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RESEARCH

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Therapeutic effects of chitosan/β-glycerophosphate/collagen hydrogel combined with MSCs on chronic achilles tendon injury via the Akt/GSK-3β pathway

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Abstract

Background Chronic Achilles tendon injuries, commonly resulting from inadequate management of acute incidents, significantly reduce patients' quality of life. Current treatments, including conservative, surgical, and regenerative approaches, often yield suboptimal results. This study investigated the therapeutic effectiveness of a chitosan/*β*-glycerophosphate/collagen (C/GP/Co) hydrogel combined with bone marrow mesenchymal stem cells (MSCs) for chronic Achilles tendon injury in a rat model.

Material & Methods A temperature-sensitive injectable C/GP/Co hydrogel was synthesized and combined with MSCs to treat a chronic Achilles tendon injury in Sprague–Dawley rats. The rats were divided into four groups receiving saline (model), C/GP/Co hydrogel, C/GP/Co/MSCs hydrogel, or normal control. After 6 weeks, morphological, biomechanical, and molecular assessments were conducted, including histology, Western blot analysis for protein expression, and the evaluation of the Akt/GSK-3*β* signaling pathway.

Results The C/GP/Co/MSCs hydrogel significantly enhanced tendon healing compared to the model and C/GP/Co groups, as evidenced by improved collagen fiber organization and an increased type I/III collagen ratio on histological analysis. Western blot results revealed activation of the Akt/GSK-3*β* pathway by the C/GP/Co/MSCs hydrogel, leading to enhanced tendon cell proliferation and reduced apoptosis, demonstrated by a decreased Bax/Bcl-2 ratio and Caspase-3 expression. Downregulation of inflammation markers CD206 and CD163 was significant. Biomechanical testing indicated that the C/GP/Co/MSCs hydrogel restored tendon tensile strength closer to normal levels.

Conclusions The C/GP/Co/MSCs hydrogel establishes a supportive microenvironment for MSC function, aiding tendon healing through the Akt/GSK-3*β* pathway. Its dual role in inflammation and apoptosis reduction, while enhancing biomechanical properties, demonstrates its potential as an innovative treatment for persistent Achilles tendon ailments. Future research endeavors should comprehensively explore the molecular pathways and assess their clinical applicability.

Keywords Chronic Achilles tendon injuries, Chitosan hydrogel, Bone marrow mesenchymal stem cells, AKT/GSK-3*β*, Tendon repair

Introduction

The Achilles tendon, the thickest tendon in the human body, connects the gastrocnemius and soleus muscles to the calcaneus, playing a crucial role in various

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physical activities. Among athletes, Achilles tendon injuries are prevalent, with chronic cases arising from untreated acute injuries or inadequate initial treatment [1]. Symptoms of chronic Achilles tendon injury include pain, swelling, and functional impairment, significantly impacting patients' well-being [2, 3]. Current treatment options encompass conservative approaches, surgery, and regenerative therapies, yet these methods often yield unsatisfactory results [4–10]. Therefore, the quest for more effective and safer treatment modalities is imperative to enhance the quality of life for individuals affected by this condition.

Biomaterials have been employed in fabricating hydrogels for Achilles tendon repair in tissue engineering, showcasing notable progress. Chitosan (CS), a natural biodegradable material, exhibits exceptional biocompatibility, biodegradability, antibacterial properties, and the ability to promote cell adhesion and proliferation [11]. Sodium β-glycerophosphate (β-GP), a phosphate buffer, provides cells with essential phosphate ions, supporting proliferation and differentiation [12]. Collagen (Col), a key constituent in tissue regeneration, offers biocompatibility, advantageous mechanical properties, and structural support for cells [13]. Additionally, bone marrow mesenchymal stem cells (MSCs), with their capacity for multi-lineage differentiation into tenoblasts, chondrocytes, and other cell types, present a promising avenue for tissue restoration [14]. The Akt/Gsk-3β signaling pathway plays a pivotal role in cellular functions such as growth, proliferation, apoptosis, and metabolism. As a biomaterial, the chitosan/β-glycerophosphate/collagen (C/GP/Col) hydrogel demonstrates exceptional biocompatibility, biodegradability, and tunability, highlighting its potential in tissue engineering and drug delivery applications. The incorporation of MSCs with the C/GP/Col hydrogel enhances cell viability and differentiation efficacy, thereby facilitating the regeneration and repair of Achilles tendon tissue.

