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# Investigation on the role of Icariin in tendon injury repair: focusing on tendon stem cell differentiation

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## Abstract

**Objective** Tendon injury is a common and frequent disease in the field of sports medicine, and tendon repair after injury is a common clinical difficulty. Repair strategy based on tendon stem cells (TDSCs) therapy is considered a promising therapeutic option for the treatment of tendon injuries. Icariin (ICA) has been shown to be an effective herbal monomer for the treatment of tendon-bone healing and may be effective in the repair of tendon injuries.

**Methods** In vitro, TDSCs isolated from C57 mice were treated with ICA (0.01–100 μM) to assess proliferation (CCK-8 assay) and tendonogenic differentiation (qRT-PCR). In vivo, 42 C57 mice with surgically induced patellar tendon defects were randomized into three groups ($n=14$/group): (1) 20 mg/kg ICA, (2) 40 mg/kg ICA, and (3) control group (DMSO), administered intraperitoneally for 14 days. Half of each group ($n=7$) underwent histopathological (hematoxylin-eosin staining, Masson staining) and molecular (qRT-PCR) analyses at 2 and 4 weeks post-surgery.

**Results** In vitro, after 7 days of ICA intervention in TDSC, the expression of Mohawk (MKX), Scleraxis (SCX), fibromodulin (FMOD), and Tenomodulin (TNC) were higher in the ICA group than in the control group. In vivo, the expression of MKX, SCX, FMOD, and TNC was higher in the 20 mg/kg ICA group and 40 mg/kg ICA group than in the control group at 2 and 4 weeks after surgery. Histological evaluation revealed superior tendon repair in both ICA-treated groups compared to controls at both 2 and 4 weeks postoperative intervals. The 20 mg/kg ICA group demonstrated a significant enhancement in tissue continuity and collagen fiber organization, exhibiting greater defect filling, fewer interstitial gaps, and reduced vascular infiltration. In contrast, control specimens exhibited disorganized collagen architecture with prominent interstitial gaps. The 40 mg/kg ICA group showed intermediate repair outcomes between the 20 mg/kg ICA and control groups.

**Conclusion** ICA can improve tendon injury repair by enhancing tendonogenic differentiation of TDSC.

**Keywords** Icariin, Tendon repair, Tendon stem cell, Tendonogenic differentiation

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## Introduction

Tendons serve as critical biomechanical connectors that facilitate musculoskeletal movement. These fibrous connective tissues consist of collagen fibers, elastic fibers, paratendon tissue, epitenon, and endotenon, each featuring distinct structural layers and compositions that collectively maintain tendon function. The primary functions of tendons are to transmit muscle force and absorb external forces, playing a crucial role in movement. Their mechanical properties are closely related to the structure and characteristics of collagen fibers [1]. Acute sports-related injuries frequently induce tendon damage, with inadequate clinical management often leading to chronic pain, functional impairment, and substantial socioeconomic burden [2–4]. While healing outcomes are influenced by anatomical location and microenvironmental factors, the canonical tendon repair process progresses through three distinct phases: inflammatory response, proliferative and remodeling phase [4–7]. Notably, emerging evidence highlights inflammation and its dysregulation as critical precursors in the pathogenesis of tendon disorders, with inflammatory processes preceding the histological manifestations of fibrosis and degenerative tissue remodeling [8, 9]. The tendon healing cascade is inherently mediated by resident tenocytes that constitutively secrete a complex milieu of inflammatory mediators, encompassing both pro-inflammatory and anti-inflammatory cytokines, coupled with an array of growth factors [10–12]. While acute-phase cytokine signaling facilitates essential repair mechanisms, persistent low-grade inflammatory microenvironments associated with chronic tendinopathy create a state of "healing failure" following acute injury [7]. In clinical conditions of overuse related to tendons and their surrounding areas, significant inflammation is uncommon and is primarily associated with tendon ruptures [13]. Light and electron microscopic evaluation of 397 ruptured Achilles tendon specimens by Kannus et al. demonstrated a complete absence of acute inflammatory infiltrates in histopathological analyses [14]. Similarly, Arner et al. demonstrated an absence of neutrophil infiltration within 24 h after tendon rupture [15]. However, a recent immunohistochemical study of acute tendon rupture specimens paradoxically identified neutrophilic infiltration in all cases, indicative of acute-phase inflammation [16]. This suggests that subsequent inflammatory responses observed in later stages represent secondary phenomena rather than primary drivers of tendon rupture. Mechanistically, collagen fibril disorganization and tenocyte necrosis may precipitate localized sterile inflammation, compromising tendon biomechanical integrity. Such inflammatory-driven matrix degradation likely exacerbates structural vulnerability, predisposing tendons to catastrophic failure under physiological loads [2]. Moreover, post-traumatic healing predominantly generates biomechanically inferior fibrotic scar tissue rather than functional tendon matrix, creating persistent challenges for athletic recovery and clinical intervention [17].

Tenocyte isolation from native tendon can be achieved through enzymatic digestion or explant culture systems; however, their clinical translation remains constrained by proliferative capacity during in vitro expansion [18, 19]. Cao et al. cultured tendon cells seeded on a polylactic acid scaffold for one week before implanting them into nude mice [20]. Histomorphometric and biomechanical evaluations revealed scaffold-guided reorganization of collagen bundles into parallel and linear architectures, demonstrating a tensile modulus comparable to native tendon tissue. When the polylactic acid scaffold seeded with tendon cells was implanted at the site of flexor tendon defects, after 12 weeks post-surgery, the tendon cells and collagen fibers were oriented longitudinally. By week 14, the engineered tendon exhibited a typical tendon structure, with a failure load reaching 83% compared to normal tendons [21]. Although the tendon cell-based tissue engineering strategy has been proven effective, it faces challenges such as limited cell sources, donor site complications, prolonged culture times, and restricted expansion potential [18, 22]. Contemporary advances in regenerative medicine have established stem cell therapy and tissue engineering as promising approaches for tendon restoration [23–26]. Comparative analyses of mesenchymal stem cell (MSC) sources, including bone marrow-derived MSCs (BMSCs), TDSCs, and embryonic stem cells (ESCs), reveal significant variations in cellular yield, proliferative capacity, and tendonogenic differentiation potential [17, 27]. Bi et al. demonstrated that TDSCs possess the capacity to generate tendon tissue and serve as cellular donors for neoplastic tendon tissue [28]. Consequently, utilizing TDSCs, which have repair potential derived from tendon tissue, may offer a superior stem cell-based therapeutic option for tendon injury repair. However, the inherent multipotent differentiation capacity of these cells necessitates precise regulatory control to optimize tendon-specific repair.

