (PE)-conjugated anti-mouse CD206 and allophycocyanin (APC)-conjugated anti-mouse CCR7 antibodies (both from eBioscience, San Diego, CA, USA) for 1 h in the dark. APC-conjugated IgG2a,κ and PE-conjugated IgG2a,κ were used as isotype controls. Cells were sorted on a Guava flow cytometer and analyzed with the accompanying software (Millipore, Billerica, MA, USA).

2.6. Real-time polymerase chain reaction (RT-PCR)

The expression of CD86 (M1 marker), CD206, BMP-2, and VEGF

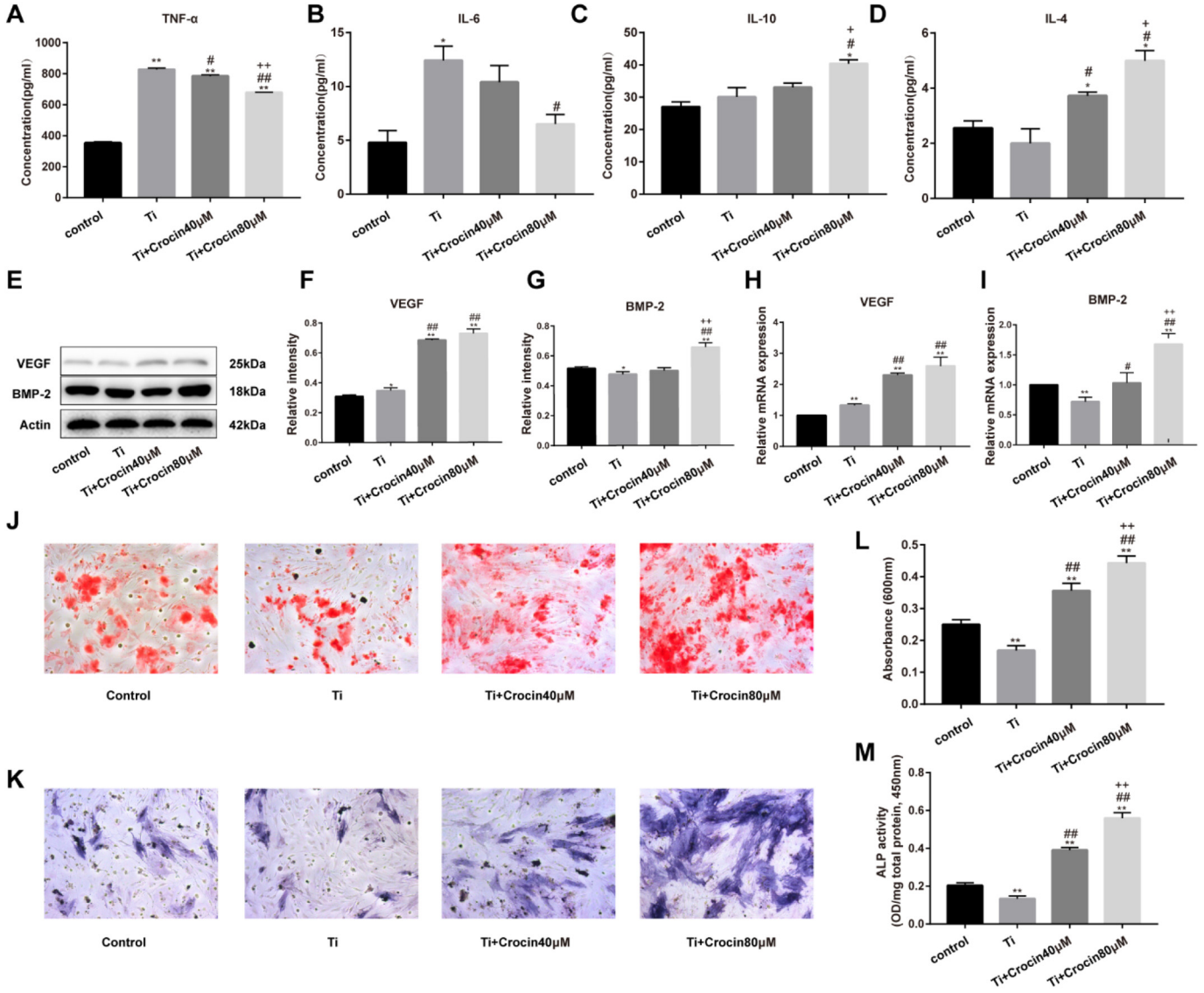

Fig. 4. Crocin creates an immunomodulatory microenvironment that promotes osteogenic differentiation. (A–D) Production TNF-α (A), IL-6 (B), IL-10 (C), and IL-4 (D) by RAW264.7 cells, as detected by ELISA. (E) Western blot analysis of osteogenic protein expression. (F, G) Quantification of signal intensity of protein bands shown in panel E. (H, I) RT-PCR detection of osteogenic gene expression. (J, K) ARS and ALP staining of BMSCs. (L) Quantitative analysis of ARS. (M) ALP activity in BMSCs. $^{*,\#,+}P < 0.05$, $^{**,\#\#,++}P < 0.01$ *vs.* Control, Ti, and Ti + Crocin40 μM, respectively.

were evaluated by RT-PCR. After 24 h of culture, RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and 500 ng were used to synthesize cDNA with the PrimeScript RT Reagent Kit (Takara Bio, Otsu, Japan). RT-PCR was performed using SYBR Premix Ex TaqII (Takara Bio) on an ABI 7900HT instrument (Applied Biosystems, Foster City, CA, USA). Expression levels of target genes were quantified with the $2^{-\Delta\Delta Ct}$ method and normalized to the mean cycle threshold value of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The