This study aimed to investigate the efficacy of a chitosan/β-glycerophosphate/collagen hydrogel combined with bone marrow mesenchymal stem cells (MSCs) for treating chronic Achilles tendon injuries in rats. The assessment encompassed morphological changes, biomechanical properties, tissue parameters, and the expression of proteins associated with the Akt/Gsk-3β signaling pathway. The objective of this study was to comprehensively understand the therapeutic mechanisms underlying MSC treatment for chronic Achilles tendon injuries.

Material and Methods

Animals

42 male Sprague–Dawley (SD) rats, aged 4 weeks and weighing approximately 100 g, were sourced from the Experimental Animal Center of China Three Gorges University. Two rats were utilized for MSC isolation, while the remaining 40 were designated for subsequent animal experiments. All animal procedures strictly adhered to the guidelines for experimental animal care and were approved by the Animal Research Ethics Committee of Yangtze University [YZLL2024-015].

Isolation and culture of bone marrow stem cells

Four-week-old Sprague–Dawley rats were euthanized via cervical dislocation, disinfected in 75% alcohol for 5 min, and sterilized. Dissecting skin, subcutaneous tissues, nerves, and blood vessels aseptically extracted both femurs. The femurs were rinsed thrice in sterile phosphate-buffered saline, and their metaphyseal ends were excised to expose the medullary cavity. Bone marrow was flushed out using a 1 mL syringe filled with complete culture medium (high-glucose medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin). The bone marrow extraction continued until the femur appeared pale. The cells were cultured in flasks at 37 °C in a 5% CO_2 environment. The culture medium was renewed after 48 h and subsequently every 3 days. Upon reaching 80% confluence, the cells were subcultured.

Preparation of C/GP/Co/MSCs hydrogel

The chitosan/β-glycerophosphate/collagen (C/GP/Col) hydrogel was prepared according to the method outlined by Yang et al. [15]. Initially, 0.2 g of chitosan powder (Bomei, Hefei, China) was sterilized through autoclaving (121 °C, 20 min), dissolved in 10 mL of 0.1 mol/L acetic acid solution to yield a 20 g/L chitosan solution, filtered to remove insoluble substances, and stored at 4 °C. In a separate process, 1 g of β-glycerophosphate (GP) was dissolved in 1 mL of 0.03 mol/L sodium hydroxide solution, filtered using a 0.22 μm membrane, and stored. The GP solution was combined with the chitosan solution at a 6:1 volume ratio while stirring on ice. A 2 mg/mL collagen type I solution (Bomei, Hefei, China) was prepared by dissolving collagen in deionized water. The C/GP/Col hydrogel was created by mixing the solutions at a 6:1:8 volume ratio under ice-bath conditions. The pH of the hydrogel was determined using a pH meter. The hydrogel was placed in a 37 °C incubator to monitor gelation time. Following this, mesenchymal stem cells (MSCs) were trypsinized, suspended in PBS, and blended with the C/GP/Col hydrogel to achieve a final cell density of 10^9 cells/300 μL.

Animal model and treatments

A chronic Achilles tendon injury model was established according to the protocol by De Cesar Netto et al. [16]. Rats were anesthetized with intraperitoneal sodium

pentobarbital injection (Rongbai, Shanghai, China) based on weight. The right hind limb was shaved and disinfected with iodophor. Thirty SD rats had their right Achilles tendon injected with a low dose of collagenase (Biosharp, Hefei, China) six times over four weeks. Another 10 SD rats had their right Achilles tendon left untreated as a control. After four weeks, 10 rats were injected with 300 μl 0.09% saline, 10 with 300 μl C/GP/Co hydrogel, and 10 with 300 μl C/GP/Co/MSCs hydrogel. In the sixth week, rats were euthanized by spinal dislocation, and their right Achilles tendons were harvested for histological, gene, protein expression, and biomechanical analyses.

Western blot

Signaling pathways and apoptosis-related protein expression were assessed using Western blot analysis. Achilles tendon samples were collected from four groups of Sprague–Dawley rats following a 6-week treatment period for total protein extraction. Protein quantification was performed with the BCA Protein Assay kit (BOSTER, Wuhan, China). Absorbance values of the samples were measured at 562 nm (540-590 nm) using an ELISA reader, with protein concentrations determined for each group. Total protein was separated by SDS-PAGE, transferred to a PVDF membrane, and incubated overnight at 4 °C with specific antibodies (BAX 1:2000, Bcl-2 1:1500, AKT 1:5000, P-AKT 1:2000, GSK 1:4000, GSK-3 β 1:2000, and β-actin 1:20,000). A horseradish peroxidase-labeled secondary antibody (1:5000) was then applied, followed by incubation at room temperature for 1 h. Protein expression levels were analyzed by measuring band area and grayscale values using Image J software, and relative protein expression levels were calculated for the four groups.