Icariin (ICA; $C_{33}H_{40}O_{15}$, molecular weight 676.67), a bioactive flavonoid isolated from *Epimedium* species, has demonstrated osteogenic and angiogenic properties in bone defect models [29]. Emerging evidence from rotator cuff studies in rats suggests ICA enhances tendon-bone interface healing through collagen matrix augmentation and neovascularization [30]. Despite these promising findings, the direct therapeutic effects of ICA on tendon parenchymal repair remain unexplored.

This investigation systematically evaluates ICA's regulatory effects on TDSC differentiation and its functional impact on tendon repair using murine models. Our results demonstrate that ICA significantly enhances

tendonogenic lineage specification in TDSCs. In vivo experiments revealed accelerated healing kinetics and improved ultrastructural organization in ICA-treated patellar tendon defects.

## Materials and methods

### Isolation and culture of TDSCs

Four-week-old C57 mice weighing 20–30 g were selected for the experiment. Isolation and culture of TDSC from mice. Mouse tails were first obtained, and the mouse tail tendons were carefully separated and placed in sterile PBS for 5 min for immersion. The caudal tendons were stopped in 1.5 ml centrifuge tubes, and they were trimmed to 1 mm × 1 mm × 1 mm sized tissues using sterile ophthalmic shears. The tissues were transferred to a sterile mixing flask, 3 mg/ml of type I collagenase (1904GR001, Biofroxx, China) was added, and the tissues were digested in the mixing flask for 1.5–2 h at 37 degrees Celsius. The digested cytosol was centrifuged at 3000 xg for 5 min and resuspended in modified DMEM/F-12 medium (10-092-CVRC, CORNING, China) containing 10% fetal bovine serum (Z7181FBS-500, ZETA LIFE, Guangzhou, China), penicillin-streptomycin (P1400-100 ml, Solarbio, Chengdu, China). The cells were incubated at 37 °C in 5% CO2 for 3 days and then the medium was changed. On day 5–6, when the cell density reaches 80%, generation 0 cells can be obtained for passage. The passages 3 cells were used for all subsequent experiments.

### Flow cytometry assay of TDSCs

P1 generation TDSC ($1 \times 10^6$) resuspended in PBS and 1 μg of PE, APC, Alexa Flour 700, PerCP-Cy5.5 were added. Incubate for 30 min away from light. After incubation, remove the supernatant after centrifugation at 2000 rpm for 5 min. Then the stained cells were resuspended with 200 μL of PBS solution and assayed on cell flow meter (FACStarPlus flow cytometer, BD Company, USA). Approximately $10^4$ events recorded per sample. The antibodies, anti-CD90 (205903, Biolegend, China), anti-CD44 (103015, Biolegend, China), anti-CD31 (102510, Biolegend, China), anti-CD44 (103129, Biolegend, China), were used in this study.

### Trilineage differentiation of TDSCs

Detection of trilineage differentiation (osteogenic, lipogenic, chondrogenic) capacity of TDSC using a trilineage differentiation kit (RASTA-90021, RASTA-90031, RASTA-90041, Cyagen, China). TDSC was inoculated into 24-well plates at a density of $5 \times 10^4$ cells/well. Then lipogenic, osteogenic and chondrogenic induction media were given for 10–14 days. After the induction was completed, the cells were fixed for 30 min with 4% paraformaldehyde (G1101-500 mL, Servicebio, China). Oil red O, alizarin red, and alisin blue staining was performed using a staining kit (G1260-100 ml, G2540-100 ml, G3280-3 × 50 ml, Solarbio, China).

### Colony formation assay

500 cells/well TDSC were inoculated into 6-well plates and cultured in complete medium for 7 days, then fixed with paraformaldehyde (G1101-500 mL, Servicebio, China) for 30 min, and stained with 1% crystal violet (G1063-10 ml, Solarbio, China) for 10 min. Photographs were taken under light microscope.

### Effect of Icariin on the proliferation of TDSC

ICA (HY-N0014, MedChemExpress, China) was dissolved in DMSO (D8371-50 ml, Solarbio, China) and configured to gradient concentrations of 0.01 μM, 0.1 μM, 1 μM, 10 μM and 100 μM, respectively. The third generation TDSC cells were taken in 96-well plates and sequentially inoculated with 2000 cells/well. Cell proliferation assay was evaluated using CCK-8 (C266180-1 ml, aladdin, China). 96-well plates were actually incubated for 2 h by adding 10 μL CCK-8 to each well. The absorbance (450 nm) of individual wells was measured after completion of incubation to assess cell viability. The absorbance values of each well were determined at 24 h, 48 h, and 72 h after the addition of ICA, respectively.

### Effect of ICA on the differentiation of TDSC into tendon cells

P3 generation TDSC grew in 12-well culture plates and intervened when the cells were completely adhered to the wall and spread all over the plates. The experimental group was added with ICA solution (HY-N0014, MedChemExpress, China) at a concentration of 1 μM, and the control group was added with an equal concentration of DMSO (D8371-50 ml, Solarbio, China) solution. Configured tendon-forming induction solution (25 μmol/L L-Ascorbic acid (HY-B0166, MedChemExpress, China) and 50 ng/ml Recombinant Human CTGF (120-19-20, PEPROTECH, China)) was added to both groups. The culture solution was changed at 3-day intervals and drug interventions were added. Levels of SCX, MKX, TNMD, and TNC were measured by qRT-PCR on day 7. GAPDH was the housekeeping gene.