following forward and reverse primers were used: CD86, 5′-TGCTCATCATTGTATGTCAC-3′ and 5′-GTCTCTCTGTCAGCGTTACT-3′; CD206, 5′-TACTTGGACGGATAGAT GGAGG-3′ and 5′-CATAGAAAGGAATCCACGCAGT-3′; BMP-2, 5′-AAC GAGAAAAGCGTCAAGCC-3′ and 5′-AGGTGCCACGATCCAGTCAT-3′; VEGF, 5′-AGGAGTACCCCGACGAGATAGA-3′ and 5′-CACATCTGCTGT GCTGTAGGAA-3′; and GAPDH, 5′-AAATGGTGAAGGTCGGTGTG-3′ and 5′-AGGTCAATGAAGGGGTCGTT-3′.

2.7. Western blotting

RAW264.7 cells were seeded in 6-well plates and pretreated with various concentrations of crocin for 8 h, followed by stimulation with Ti particles for predetermined times. The cells were lysed in radioimmunoprecipitation assay lysis buffer for 30 min on ice and total protein was extracted, quantified, resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and transferred to a polyvinylidene difluoride membrane that was blocked in 5% skim milk for 1 h and then incubated overnight at 4 °C with primary antibodies against inducible nitric oxide synthase (iNOS), Arg-1, VEGF, BMP-2, p38, phosphorylated (p-)p38, JNK, p-JNK, and β-actin. The membrane was washed three times and incubated with appropriate secondary antibodies, and protein bands were detected with enhanced chemiluminescence reagent (Millipore). Signal intensity was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.8. Enzyme-linked immunosorbent assay (ELISA)

After culturing for 2 days, the culture medium of RAW264.7 cells was collected and centrifuged and TNF-α, IL-4, IL-6, and IL-10 concentrations in the supernatant were evaluated with ELISA kits (Anogen-Yes Biotech Laboratories, Mississauga, ON, Canada) according to the