Hematoxylin & Eosin (H&E) staining

The Achilles tendon tissue was fixed in 40 g/L paraformaldehyde for 48 h, dehydrated using gradient ethanol and xylene, and embedded in paraffin. Sections of 5 μm thickness were cut from the paraffin blocks. After dewaxing in xylene and hydration in gradient ethanol, the sections were stained with hematoxylin for 12 min, followed by a 1-min wash in running water. Differentiation was achieved using 1% hydrochloric acid ethanol for 1 min, followed by a 2-min wash in distilled water. Subsequently, the sections were blued with 1% ammonia water for 1 min, counterstained with eosin for 5 min, dehydrated, cleared with distilled water, and sealed with neutral gum. Tissue changes were examined using a microscopic imaging system at 20 and 40 magnification.

Masson trichrome staining

The tendon tissues were fixed in formaldehyde, dehydrated, paraffin-embedded, deparaffinized in xylene, and rehydrated in ethanol concentrations. Subsequently, they were immersed in a potassium dichromate solution overnight and then stained with hematoxylin for blue coloration. Ponceau S (Servicebio Wuhan China) was applied for 10 min, rinsed with distilled water, and then Toluidine blue (Servicebio Wuhan China) for 2 min to visualize the collagen fibers. The sections were dehydrated in graded ethanol, cleared in xylene, and mounted with neutral gum.

Immunohistochemical staining

The rat Achilles tendon tissues were embedded in paraffin, followed by sectioning, baking, dewaxing, and hydration. Antigen retrieval was conducted using a citrate antigen retrieval solution (G1202, Servicebio Wuhan, China). Subsequently, the sections were treated with a 3% hydrogen peroxide solution to inhibit endogenous peroxidase activity. Blocking was achieved by incubating the sections with normal goat serum (G1208-5ML, Servicebio Wuhan China) for 30 min at room temperature. Overnight incubation with rabbit anti-Collagen I, Collagen III, and Caspase-3 antibodies (all from Servicebio Wuhan, China) followed. This was succeeded by incubation with goat anti-rabbit IgG for 50 min at room temperature. The sections were stained with DAB chromogen solution (G1212, Servicebio Wuhan China), counterstained with hematoxylin, dehydrated using an ethanol gradient, and mounted. Microscopic observation, image collection, and analysis were performed using a Nikon E100 microscope (Japan).

Immunofluorescence staining

Paraffin-embedded tissue sections were washed with PBS, permeabilized, and stabilized using 0.3% Triton X-100 (Servicebio, Wuhan, China) for 20 min to prevent nonspecific binding. Primary antibodies (CD206 antibody 1:1,000, CD163 antibody 1:1,000) were diluted to appropriate concentrations, with 50 μL applied to each section. The sections were incubated overnight at 4 °C, followed by three 10-min PBS washes. Subsequently, HRP-labeled secondary antibodies (all from Servicebio, Wuhan, China) were applied and incubated at room temperature for 50 min. After another three PBS washes, the sections were treated with DAPI at room temperature for 30 min. Images were acquired and analyzed using ImageJ software with a fluorescence microscope.

Biomechanical testing

For subsequent biomechanical testing, three Achilles tendons were selected from each group, spanning the calf muscle segment (0.5 cm above the tendon juncture), the Achilles tendon itself, and the calcaneus. The tendon was immobilized after excision of surrounding muscles and blood vessels. Tension changes and maximum tension during stretching were monitored as the tendon was subjected to a stretching rate of 5 mm/min until rupture.

Statistical analysis

Experimental data were presented as mean ± SD and analyzed using SPSS26.0. *T*-tests compared differences between two groups, while one-way analysis of variance assessed differences among multiple groups. Significance was set at $P < 0.05$.

Results

4.1 pH and gelation time of C/GP/Co hydrogel

At room temperature, the C/GP/Co solution remained in a liquid state (Fig. 1A). Upon heating to 37 °C, the solution underwent gelation, transitioning into a solid state (Fig. 1B–1C) and returning to a liquid state after 6 weeks at room temperature (Fig. 1D). The pH of the C/GP/Co solution was measured at 7.30, with a gelation time of approximately 8 min. The solution's color changed from transparent to opaque after gelation. The gelation time of approximately 8 min was suitable for manipulating bone marrow mesenchymal stem cells (Fig. 1E) when combined with the C/GP/Co solution for subsequent in vivo injection.