### qRT-PCR

RT-PCR was performed by quantitative reverse transcription polymerase chain reaction (qRT-PCR) for gene expression of SCX, MKX, FMOD and TNC after 7 days of co-culture ($n = 3$). Total RNA was extracted from cells using TRIzol reagent (9109, TaKaRa, China). Full-length cDNA was then reverse transcribed using a cDNA synthesis kit (RK21203, Abclonal, China). The qRT-PCR was performed according to the manufacturer's instructions (RK21203, Abclonal, China). GAPDH was the

housekeeping gene. PCR primer sequences are shown in Table 1.

### Mice and experimental design

Forty-two C57 mice aged 4 weeks and weighing 20–30 g were used for the experiment. Forty-two mice were randomly divided into 2 groups: (1) 20 mg/mL ICA group; (2) 40 mg/mL ICA group; and (3) DMSO group (control group). Animals in all 3 groups had intraperitoneal administration of the drug once a day for 14 days and then stopped. In total, $n=7$, half were used for histological analysis and half for PCR analysis. All animals were executed at 2 and 4 weeks after surgery.

### Surgical procedure

All animal experiments were conducted in the Animal Experiment Center of the Affiliated Hospital of Traditional Chinese Medicine of Southwest Medical University.

Mice were anesthetized intraperitoneally using 0.5% sodium pentobarbital, and the skin of the knee joint was incised aseptically to expose the patellar tendon. Two cuts are made parallel to the tendon in the center of the patellar tendon, and then the Ophthalmic scissors are placed below the tendon for support. A full-thickness partial transection was formed in the patellar tendon using a 0.5-mm-diameter biopsy punch (Fig. 1). Both patellar tendons were constructed as described above to model patellar tendon injury (Punch model). Finally, the skin was sutured, and the mice were allowed to move freely in the cage. After 2 and 4 weeks after injury, all animals were euthanized, and the tendons were collected for experiments.

### Tissue qRT-PCR

After obtaining the patellar tendon tissue, 1 cm of patellar tendon tissue above and below the injury site was taken for RNA extraction. Tissue RNA was extracted using the method described above. Briefly, the patellar tendon tissue was crushed (the size of a sesame seed grain) to extract the tissue RNA with Trizol hair. The gene expression of SCX, MKX, TNMD, and TNC was determined at the site of the patellar tendon injury in the animals at 2 and 4 weeks after the surgery, respectively, by fluorescence quantitative PCR. Gene expression. PCR primer sequences are shown in Table 1.

**Table 1** Reverse transcription–polymerase chain reaction primer sequences

| Genes | Forward Primer Sequences | Reverse Primer Sequences |
|---|---|---|
| SCX | GAGAACACCCAGCCCAAACA | TGTCACGGTCTTTGCTCAAC |
| MKX | ACGCTAGTGCAGGTGTCAAA | AGCGTTGCCCTGAACATACT |
| FMOD | CAGACTTGCACACTCTCCGT | ACTGCATTTTTGTCTCTTGGGT |
| TNC | CACGGCTACCACAGAGGC | GTCCAGCAGCTTCCCAGAAT |
| GAPDH | TGACTTCAACAGCAACTC | TGTAGCCATATTCATTGTCA |

Primer sequences were given in 5′ to 3′ direction

### Histopathological analysis

Hematoxylin and eosin staining (HE staining) and Masson staining were performed on the specimens obtained. Specimens were fixed in 4% paraformaldehyde for 24 h, dehydrated in graded alcohol, clear, immersed in wax, and the tissue was embedded using paraffin. After the embedding was completed, sections were made in the coronal plane along the longitudinal axis of the tendon (6 μm). HE staining (G1120-100, Solarbio, Chongqing, China) and Masson staining (G1343-7 × 100 ml, Solarbio, Chongqing, China) were performed according to the manufacturer's recommended standard staining protocol. An established histological scoring system was used to analyze changes in scores on HE-stained slides after treatment [31].

### Statistical analysis

All data are expressed as mean ± standard deviation. T-test was used to test the difference between the two groups. Statistical analysis was performed using SPSS. Bar and dot plots were generated using GraphPad Prism 9 and mean ± S.D was displayed.

## Results

### Characterization of TDSCs

Methods Sexual TDSC were isolated as described in Materials and Methods. The P0-P2 generation of cells is shown (Fig. 2A). The P0 generation of cells exhibited clonality and proliferation, with one generation passed every 2–3 days. Finally, the P3 generation cells showed a uniform long shuttle cell morphology. TDSC also had some clonal reproduction ability as shown in (Fig. 2B). Expression of surface markers on TDSC was detected using flow cytometry to confirm tendon-derived cells as stem cell types. Results showed the presence of CD90 (93.6%) and CD44 (99.3%) markers but were negative for CD31 and CD45 (Fig. 2D). It was shown in an in vitro induction model that TDSC can successfully differentiate into adipogenic, osteogenic or chondrogenic cell lines (Fig. 2C).

### ICA promotes TDSC proliferation

After using 0.01 μM, 0.1 μM, 1 μM, 10 μM, and 100 μM of ICA to act on TDSC, studies at 24 h, 48 h, and 72 h by the CCK-8 assay showed that 1 μM of ICA promoted and 100 μM inhibited the proliferation of TDSC. Moreover, the effect of 1 μM was more significant. Therefore, 1 μM ICA was chosen to continue the experiment (Fig. 3A).