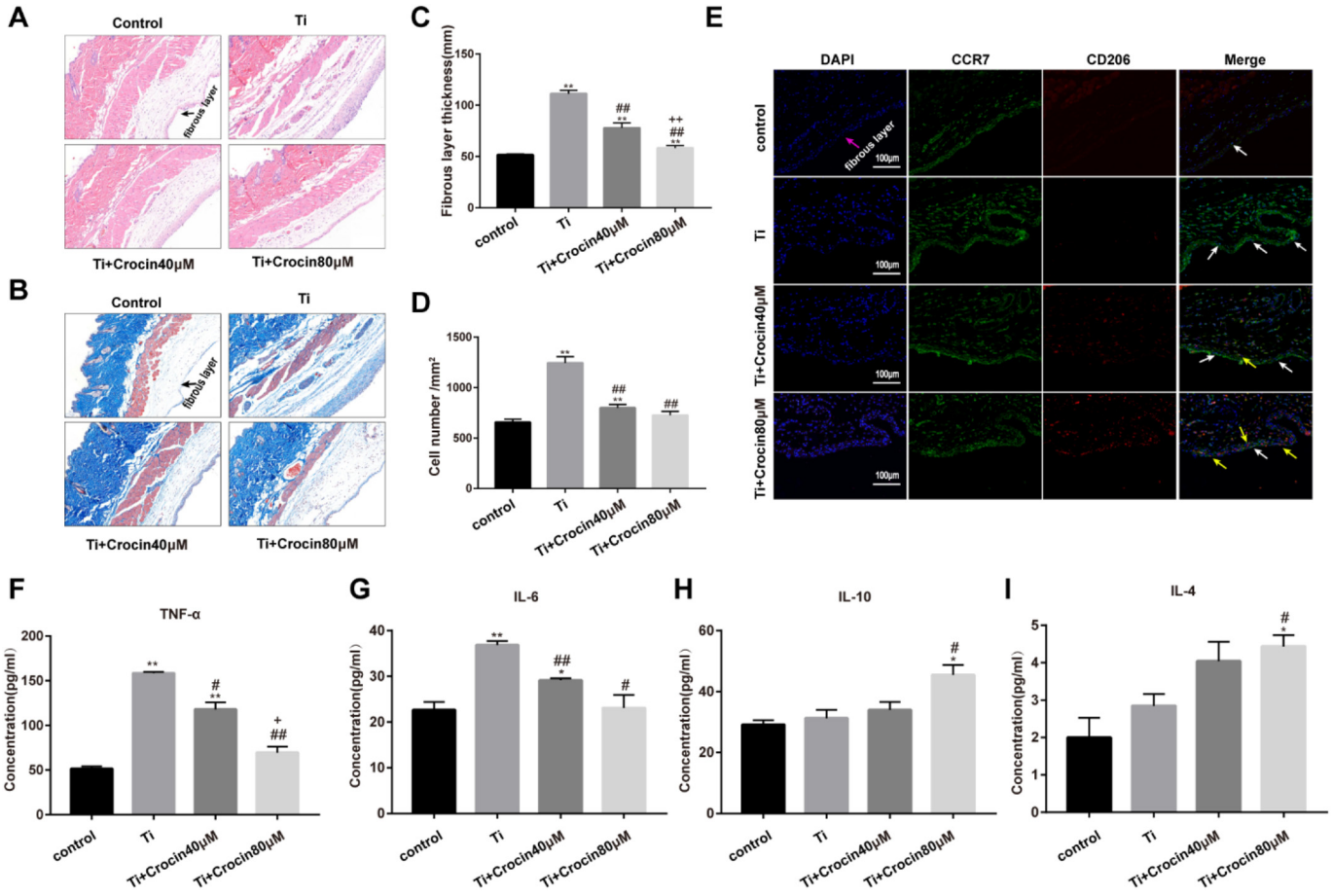

Fig. 5. Crocin inhibits Ti particle-induced inflammation and induces M2 macrophage polarization *in vivo*. (A, B) Image of HE (A) and Masson's trichrome (B) staining of air pouch tissue. (C) Thickness of the fibrous layer. (D) Number of infiltrated cells. (E) Immunofluorescence labeling of CCR7 (green; white arrows) and CD206 (red; yellow arrows) in air-pouch tissue; nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI; blue). Scale bar: 100 μm. (F—I) Detection of TNF-α (F), IL-6 (G), IL-10 (H), and IL-4 (I) in air-pouch exudates by ELISA. $^{*,\#,+}P < 0.05$, $^{**,\#\#,++}P < 0.01$ *vs.* Control, Ti, and Ti + Crocin40 μM, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

manufacturer's instructions.

2.9. Bone mesenchymal stem cell (BMSC) isolation and culture

BMSCs were isolated and cultured as previously described [22]. Briefly, primary cells were isolated from mouse BM under sterile conditions and cultured in DMEM containing 10% FBS and 1% penicillin/streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C.

