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Human umbilical cord mesenchymal stem cell exosomes promote elastin production and acute skin wound healing via TGFβ1-Smad pathway

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Abstract

Background Skin wound healing is a complex physiological process influenced by multiple factors, including the patient's overall health status. Exosomes derived from human umbilical cord mesenchymal stem cells (hUCMSC-exos) have demonstrated considerable potential in enhancing wound repair. This study investigates the mechanisms through which hUCMSC-exos facilitate skin wound healing and evaluates their potential application in combination with hydrogels for clinical treatment.

Method Human foreskin fibroblasts (HFF-1) were treated with varying concentrations of hUCMSC-exos to evaluate their impact on cell proliferation, assessed via the CCK-8 assay. Exosome uptake by HFF-1 cells was visualized using PKH26 dye staining, while flow cytometry was employed to analyze cell cycle changes. Cell migration was evaluated through scratch and Transwell assays. Gene expression levels of Collagen I, Elastin, and Fibronectin were quantified by qRT-PCR, while Elastin secretion was measured by ELISA. Western blotting was used to examine proteins in the TGFβ1-Smad signaling pathway. The role of SP1 in regulating Elastin gene expression was investigated by testing the SP1 inhibitor Plicamycin and examining hUCMSC-exos' ability to counteract its effect. Additionally, a chromatin immunoprecipitation (ChIP) assay was performed to analyze SP1 binding at the Elastin gene promoter. In vivo, the efficacy of hUCMSC-exos combined with hydrogels in promoting wound healing was assessed using a mouse skin wound model.

Results hUCMSC-exos significantly enhanced HFF-1 cell proliferation at concentrations exceeding 1×10^9 particles/mL and increased the proportion of cells in the S and G2/M phases. HFF-1 cells readily absorbed these exosomes, leading to improved cell migration. Treatment with hUCMSC-exos upregulated the gene expression of Collagen I, Fibronectin, and Elastin. The SP1 inhibitor Plicamycin reduced Elastin gene expression, an effect that was reversed by hUCMSC-exos. In vivo, the combination of hUCMSC-exos and hydrogels accelerated wound healing, enhanced collagen organization, and promoted the formation of elastic fibers and blood vessels.

Conclusions hUCMSC-exos facilitate skin wound healing by promoting SP1 binding to the Elastin gene promoter, thereby upregulating Elastin expression and supporting extracellular matrix remodeling. These findings suggest a promising therapeutic role for hUCMSC-exos in clinical applications for wound healing.

1. Background

Mesenchymal stem cells (MSCs) are multipotent adult stem cells originating from the mesoderm, with the capacity to differentiate into various cell types, including adipocytes, osteocytes, and chondrocytes. They can be isolated from diverse sources such as bone marrow, adipose tissue, placenta, and umbilical cord blood, making them readily accessible for therapeutic applications[1]. MSCs play a crucial role in tissue repair through the secretion of various cytokines and growth factors, such as epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), which exhibit anti-inflammatory, anti-apoptotic,

and angiogenic properties, thereby regulating cell proliferation, differentiation, and migration[2][3][4]. Among these sources, human umbilical cord mesenchymal stem cells (hUC-MSCs) are particularly advantageous due to their abundant availability and ease of isolation[5].

Beyond their regenerative properties, MSCs secrete exosomes—extracellular vesicles that mediate intercellular communication[6][7]. Exosomes, typically measuring 50 to 150 nm in diameter, form via plasma membrane invagination, resulting in early endosome formation[8]. These endosomes can either fuse with lysosomes for degradation or with the plasma membrane to release their intraluminal vesicles as exosomes[9]. The contents of these exosomes, which include proteins, lipids, and RNA, reflect the characteristics of their cells of origin[10]. Upon release, exosomes can affect distant tissues through the bloodstream or support paracrine signaling locally[6]. Notably, exosomes derived from stem cells contain specific proteins and nucleic acids that promote cell proliferation and modulate immune responses, offering an appealing alternative to direct stem cell therapies[11]. They address several limitations of traditional stem cell applications, such as reduced viability and efficacy after extended culture, high costs, and potential tumorigenicity[12][13]. Additionally, their low immunogenicity and ease of administration enhance their clinical potential, particularly in wound healing applications[14].