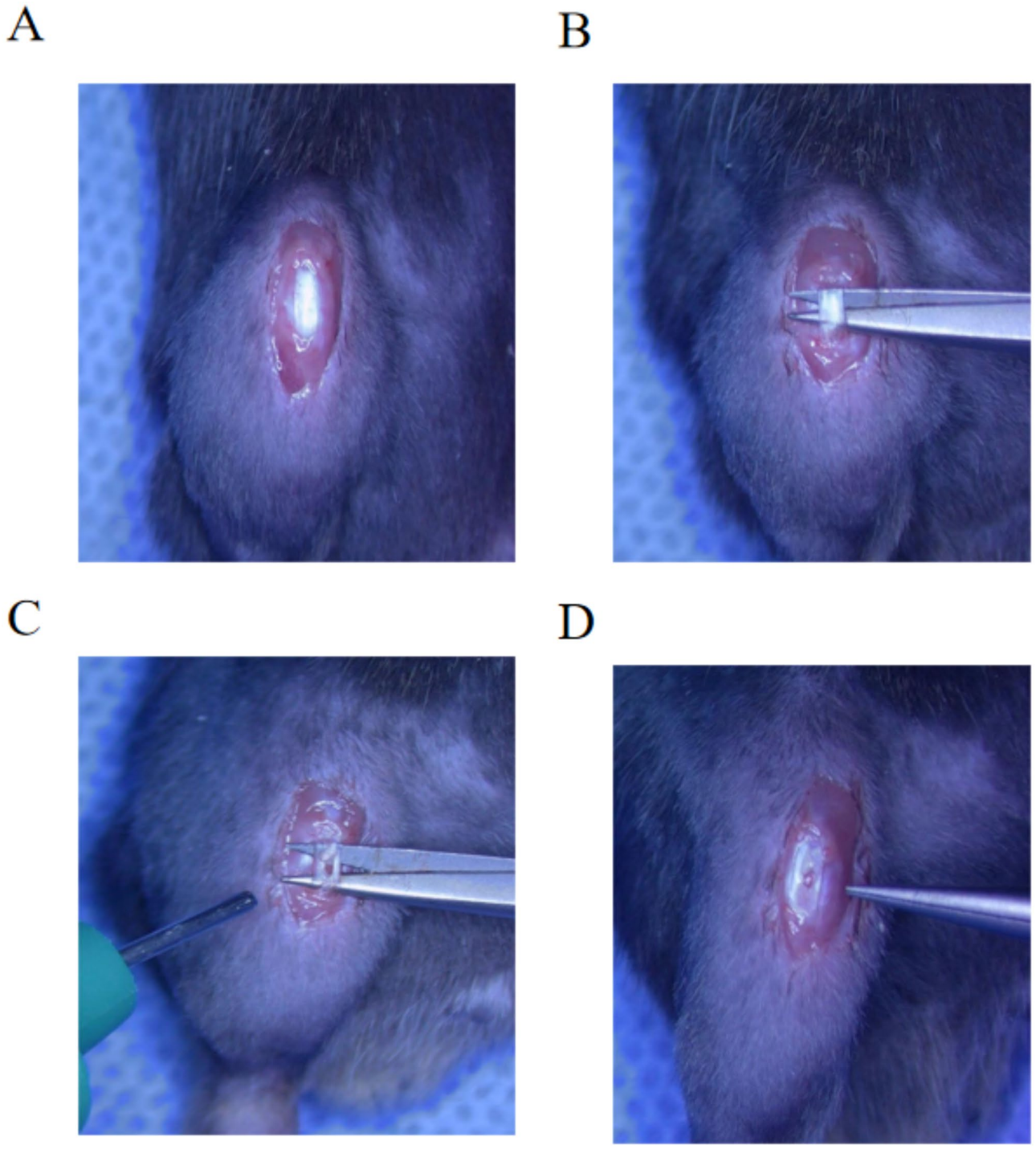


**Fig. 1** An animal model of repairing Patellar tendon defects. (**A**) The skin is incised to expose the patellar tendon. (**B**) The patellar tendon is revealed with scissors. (**C**) A 0.5 mm diameter defect was created in the patellar tendon using a skin sampler. (**D**) The skin was sutured after the molding was completed

#### ICA promotes tendonogenic differentiation of TDSC

The effect of ICA on TDSC in gene expression profiles was analyzed by qRT-PCR after using 1 μM of ICA to act on TDSC for 7 days. The expression levels of SCX, MKX, FMOD, and TNC were up-regulated in the ICA group compared with the control group (Fig. 3B).

#### ICA promotes the expression of TDSC tendonogenic genes in the region of patellar tendon injury in mice

After obtaining specimens from the injured region of the tendon, gene expression profiles of the injured region were analyzed by qRT-PCR at 2 and 4 weeks. The expressions of SCX, MKX, FMOD, and TNC were higher in the