2.10. Co-culture of BMSCs and RAW264.7 cells

BMSCs were seeded in 24-well tissue culture plates containing an 8-μm pore size filter in DMEM at a density of 2×10^4 cells/well. After culturing with Ti particles without or with crocin for 2 days, the cells were transferred to a transwell plate and exposed to BMSC-conditioned medium, which allowed BMSCs and RAW264.7 cells to grow in the same medium without direct contact.

2.11. Osteogenic differentiation of BMSCs

Alkaline phosphatase (ALP) staining and Alizarin red staining (ARS) were used to evaluate ALP expression and extracellular matrix (ECM) mineralization, respectively. After co-culture for 2 weeks, BMSCs were fixed and stained with ALP or Alizarin red dye (Beyotime Institute of Biotechnology, Shanghai, China), and the cells were observed under a light microscope (Leica). ALP activity at 14 days was determined with the ALP kit (Beyotime Institute of Biotechnology). The optical density value of the solution at 600 nm was measured after dissolving the deposited calcium with 10% cetylpyridinium chloride for quantification of ARS.

2.12. In vivo air pouch model

A total of 24 male C57BL/6 mice weighed approximately 25 g (8 weeks old) were used for the experiment. The air pouch model was established according to a previously described method [23]. The mice were divided into 4 groups, with different treatments including control group, Ti-treated group, Ti with low concentration of crocin treated group and Ti with high concentration of crocin treated group. The air pouches were injected with 0.5 ml PBS without or with Ti particles as well as various concentrations of crocin, which was injected daily until the animals were sacrificed five days after the initial injection. The pouch membrane was harvested and inflammatory exudate was collected by repeatedly washing the air pouch cavity with 2 ml of PBS. The exudate was centrifuged and stored at −80 °C for ELISA. The fixed air pouch membrane was embedded and cut into 4-μm sections that were stained with hematoxylin and eosin (HE) and Masson's trichrome and observed under a light microscope to assess inflammation. Immune cell infiltration and membrane thickness were analyzed using Image-Pro Plus software (Media Cybernetics, Rockville, MD, USA). CCR7- and CD206-positive cells were detected by immunofluorescence labeling. Animal experiments were approved by the Animal Care and Experiment

Committee of Shanghai Sixth People's Hospital affiliated with Shanghai Jiao Tong University.

2.13. Statistical analysis

Data are presented as mean ± SD and were analyzed with SPSS v.18.0 software (SPSS Inc., Chicago, IL, USA). Differences among groups were evaluated by one-way analysis of variance and the *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Crocin regulates macrophage polarization in vitro

We first assessed the toxicity of Ti particles to murine macrophage RAW 264.7 cells in the absence or presence of crocin at different concentrations. There were no statistically significant differences in the viability of cells treated with Ti particles with or without crocin (20–140 μM) for 24 h, as determined with the CCK-8 assay (Fig. 1A). Crocin treatment decreased the mRNA expression of CD86 (M1 marker) and increased that of CD206 (M2 marker), as determined by RT-PCR (Fig. 1B, C). Similar trends were observed for the protein levels of iNOS (M1 marker) and Arg-1 (M2 marker) by western blotting (Fig. 1D–F).

The effect of crocin on RAW264.7 cell polarization toward the M1 and M2 phenotypes was assessed by immunofluorescence detection of CCR7 and Arg-1, respectively. Crocin increased Arg-1 expression in the rank order of Ti + Crocin80 μM > Ti + Crocin40 μM > control > Ti (Fig. 2A). Meanwhile, CCR7 showed the opposite trend (Ti > Ti + Crocin40 μM > Crocin80 μM > control). The M1 and M2 fractions were quantified by detecting CCR7- and CD206-positive cells by flow cytometry. The percentage of CCR7-positive cells decreased from 23.61% in the Ti group to 7.31% in the Ti + Crocin80 μM group, which also had the largest proportion of CD206-positive cells (13.44%) among the four groups (Fig. 2B–E). These results indicate that crocin induces macrophage polarization toward the M2 phenotype.