The wound healing process is intricate, encompassing phases such as inflammation, cell proliferation, migration, extracellular matrix (ECM) synthesis, angiogenesis, and remodeling[15][16]. Each phase requires precise coordination among various cell types and is influenced by intrinsic and extrinsic factors that can impede healing. Conventional wound management typically involves initial disinfection and debridement, followed by the application of topical agents, including recombinant EGF and mupirocin ointment. However, these treatments may exhibit limited efficacy, particularly in complex wounds or unique physiological conditions, highlighting the need for innovative strategies that accelerate healing and reduce scarring.

Recent research has increasingly focused on exosomes derived from various MSC sources, as well as functional dressings, to enhance wound healing. Studies demonstrate that MSC-derived exosomes facilitate skin wound repair through mechanisms such as modulation of inflammation, immune protection, angiogenesis, and ECM remodeling[1]. Functional dressings, particularly moist wound dressings, offer essential support by providing a conducive environment for healing. Furthermore, synergistic effects have been observed when exosomes are combined with specialized dressings[17]. For instance, gelatin hydrogels loaded with bone marrow MSC-derived exosomes have been shown to enhance healing in rat skin injuries[18], while the use of adipose-derived stem cell-conditioned medium with injectable hydrogels has effectively inhibited hypertrophic scarring in rabbit ear wound models[19].

The skin consists of three primary layers: the epidermis, dermis, and subcutaneous tissue[20]. The epidermis is primarily composed of keratinocytes, while the dermis contains fibroblasts essential for the synthesis and remodeling of ECM components[21][22]. Injury to the skin results in the loss of both ECM proteins and cells, necessitating cellular proliferation and ECM remodeling to restore skin integrity[23][24].

Scar formation, marked by excessive collagen deposition and reduced cellularity, often results from healing processes and lacks the elasticity of normal tissue[25].

In this study, we observed that exosomes, either alone or in combination with hydrogel, significantly promoted wound healing in Kunming mice. After 15 days, the exosome-treated group exhibited markedly improved wound closure, collagen deposition, elastin levels, and angiogenesis compared to the control group. In vitro, exosomes enhanced HFF-1 cell proliferation and migration through key processes, including cell proliferation, scratch assays, and upregulation of several crucial ECM proteins. Exosomes significantly upregulated gene expression of Collagen I, Fibronectin, and Elastin, with the most notable enhancement observed in Elastin transcription. Although elastin synthesis typically ceases in post-embryonic adults, it can be reactivated in response to injury or disease[26][27]. Factors such as tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) are known to influence this process[26]. Elastin not only provides mechanical support for skin but also plays a role in cell signaling, which can induce cell proliferation and migration, enhance cellular adhesion, and thus support skin wound healing. Additionally, elastin reduces wound contraction and promotes dermal regeneration[28][29].

Further analysis suggests that these effects may be mediated through the TGFβ1-Smad signaling pathway, as confirmed by chromatin immunoprecipitation (ChIP) experiments. These findings indicate that the TGFβ1-Smad pathway ultimately influences SP1 binding to the GC-rich region of the Elastin promoter, thereby enhancing Elastin transcription.

In summary, hUCMSC-exos enhance elastin transcription and facilitate wound healing, representing a promising therapeutic advancement for improving wound healing outcomes through targeted molecular strategies.