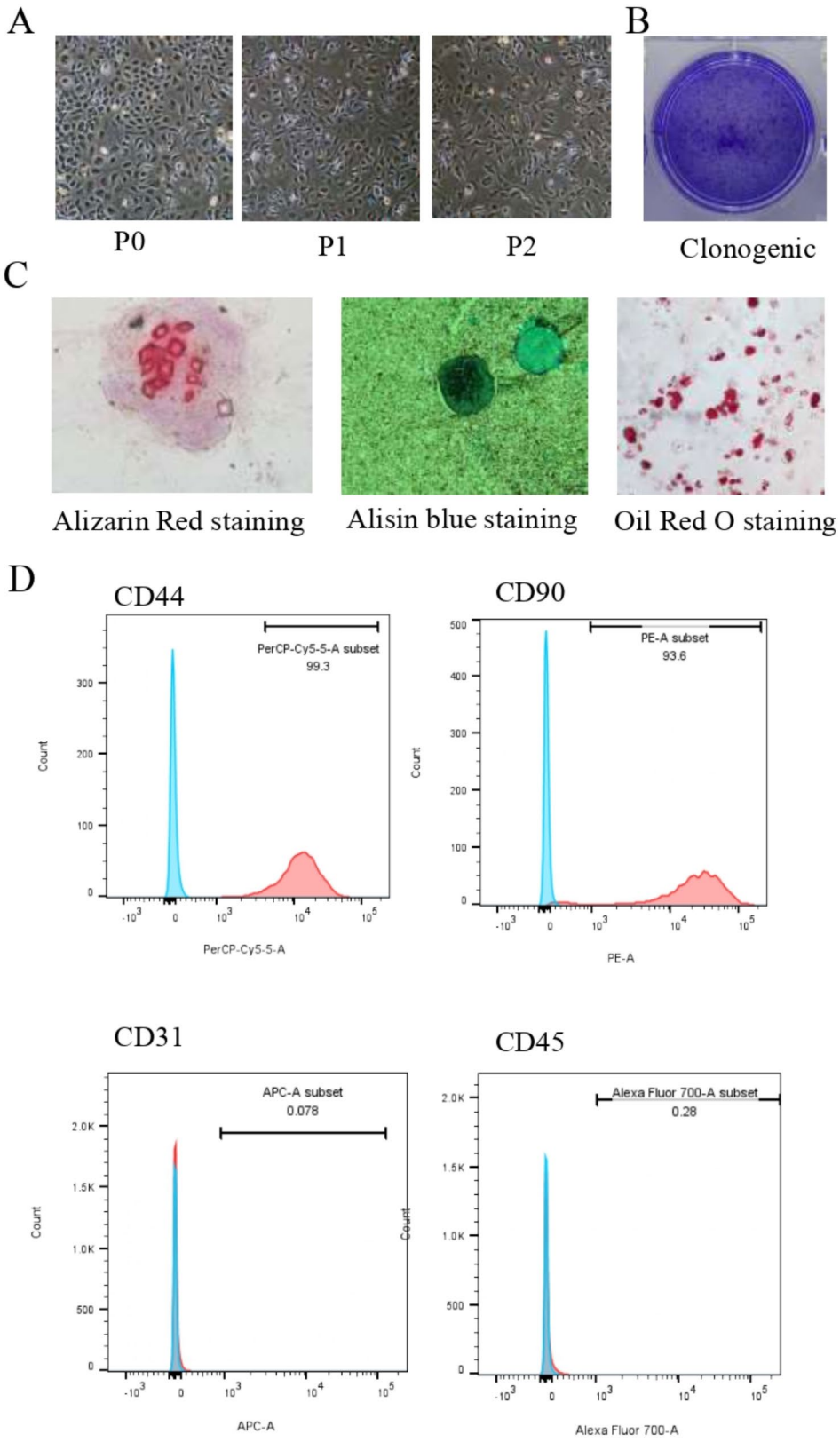


**Fig. 2** Characterization of TDSCs. (**A**) The cells presented spindly like. (**B**) Clonogenesis of TDSCs, using crystal violet staining. (**C**) Adipogenesis, osteogenesis, cartilage-like, using Oil red O, Alizarin red, and Alisin blue staining, respectively. (**D**) TDSCs were positive for CD44, and CD90 but negative for CD31 and CD45