3.2. Crocin modulates M2 macrophage polarization by inhibiting p38 and JNK phosphorylation

To clarify the mechanism by which crocin induces macrophage polarization, we examined the activation of the p38 and JNK signaling pathways. RAW264.7 cells were treated with Ti particles at various concentrations and p38 and JNK phosphorylation was detected by western blotting. We found that Ti particles activated p38 and JNK pathways, as evidenced by the dose-dependent increase in p38 and JNK phosphorylation (Fig. 3A–C). When RAW264.7 cells were pretreated with crocin for 8 h followed by stimulation with Ti particles, p38 and JNK phosphorylation was decreased relative to cells treated with Ti only (Fig. 3D–F). These results indicate that crocin regulates macrophage plasticity and polarization *via* inhibition of p38 and JNK signaling.

3.3. Crocin creates an immunomodulatory microenvironment that promotes osteogenic differentiation

The effect of crocin on TNF-α, IL-4, IL-6, and IL-10 production by RAW264.7 cells was evaluated by ELISA. The secretion of the pro-inflammatory cytokines TNF-α and IL-6 was lower and that of the anti-inflammatory cytokines IL-4 and IL-10 was higher in the Ti + Crocin80 μM group than in the other groups (Fig. 4A–D). We co-cultured BMSCs with RAW264.7 cells to evaluate the immunomodulatory effect of crocin on osteogenic differentiation and found that crocin enhanced the protein and mRNA expression of osteogenesis-related factors including VEGF and BMP-2, as determined by western blotting (Fig. 4E–G) and RT-PCR (Fig. 4H, I), respectively. ALP staining and ARS showed that the Ti + Crocin80 μM group had the largest area of ECM mineralization and the highest expression of ALP (Fig. 4J, K), which was confirmed by the optical density values of ECM and ALP activity in co-cultured BMSCs (Fig. 4L, M).

3.4. Crocin inhibits Ti particle-induced inflammation and induces M2 polarization in vivo

Air pouch tissue from mice was stained with HE and Masson's trichrome and inflammation was evaluated based on the thickness of the fibrous layer and immune cell infiltration into the pouch membrane. The Ti + Crocin80 μM group had the thinnest fibrous layer and the fewest infiltrated cells, indicating that crocin attenuates Ti particle-induced inflammation (Fig. 5A–D). Immunofluorescence analysis of the fibrous layer revealed that CD206 was highly expressed in the Ti + Crocin80 μM group, whereas more CCR7-positive cells were observed in the Ti group (Fig. 5E). We also evaluated the cytokine concentration in inflammatory exudates by ELISA and found that injection of crocin reduced the levels of TNF-α and IL-6 and increased those of IL-4 and IL-10 relative to the Ti group (Fig. 5F–I). These results demonstrate that crocin induces M2 macrophage polarization and suppresses Ti particle-induced inflammation *in vivo*.

4. Discussion

PIO is the major cause of aseptic loosening leading to failure of TJA. Wear particles generated from prosthetic implants can activate innate immune responses, causing inflammation and enhancing bone resorption [3,24,25]. Multiple cell types are involved in the pathogenesis of osteolysis including fibroblasts, macrophages, osteoblasts, osteoclasts, osteocytes, and MSCs [6]. Macrophages play a critical role in the pathogenesis of osteolysis as they secrete inflammatory cytokines and differentiate into osteoclasts [26,27]. Thus, therapeutic strategies that target wear particle-induced inflammation are presumably effective for mitigating PIO. Treatment with bisphosphonates has not been completely successful, and natural compounds are becoming more attractive alternatives owing to their biosafety and multifunctionality.

Crocin can inhibit oxidative stress as well as inflammation [16], which has been demonstrated in the context of some inflammatory disorders [28–30]. It was also shown to alleviate coronary atherosclerosis by inducing M2 macrophage polarization [20]. The present study investigated the immunomodulatory effect of crocin on Ti particle-induced osteolysis and subsequent bone dynamics both *in vitro* and *in vivo*, which has not been previously reported.