2. Methods

2.1 Exosome sequestering and identification

hUCMSCs were cultured in T125 flasks (NEST, China) until reaching 80% confluence. The spent medium was then removed, and the cells were gently washed twice with Dulbecco's Phosphate-Buffered Saline (DPBS). Cells were subsequently cultured in a serum-free medium for 24 hours to collect the supernatant. The collected supernatant underwent centrifugation at 300 g for 5 minutes, followed by a secondary centrifugation at 2000 g for 20 minutes to eliminate cell debris. To further purify the exosomes, the supernatant was subjected to centrifugation at 10,000 g for 50 minutes to remove organelles and microvesicles, followed by filtration through a 0.22 μm membrane filter. The filtered supernatant was then transferred to ultracentrifuge tubes and centrifuged at 100,000 g for 2 hours at 4°C. The supernatant was discarded, and the resulting pellet was resuspended in PBS by gentle pipetting. The prepared exosome solution could be used immediately or stored at -80°C for future use. Exosome morphology was assessed via transmission electron microscopy, while size and concentration

were determined using nanoparticle tracking analysis. Expression of specific exosomal markers, including CD9, CD63, and TSG101, was verified by Western blotting.

2.2 Establishment of an Animal Wound Model

Twenty female Kunming mice, aged 8 weeks and weighing approximately 40 g, were housed under controlled environmental conditions (relative humidity of 60 ± 5%, temperature of 22 ± 2°C, and a 12-hour light-dark cycle). All experimental procedures adhered to ethical guidelines for animal research and received approval from the Ethics Committee of Yanbian University Experimental Animal Center.

The mice were randomly assigned to one of four groups: PBS group, hydrogel group, hUCMSC-Exos group, and hydrogel + hUCMSC-Exos group, with five mice per group. Anesthesia was induced via intraperitoneal administration of a 2.5% Avertin solution at a dosage of 0.15 mL/10 g body weight. After anesthesia, fur on the mice's backs was removed with a hair clipper, followed by application of depilatory cream using a cotton swab until complete fur removal. The exposed area was then cleaned multiple times with sterile water. A circular full-thickness skin wound model, approximately 8 mm in diameter, was created on the back of each mouse using an 8 mm circular skin punch.

Each group received a different treatment applied to the wound: 100 μL of PBS, 25% hydrogel, 1×10^9 particles/mL exosomes, or a combination of 25% hydrogel and 1×10^9 particles/mL exosomes. Immediately following surgery, each mouse was photographed and labeled, then housed individually to prevent mutual aggression that could interfere with wound healing. Wound status was documented photographically every three days, and the respective treatment (PBS, hydrogel, hUCMSC-Exos) was reapplied as necessary. Wound area was measured using ImageJ software, with the healing rate on day *n* calculated as follows: Healing Rate on Day n=(Initial Wound Area-Wound Area on Day nInitial Wound Area)/ Initial Wound Area×100%. On day 15 post-surgery, the mice were euthanized using an overdose of anesthesia, and skin tissue from the wound area was excised, trimmed to approximately 1 cm in diameter, and fixed in 4% paraformaldehyde.

2.3 Immunohistochemistry assays

After 24 hours of fixation, skin tissue samples were processed for routine paraffin embedding and sectioned at a thickness of 5 μm. Following the manufacturer's protocols (Solarbio, China), the tissue sections were stained using Hematoxylin and Eosin (H&E), Masson's trichrome, and a modified Gomori's aldehyde fuchsin method. Additionally, sections were immunohistochemically stained for CD31 (Abcam, UK) to assess angiogenesis. These histological evaluations were conducted to analyze wound healing by examining cellularity, extracellular matrix composition, and angiogenesis at the wound site. Staining results were captured using a CNOPTECCOM optical microscope (Aotai, China).

2.4 Cell culture

The standard cell culture medium consisted of DMEM supplemented with 15% fetal bovine serum (FBS,Gibco,USA)and 1% penicillin-streptomycin. To evaluate the effects of varying concentrations of hUCMSC-Exos on HFF-1 cells, appropriate volumes of exosome stock were added to DMEM to achieve

final concentrations of Exos1 at 1 × 10^8 particles/mL and Exos2 at 1 × 10^9 particles/mL. Additionally, to investigate the transcriptional effects of Plicamycin (MCE, USA) on HFF-1 cells, Plicamycin powder was dissolved in DMSO to prepare a 1 mM stock solution, which was subsequently diluted in DMEM.