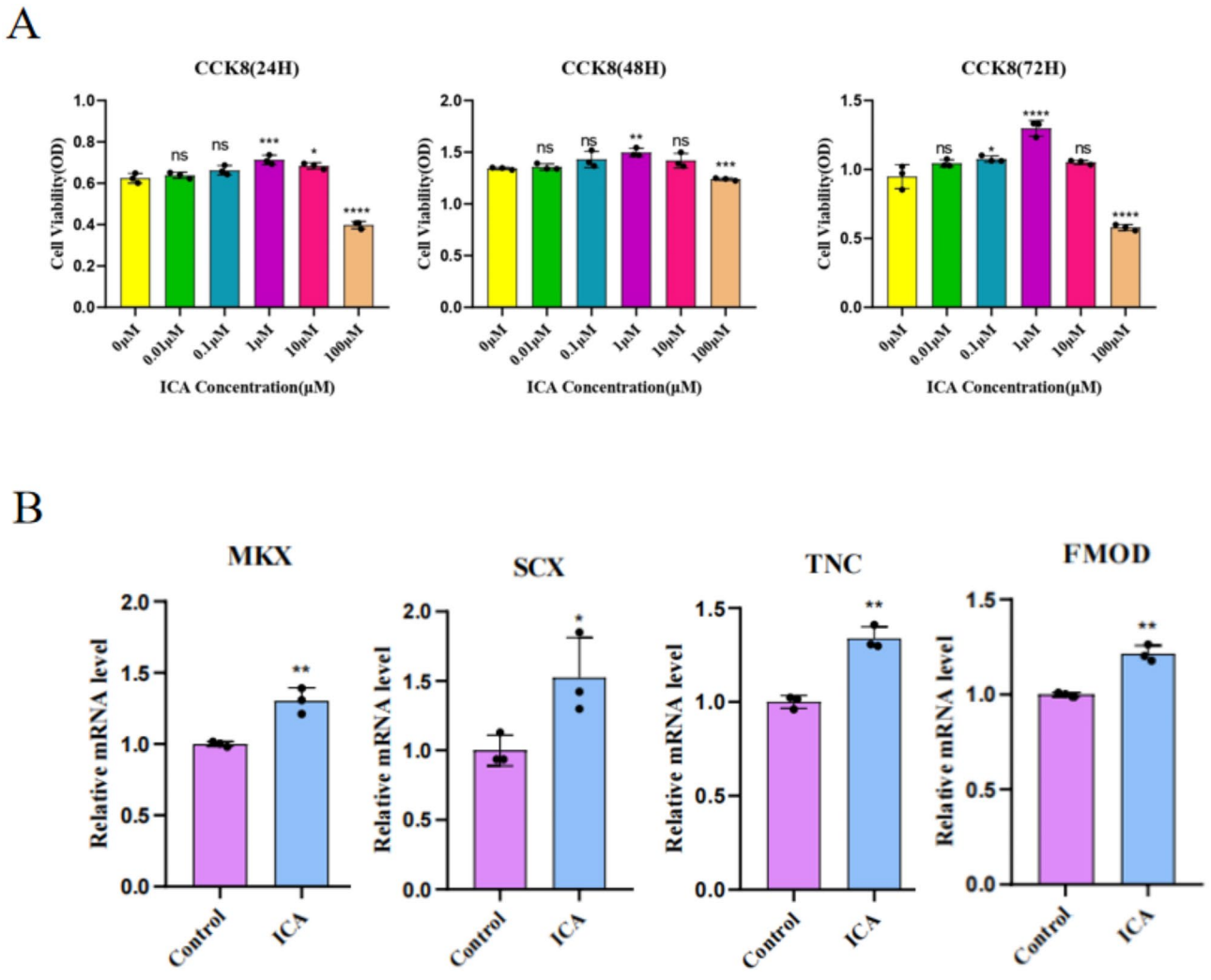


**Fig. 3** ICA promoted cell proliferation and tendonogenic differentiation of tendon-derived stem cells (TDSCs). (**A**) CCK-8 showed that 1 μM of ICA promoted TDSC value-addition and 100 μM inhibited TDSC proliferation during ICA intervention in TDSC at 24 H, 48 H and 72 H. (**B**) Total RNA was extracted on days 7 and the expression of genes related to tendonogenic differentiation were detected by quantitative real-time qPCR. (Compared to the control group, ns indicates no statistically significant difference; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$)

20 mg/kg ICA group and 40 mg/kg ICA group compared to the control group at 2 and 4 weeks. And the expression of the genes of SCX, MKX, FMOD and TNC was higher in the 20 mg/kg ICA group than in the 40 mg/kg ICA group at 2 and 4 weeks (Fig. 4).

### ICA improves patellar tendon healing by gross and histologic observations

During the animal experiments, all mice showed no complications related to anesthesia or surgery and no infections or appetite abnormalities. All mice were free to move around in the mouse cages. Gross observations showed that the patellar tendon defect site was gradually repaired at 2 and 4 weeks postoperatively, and no significant infection or postoperative tendon adhesion was observed (Fig. 5A). HE staining (Fig. 5B) and Masson's (Fig. 5C) results showed that the tendon alignment was more continuous and regular in the 20 mg/kg ICA and 40 mg/kg ICA groups at 2 and 4 weeks postoperatively compared with the disorganized tendons in the control group. The tendon defects in the 20 mg/kg ICA group had more filling and fewer voids with a small amount of small-vessel infiltration, whereas the DMSO group showed the opposite with more disorganized arrangement, and the 40 mg/kg ICA group was in the middle.

## Discussion

In the present study, we examined the role of ICA in tendon repair using a patellar tendon defect model (Punch model). Previous studies have demonstrated that ICA exhibits osteogenic and vasculogenic properties conducive to tendon-bone healing [30, 32]. Therefore, it is reasonable to hypothesize that ICA may also facilitate the healing of tendon injuries. We conducted in vitro