Western blot analysis of related protein expression

Western blot analysis demonstrated decreased levels of p-AKT and p-GSK-3β in the model group compared to the control group, indicating impaired tendon healing. Levels of p-AKT and p-GSK-3β were significantly higher in the C/GP/Co hydrogel group compared to the model group, suggesting that the hydrogel enhances tendon healing through the AKT/GSK-3β pathway. The C/GP/Co/MSCs hydrogel group exhibited the highest levels of p-AKT and p-GSK-3β, surpassing those in the C/GP/Co hydrogel group and the model group significantly (Fig. 2A, 2B, and 2D), indicating that the addition of MSCs further enhances the therapeutic efficacy of the hydrogel through this pathway. Analysis of apoptosis marker protein expression in western blotting revealed increased expression of the anti-apoptotic protein Bcl-2 and decreased expression of the pro-apoptotic protein Bax in the C/GP/Co/MSCs hydrogel group compared to the model group (Fig. 2A and 2C). These results suggest that the C/GP/Co/MSCs hydrogel may alleviate apoptosis in Achilles tendon tissue.

Histological analysis

Hematoxylin and eosin (H&E) staining indicated disrupted collagen alignment in the model and the C/GP/Co hydrogel groups, characterized by irregularly shaped tendon fibers. Conversely, the C/GP/Co/MSCs hydrogel and control groups demonstrated well-aligned tendon fibers with collagen bundles running parallel to the tendon axis, resembling the structure of normal tendons. Masson's trichrome staining revealed an increase in disorganized collagen fibers (stained blue) in the model group, with partial improvement observed in the C/GP/Co hydrogel group. The C/GP/Co/MSCs hydrogel group exhibited enhanced collagen fiber organization and density compared to the C/GP/Co hydrogel group, featuring predominantly myofibril-like collagen structures. The control group displayed dense and neatly arranged collagen fibers, primarily in myofibril form (Fig. 3A).

Immunohistochemical analysis

Immunohistochemical staining of tendon sections was conducted to evaluate the therapeutic effects of C/GP/Co/MSCs hydrogel. Caspase-3, a tendinopathy marker, showed reduced staining intensity in both the C/GP/Co hydrogel and C/GP/Co/MSCs hydrogel groups compared to the model group, with the C/GP/Co/MSCs hydrogel group exhibiting a more significant decrease (Fig. 4A, D). In the control group, collagen type I (Col 1) was prominently expressed, while collagen type III (Col 3) levels were minimal, indicative of normal tendon structure. The model group displayed disrupted collagen fibers, increased Col 3 expression, and decreased

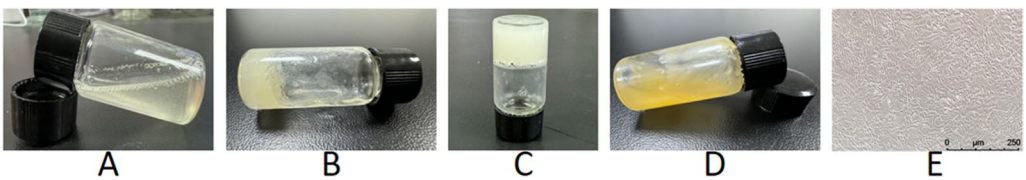

Fig. 1 Preparation and in vitro degradation of C/GP/Co hydrogels. **A** The C/GP/Co solution remains in liquid form at room temperature; **B**, **C** the solution transitions into a gel at 37 °C; **D** the solution returns to liquid form at room temperature after 6 weeks; **E** Bone marrow mesenchymal stem cells mixed with the C/GP/Co solution