Macrophages are the main cells involved in the host response to prosthetic implants [31,32]. Macrophage polarization is plastic and is induced by various signals from the surrounding tissue [8]. Our immunocytochemical analysis revealed that crocin increased the expression of the M2 marker Arg-1 and decreased that of the M1 marker CCR7. In addition, the flow cytometry results confirmed that the proportion of CD206-positive cells was higher in the crocin group, indicating that crocin induces M2 macrophage polarization. M1 and M2 macrophages secrete pro- and anti-inflammatory cytokines, respectively, with the latter promoting tissue repair [33–35]. We determined by ELISA that crocin stimulated the production of anti-inflammatory cytokines (IL-4 and IL-10) and suppressed that of pro-inflammatory cytokines (TNF-α and IL-6) by RAW264.7 cells. Thus, crocin inhibits Ti particle-induced inflammation by promoting M2 macrophage differentiation.

Macrophage activation and consequent osteoclast formation play important roles in PIO; the functions of osteoblasts and their progenitor cells in osteogenesis are also closely linked to osteolysis [36–38]. Bone turnover at the bone-implant interface is a balance between osteoclastic bone resorption and osteoblastic bone formation. Inflammation influences bone dynamics; for example, anti-inflammatory M2 macrophages can create an immunomodulatory environment that favors osseointegration [13]. VEGF and BMP-2, which contribute to bone formation and

angiogenesis, were upregulated by crocin treatment; the increased concentration of anti-inflammatory cytokines secreted by macrophages also promotes bone formation. Additionally, ALP staining and ARS showed that osteogenic differentiation of BMSCs was enhanced by co-culture with crocin pre-treated macrophages. Thus, crocin promotes the M2 macrophage phenotype and thus, osteogenic differentiation.

The mouse air pouch model is useful for investigating the *in vivo* anti-inflammatory effect of a drug on inflammation caused by an exogenous substance [39,40]. Here we used an air pouch model to investigate the effect of crocin on Ti particle-induced inflammation and macrophage polarization *in vivo*. Mice treated with crocin had a thinner fibrous layer and fewer infiltrating cells than those injected with Ti particles alone, suggesting that crocin has anti-inflammatory effects. We confirmed by immunofluorescence analysis that crocin induced M2 macrophage polarization *in vivo* and also reduced inflammation, as determined by ELISA analysis of exudates from air pouches. Thus, the *in vivo* results were consistent with those obtained using cell cultures.

To investigate the molecular basis for the effect of crocin on macrophage polarization, we examined the activation of different signaling pathways. Some studies have reported that the downregulation of p-JNK induces macrophage polarization toward the M2 phenotype [41–43], while M0 macrophages can be induced to differentiate into M1 macrophages *via* activation of p38 signaling [44–46]. Ti particles drove macrophage differentiation toward the M1 phenotype, which is related to excessive inflammation. In this study Ti particles activated the p38 and JNK pathways, which promoted the inflammatory (M1) macrophage state. Crocin attenuated p38 and JNK activation in Ti particle-treated macrophages, suggesting that it suppresses wear particle-induced inflammation by inducing M2 macrophage polarization *via* inhibition of the p38 and JNK pathways.

Our study had several limitations. Firstly, we used Ti instead of other types of wear particle such as ultra-high molecular weight polyethylene or cobalt chromium particles that more closely mimic actual wear particles. It is unclear whether crocin has similar effects on inflammation induced by different types of wear particle. Secondly, we did not demonstrate with the air pouch model that crocin-induced macrophage polarization promotes osseointegration *in vivo*. Thirdly, the precise mechanisms underlying the immunomodulatory function of crocin have yet to be elucidated.

In conclusion, our study demonstrates that crocin plays an immunomodulatory role in Ti particle-induced inflammation and bone dynamics. Findings from *in vitro* and *in vivo* experiments indicate that crocin induces M2 macrophage polarization and stimulates the secretion of anti-inflammatory and osteogenic cytokines to suppress inflammation and enhance osseointegration, respectively. These effects are likely mediated *via* inhibition of p38 and JNK signaling. Taken together, our results suggest that crocin is a promising and effective therapeutic agent for preventing PIO and aseptic loosening and improving the success rate of TJA.

Declaration of competing interest

The authors declare no conflict of interest.

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