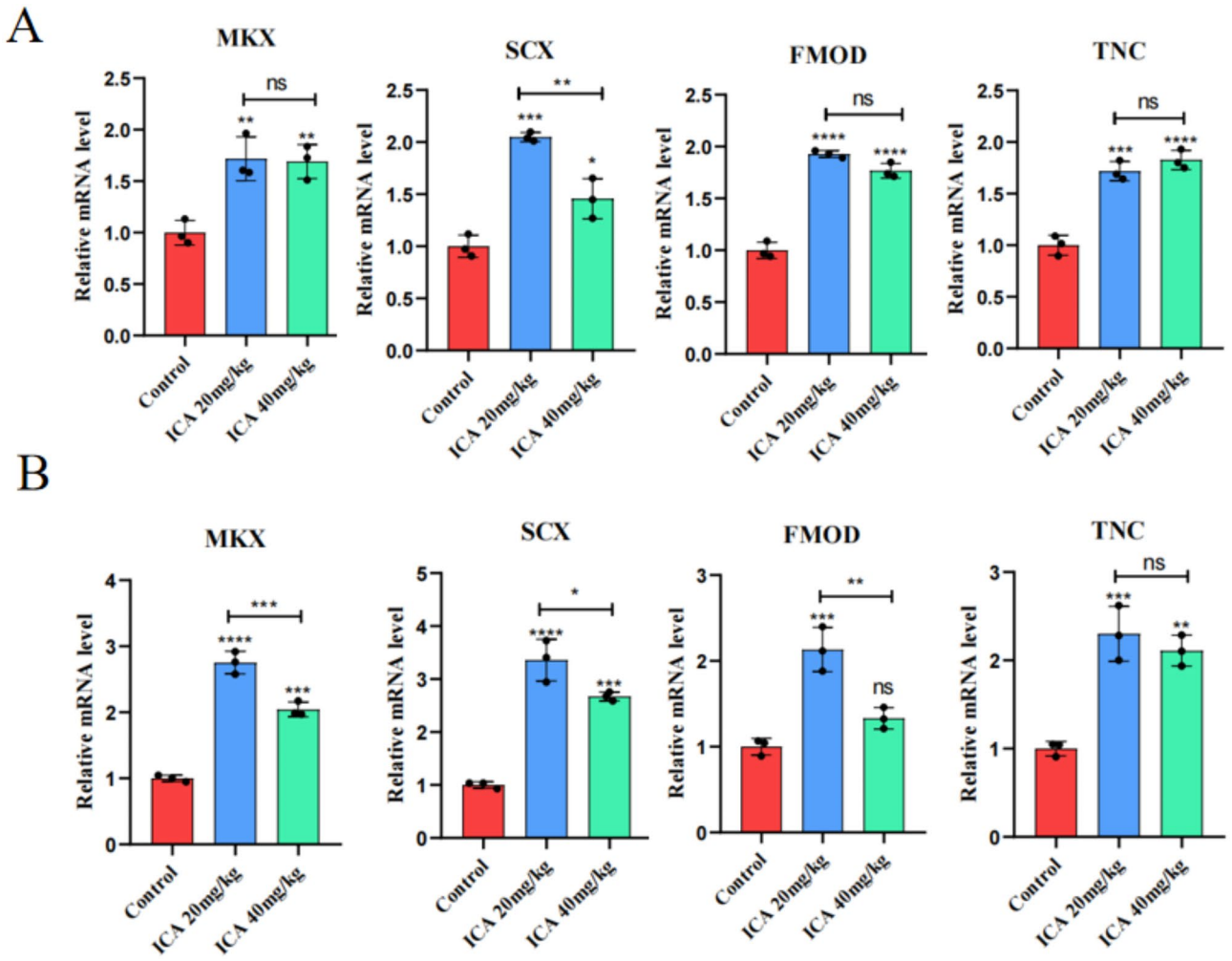


**Fig. 4** ICA promotes tendonogenic differentiation of stem cells at the site of patellar tendon injury. (**A**) Total RNA was extracted on days 14 and the expression of genes related to tendonogenic differentiation were detected by quantitative real-time qPCR. (**B**) Total RNA was extracted on days 28 and the expression of genes related to tendonogenic differentiation were detected by quantitative real-time qPCR. (ns indicates no statistically significant difference; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$)

experiments to assess the impact of ICA on the proliferation and differentiation of mouse TDSCs, while in vivo studies evaluated the effects of ICA on tendon injury repair through histological analysis and other methodologies. Our findings align with prior research and suggest a complementary role for ICA in promoting tendon-bone healing.

In biological organisms, feedback regulation governs the response of cells and tissues to drugs. At low doses, ICA effectively stimulated TDSCs, enhancing the expression of tendon-specific markers including Scx, Mkx, and Fmod through receptor-mediated signaling activation. This anabolic response diminished at elevated doses, suggesting the engagement of homeostatic counter-regulatory mechanisms analogous to endocrine negative feedback systems. For example, excessive thyroid hormone production suppresses thyrotropin-releasing hormone and thyroid-stimulating hormone through receptor-mediated feedback inhibition [33]. In this study, high doses of ICA may have triggered a similar feedback mechanism, resulting in lower expression levels of genes compared to the low-dose group. Cellular responses followed classic ligand-receptor interaction kinetics, where low-dose ICA (sub-saturation concentrations) permitted optimal receptor occupancy and downstream pathway activation. At high doses, once the receptors are saturated, excess drug cannot continue to exert its effects, and cells may undergo adaptive changes that downregulate the activity of relevant signaling pathways, leading to decreased gene expression. In the case of insulin and its receptor, when insulin concentration is excessively high and the receptors are saturated, cells will reduce the number of receptors or decrease receptor sensitivity, diminishing the effects of insulin [34]. Our CCK-8 proliferation assays revealed significant growth inhibition at 100 μM ICA, indicating concentration-dependent