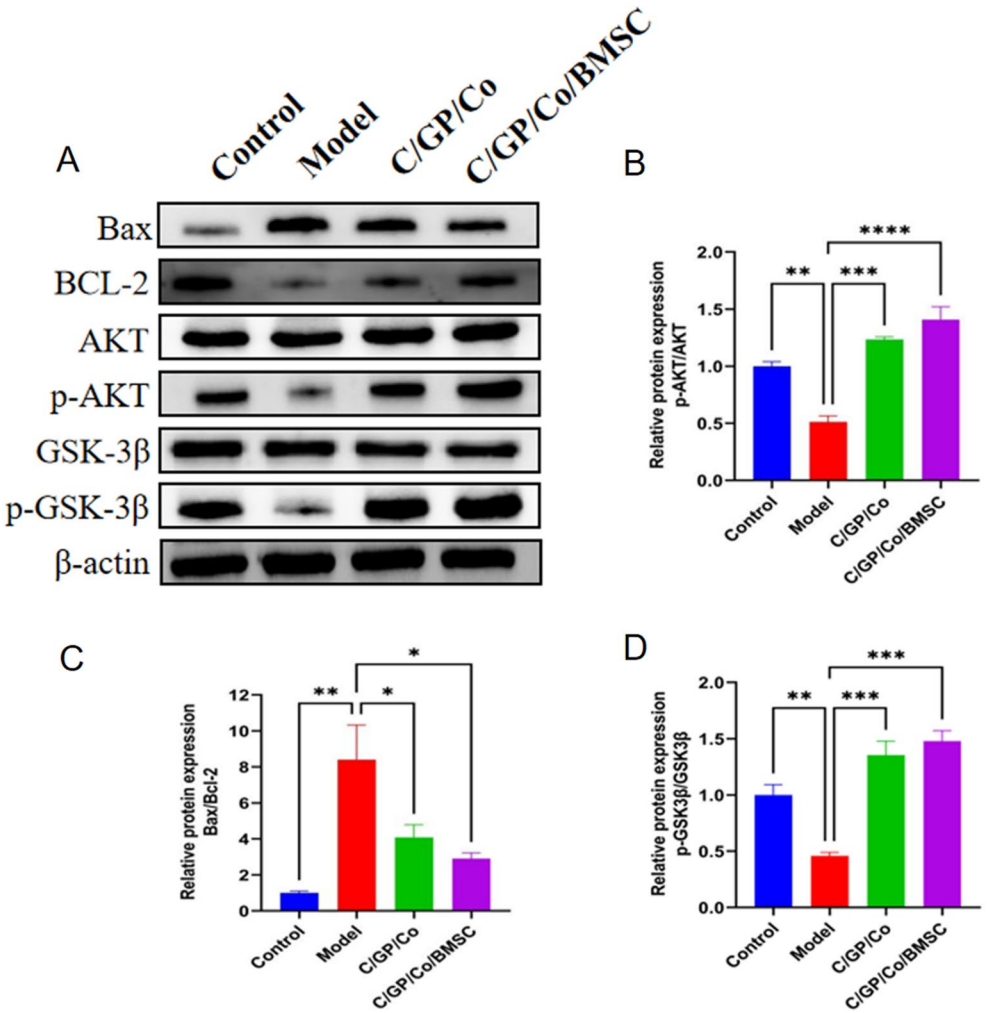

Fig. 2 C/GP/Co/MSCs hydrogel attenuates Achilles tendon tissue apoptosis through the AKT-GSK-3β pathway. **A** Western blot analysis of BAX, Bcl-2, AKT, p-AKT, GSK-3*β*, p-GSK-3*β*, and *β*-actin levels in the four groups. **B**–**D** Quantification of protein expression levels. Data are shown as mean ± SD ($N = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Col 1 expression. Treatment with the C/GP/Co hydrogel enhanced collagen alignment raised Col 1 levels, and reduced Col 3 expression. The C/GP/Co/MSCs hydrogel group showed the most substantial improvement, with elevated Col 1 and decreased Col 3 expression (Fig. 4A–C).

Immunofluorescence analysis

Immunofluorescence staining revealed a significant increase in the expression of inflammatory markers CD163 and CD206 in the model group compared to the control group, indicative of inflammation. Treatment with the C/GP/Co hydrogel notably decreased CD163 and CD206 expression levels ($p < 0.01$) relative to the model group. Furthermore, the incorporation of MSCs into the hydrogel (C/GP/Co/MSCs group) led to a further reduction in both markers ($p < 0.001$), demonstrating a synergistic anti-inflammatory impact of the hydrogel and MSCs (Fig. 5A–C).

Biomechanical testing

Biomechanical testing of the Achilles tendon indicated that the model group showed the lowest maximum tensile strength. The C/GP/Co hydrogel group exhibited enhanced tensile strength compared to the model group (Fig. 6A, B). Notably, the C/GP/Co/MSCs hydrogel group demonstrated a significant increase in tensile strength compared to the C/GP/Co hydrogel group. The control group showed the highest tensile strength (Fig. 6C).

Discussion

This study demonstrated that C/GP/Co/MSCs hydrogels effectively suppress inflammation and apoptosis in chronic Achilles tendon tissue through the AKT/GSK-3*β*

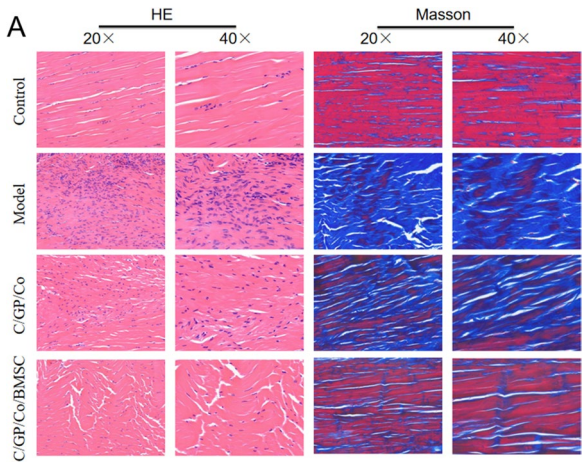

Fig. 3 Evaluation of tendon inflammation, fiber arrangement, and collagen structure in rats with chronic Achilles tendon injury treated with C/GP/Co/MSCs hydrogel. **A** Tendon macroscopic images, H&E staining, and Masson's trichrome staining of the four groups

signaling pathway. Histological analyses indicated that the anti-apoptotic and tendon repair properties of C/GP/Co/MSCs hydrogels surpassed those of C/GP/Co hydrogels. Furthermore, treatment with C/GP/Co/MSCs hydrogels significantly enhanced the tensile strength of Achilles tendons in rats with tendinopathy. These results suggest that the therapeutic action of C/GP/Co/MSCs hydrogels may involve the activation of the AKT/GSK-3β pathway.

The C/GP/Co composite scaffold promotes the adhesion, proliferation, and differentiation of MSCs, establishing an optimal microenvironment for tissue regeneration. This scaffold improves extracellular matrix (ECM) deposition and remodeling, enhancing tissue strength and elasticity. MSCs secrete growth factors and cytokines that stimulate Achilles tendon cell proliferation and differentiation, accelerating tissue regeneration. Collagen, a vital ECM component, is crucial for maintaining tissue integrity [17]. Our study illustrated that the C/GP/Co/MSCs hydrogel increased collagen deposition and enhanced the type I/III collagen ratio, improving tendon biomechanical properties. The maximum tensile strength in the C/GP/Co/MSCs hydrogel group significantly exceeded that of the model and C/GP/Co hydrogel groups, demonstrating the effectiveness of this treatment in enhancing the

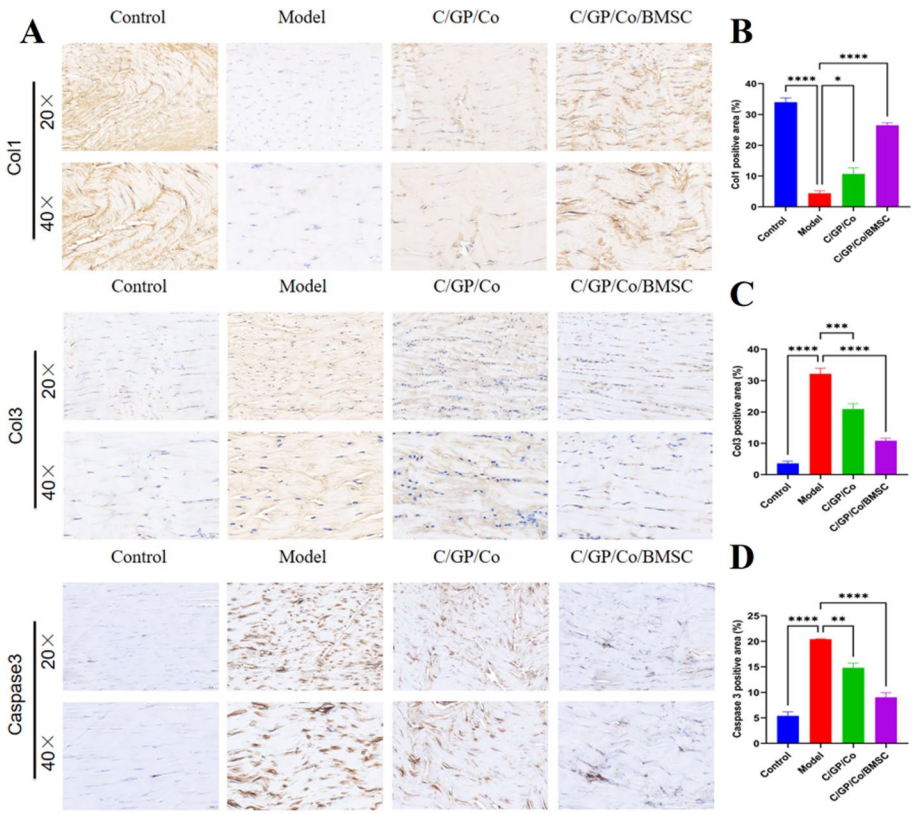

Fig. 4 Repair effects of C/GP/Co/MSCs hydrogel on rats with chronic Achilles tendon injury. **A** Immunohistochemical staining of COL1, COL3, and Caspase-3 in the four groups. **B**–**D** Quantitative analysis of positive staining areas for COL1, COL3, and Caspase-3 using ImageJ. Data are shown as mean ± SD ($N = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