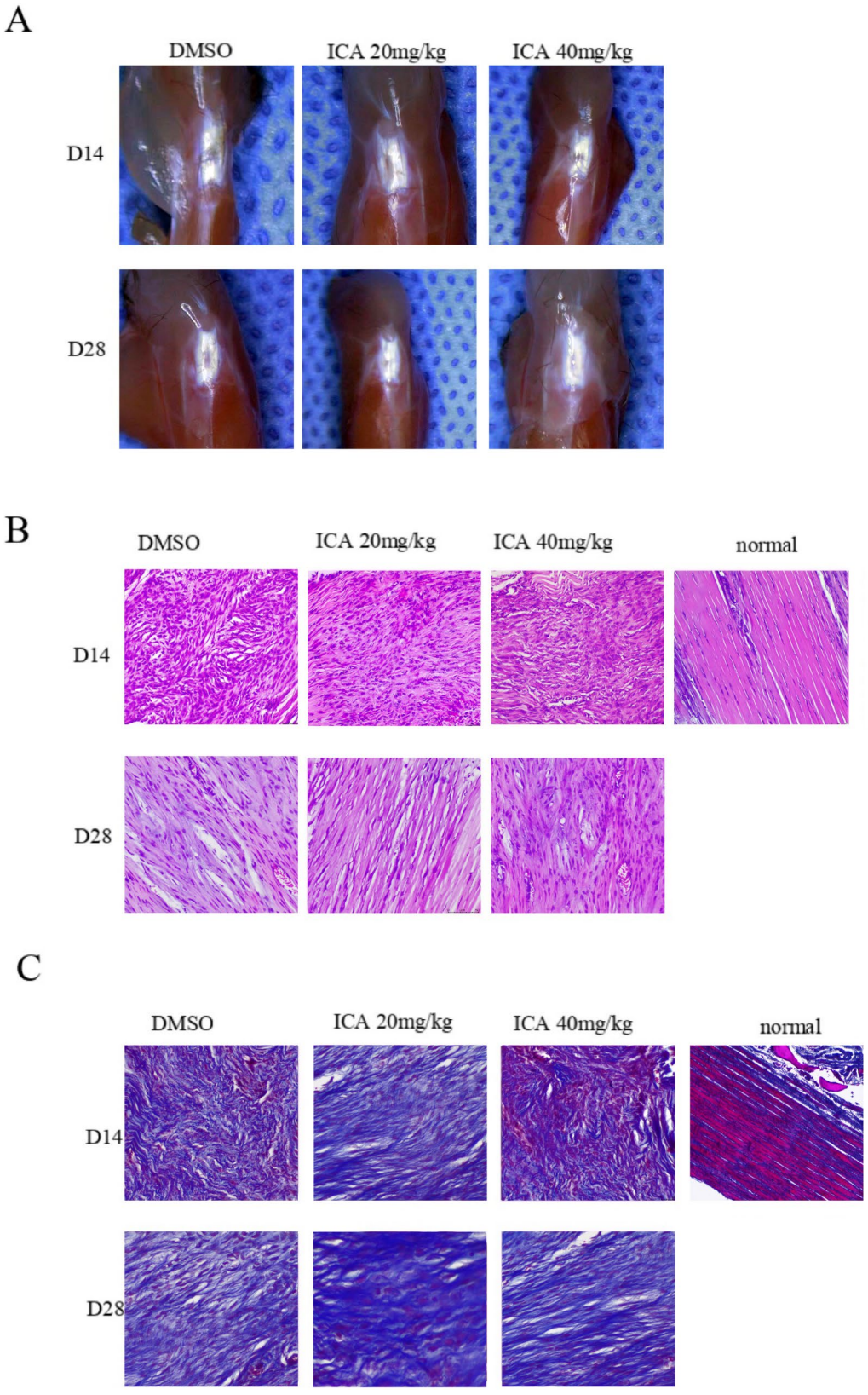


**Fig. 5** ICA has a role in promoting patellar tendon injury repair. (**A**) Gross observations of patellar tendon defect repair at 14 and 28 days. (**B**) Histological sections of patellar tendon defects from the blank defect or ICA intervention groups were stained with hematoxylin and eosin at 14 and 28 days. (**C**) Histological sections of patellar tendon defects from the blank defect or ICA intervention groups were stained with Masson at 14 and 28 days

cytotoxicity. High doses of ICA may pose potential toxicity to cells, affecting their normal functions and gene expression.

The strategy of utilizing stem cells' pluripotent differentiation capacity to enhance the repair of tendon injuries has shown promising outcomes [24, 25, 35, 36]. Whereas Sakaguchi et al. demonstrated that differences in MSC properties depend on the source of the cells, TDSC may be a more desirable source or subpopulation of stem cells targeting tendon injury repair [23, 37]. Numerous studies have demonstrated that targeting TDSCs can effectively enhance the repair of tendon defects by regulating the proliferation, migration, and differentiation of stem cells [3]. Yu et al. showed that MSCs-derived exosomes (BMSCs-exo) can regulate the behavior of TDSCs and promote TDSC proliferation, migration, and tendon differentiation, and thus the expression of MKX, TNMD, and type I collagen can be up-regulated by means of locally injected BMSCs-exo, which promotes regenerative repair of tendon injury [3]. Xu et al. showed that the expression of type I/III collagen, TNC, and SCX in TDSC could be upregulated by transfecting bone morphogenetic protein 12 (BMP12) and connective tissue growth factor (CTGF) into TDSC, and the results showed that the repair of the patellar tendon window defects in rats was facilitated after transplantation of this TDSC in vivo [25]. Therefore, factors that can modulate the differentiation of TDSC to adult tendons (including various biological factors, mechanophysical properties, and chemotherapeutic molecules) may be therapeutic strategies to promote tendon repair [38–40]. Our results showed that ICA intervention increased the expression of SCX, MKX, FMOD, and TNC in TDSC, regulated the differentiation of TDSC toward tendon-origin, and also promoted injury repair of the tendon in a mouse patellar tendon window defect model, which is similar to the findings described above.

In the past, Epimedium was used in the treatment of fractures and fracture delivery can achieve good efficacy and has not been found to have significant adverse effects. ICA, the main extracted bioactive of Epimedium, has also been found to have bone-enhancing properties and is therefore utilized for the treatment of bone disorders by utilizing its bone regenerating ability [29, 41, 42]. Qin et al. showed that ICA could promote the proliferation of rat BMSCs by activating ERK and p38 MAPK signaling, and 320 μg/L was the optimal concentration for it to promote the proliferation of BMSCs [43]. Ye et al. showed that mice receiving ICA gavage detected more COL-1, COL-2, and VEGF-positive cells at the tendon-bone injury interface, which facilitated tendon-bone healing [30]. Similarly, Zheng et al. used ICA as a topical application and observed well-organized collagen fibers at the tendon-bone healing interface, as well as better-healed tendon-bone interface tissue [32]. In addition, many studies have demonstrated the safety of ICA [41, 43, 44]. Xiao et al. used ICA 300 mg/day in psychiatric patients and no significant side effects were observed after 8 weeks of continuous dosing [45]. Therefore, ICA can be considered as a safe pharmaceutical preparation.

However, our study still has some limitations. First, we used ICA intraperitoneal administration, which is different from local administration and oral administration in the conventional sense, and therefore, it cannot be ruled out that this will reduce the translation to future clinical applications. Second, we evaluated the effect of ICA on the repair of patellar tendon window defect tendon injuries (2 and 4 weeks), but long-term clinical outcome assessment is lacking. Third, we used C57 mice for patellar tendon defect modeling experiments, which are different from the common type of tendon injury in humans. However, despite this limitation, our results also suggest that ICA has a positive effect on repair after tendon injury. In the future, larger animal models as well as longer periods of time can be studied to validate the effects of ICA on tendon repair.

## Conclusions

ICA promotes the proliferation and tendonogenic differentiation of TDSC. In a patellar tendon defect model, the repair of patellar tendon injuries can be promoted by intraperitoneal injection of ICA. Consequently, this study identifies ICA as a valuable TCM drug component that can promote tendon injury repair. A limitation of this study is the short follow-up period, which did not allow for the evaluation of the long-term effects of ICA on tendon injury healing.

#### Author contributions

Zhenhong He: Conceptualization, Project administration, Investigation, Writing - original draft, Visualization, Writing - review & editing. Shengqiang Zeng: Investigation, Writing - original draft, Writing - review & editing. Bo Qin: Writing - review & editing. Gang Liu: Writing - review & editing. Writing - review & editing. Huan Liu: Conceptualization, Supervision, Project administration, Writing - review & editing. Dingsu Bao: Supervision, Project administration, Funding acquisition, Writing - review & editing.

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#### Data availability

No datasets were generated or analysed during the current study.

## Declarations

#### Ethics approval and consent to participate

The Animal Research Ethics Committee of Traditional Chinese Medicine Hospital of Southwest Medical University in China had approved all experiments (SWMU20210053).

#### Competing interests

The authors declare no competing interests.

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