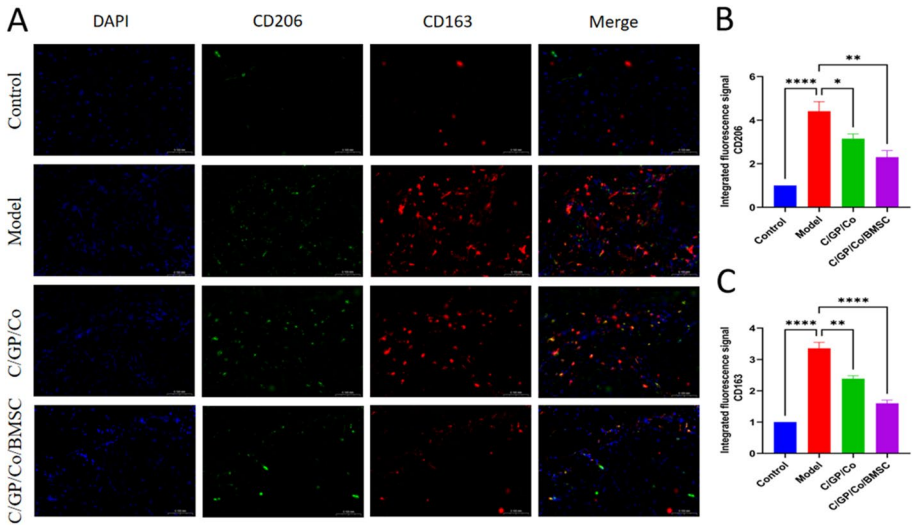

Fig. 5 Anti-inflammatory effects of C/GP/Co/MSCs hydrogel in rats with chronic Achilles tendon injury. **A** Fluorescence images of CD163 and CD206 expression in the four groups. **B**, **C** Quantification of fluorescence intensity. Data are shown as mean ± SD ($N = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

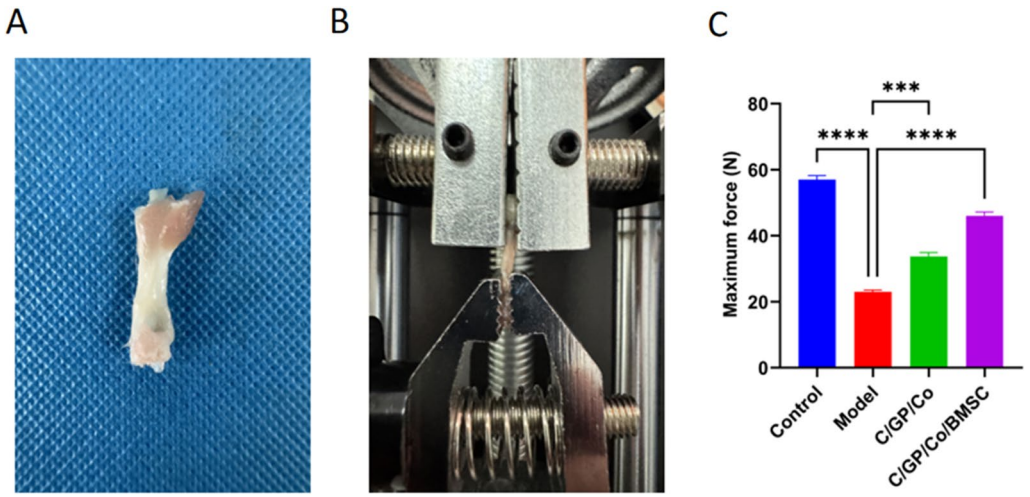

Fig. 6 Biomechanical evaluation of the Achilles tendon. **A** Tendon samples, including the calf muscle, Achilles tendon, and calcaneus. **B** Tendon mounted on a biomechanical testing machine. **C** Maximum tensile strength of the four groups. Data are expressed as mean ± SD ($N = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

mechanical properties of injured tendons towards normal tendon characteristics.

Akt and GSK-3β are pivotal intracellular signaling molecules involved in cellular processes such as proliferation, differentiation, apoptosis, and metabolism [18, 19]. The AKT/GSK-3β pathway plays a crucial role in tendon repair by modulating MSC function. MSCs secrete growth factors and cytokines, which, combined with mechanical hydrogel stimulation, trigger the PI3K/AKT signaling pathway in tendon cells. Akt activation results in GSK-3β phosphorylation and deactivation, a key step in tendon repair[20–22]. Active GSK-3β inhibits cell proliferation and differentiation, induces apoptosis, and impedes tissue regeneration. By activating the AKT/GSK-3β pathway, MSCs proliferate and differentiate into tendon cells, acting as a cellular reservoir for tendon repair [23, 24]. Furthermore, pathway activation boosts extracellular matrix (ECM) synthesis, including collagen production, thereby supporting tissue repair and remodeling.

Chronic inflammation hinders tendon healing, with the AKT/GSK-3β pathway known to regulate inflammatory responses and aid tissue repair by decreasing inflammatory factor production. Additionally, angiogenesis is essential for supplying nutrients and oxygen to damaged tissues, and the AKT/GSK-3β pathway may boost angiogenesis by increasing vascular endothelial growth factor (VEGF) expression. Previous studies have shown that modulating the AKT/GSK-3β/β-catenin signaling pathway can improve tendon function and reduce issues like excessive fat infiltration in rotator cuff muscles [25]. This study indicates that the C/GP/Co/MSCs hydrogel likely promotes MSC proliferation and differentiation by creating a favorable microenvironment and activating the AKT pathway directly or indirectly. This activation inhibits GSK-3β, decreasing apoptosis and supporting tendon repair, underscoring the potential of the AKT/GSK-3β pathway as a therapeutic target for treating chronic tendinopathy.

Apoptosis plays a critical role in regulating cell proliferation and maintaining cellular homeostasis. Excessive apoptosis in tendon cells is implicated in tendon diseases [26]. Kan et al. demonstrated a hydrogel-based microneedle system's long-term anti-inflammatory and anti-apoptotic effects [27]. Our study found that chronic Achilles tendon injury led to increased expression of Caspase-3 and the Bax/Bcl-2 ratio, indicating heightened tissue apoptosis. Treatment with C/GP/Co/MSCs hydrogel decreased Caspase-3 expression and Bax/Bcl-2 ratio, suggesting its potential to alleviate apoptosis in injured tissues.

Inflammation significantly contributes to the progression of chronic Achilles tendon injuries. Elevated levels of CD206 and CD163 in the model group indicate inflammatory responses and disruption of tendon structure. Treatment with C/GP/Co/MSCs hydrogel reduced the expression of these markers, suppressed inflammation, and improved tendon structural integrity. Studies have shown that prolonged chronic inflammation can stem from insufficient clearance of apoptotic cells, leading to fibrosis and further damage [27–29]. C/GP/Co/MSCs hydrogels promoted tendon healing in this study by mitigating inflammation and apoptosis.

This study demonstrated the efficacy of temperature-sensitive, injectable C/GP/Co hydrogels combined with MSCs for treating chronic Achilles tendon injury in rats. Furthermore, it provided preliminary evidence suggesting the involvement of the AKT/GSK-3β signaling pathway in mediating this therapeutic effect. These findings indicate that the hydrogel system represents a promising approach for managing chronic Achilles tendinopathy and improving patient outcomes.

This study, while promising, presents limitations that warrant further investigation. Specifically, our analysis of the AKT/GSK-3β signaling pathway was conducted at a correlative level. Future research should incorporate mechanistic inquiries, such as employing gene knockout models or specific inhibitors, to enhance our understanding of the pathway's function. Furthermore, the small sample size and short study duration necessitate further investigation with a larger cohort and longer follow-up period to confirm the long-term therapeutic efficacy of the Chitosan/β-Glycerophosphate/Collagen hydrogel and MSCs in chronic Achilles tendon injury and fully elucidate the involvement of the Akt/GSK-3β pathway. These further investigations are necessary to refine clinical applications and bolster the theoretical basis for treating tendon injuries.

Conclusions

This study examined the therapeutic efficacy of C/GP/Co hydrogels in conjunction with MSCs for chronic Achilles tendon injury. The outcomes reveal that this hydrogel formulation elicits anti-inflammatory, anti-apoptotic, and tendon-repair responses via the AKT/GSK-3β signaling cascade. Moreover, the C/GP/Co/MSCs hydrogel not only ameliorated the histological profile of the tendon injury but also notably augmented the mechanical robustness of the Achilles tendon. These results underscore the potential clinical utility of the C/GP/Co/MSCs hydrogel for addressing chronic Achilles tendinopathy.

Abbreviations

C	Chitosan
β-GP	β-Glycerophosphate
Co	Collagen
MSCs	Bone marrow mesenchymal stem cells

Authors' contributions

Songlin Liu conducted the implementation experiments, data collection and data analysis, and wrote the paper; Ma Liang conceptualized, designed and revised the paper. All authors reviewed the results and finalized the final version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval

The experimental animals were handled in strict accordance with the guidelines for the treatment and handling of experimental animals, and the experimental procedures were approved by the Animal Research Ethics Committee of Yangtze University [YZLL2024-015].

Consent for publication

All patients provided written informed consent for the publication of their identifying photographs.

Competing interests
The authors declare no competing interests.

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