2.5 Uptake Experiment of hUCMSC-Exosomes

hUCMSC-Exos were labeled with PKH-26 (Sigma, USA) and mixed with DMEM before being applied to cells at approximately 40% confluence. Following a 24-hour incubation, the DMEM was removed, and the cells were washed thoroughly. The cells were then fixed in 4% paraformaldehyde and subsequently incubated with DAPI solution (Abcam, UK) for 2 hours. Fluorescence microscopy (Olympus, Japan) was utilized to observe and document the cells through imaging.

2.6 CCK-8 assays

The effect of hUCMSC-Exos on HFF-1 cell proliferation was evaluated using a CCK-8 assay kit (APExBIO, USA). HFF-1 cells (5 × 10^3 cells per well) were seeded into a 96-well plate and cultured in complete medium for 24 hours. The medium was then discarded, and the cells were starved in DMEM for 4 hours. After starvation, the medium was removed, and the cells were washed once with PBS. Each group was subsequently treated with DMEM containing 1% penicillin-streptomycin and the corresponding concentration of exosomes. The cells were incubated for 24, 48, and 72 hours, after which the medium was discarded and the cells were washed twice with PBS. Subsequently, 200 μL of DMEM containing 1% CCK-8 reagent was added to each well, followed by incubation at 37°C for 2 hours. The optical density (OD) at 450 nm was then measured for each well.

2.7 Cell Cycle Analysis

HFF-1 cells (3 × 10^5 cells) were seeded in each 6 cm culture dish and cultured in complete medium for 24 hours. The medium was then discarded and replaced with DMEM containing the designated concentration of exosomes. After 24 and 48 hours of incubation, cells were digested with trypsin, and the supernatant was removed by centrifugation at 1500 g for 5 minutes. The resulting cell pellet was resuspended in 2 mL of PBS and washed by centrifuging again at 1500 g for 5 minutes, a step repeated once. Cells were subsequently resuspended in 250 μL of pre-chilled PBS and gradually added to 750 μL of pre-chilled absolute ethanol, gently mixed, and fixed overnight at 4°C.

Following fixation, cells were centrifuged at 1000 g for 5 minutes to remove the supernatant, then washed by resuspension in 1 mL of pre-chilled PBS and centrifuged again at 1000 g for 5 minutes. Each tube received 500 μL of PI/RNase solution (Thermo, USA), and the cells were gently mixed and incubated in the dark at 37°C for 30 minutes. Flow cytometry (Beckman Coulter, USA) was then performed to analyze the samples.

2.8 Scratch wound assays

When the cell confluence in the six-well plate reached approximately 80%, a vertical scratch was created at the bottom of each well using a 200 μL pipette tip. The wells were washed multiple times with PBS to

remove any detached cells from the scratched area. Serum-free DMEM containing the designated concentration of hUCMSC-Exos was then added to each group. Cell migration was observed and recorded through photography at 0, 12, 24, and 36 hours. The scratch area was analyzed using ImageJ software, with the migration rate at hour n calculated as follows : Migration Rate at Hour n=(Initial Scratch Area − Scratch Area at Hour n)/Initial Scratch Area×100%.

2.9 Transwell assays

In a 24-well plate, 100 μL of a cell suspension containing 3 × 10^4 cells was added to the upper chamber of Transwell inserts (Merck Millipore, USA), while the lower chamber was filled with DMEM containing the designated concentration of hUCMSC-Exos. After 12 hours of incubation, the Transwell inserts were removed, and a cotton swab was used to gently remove cells from the upper membrane surface. The inserts were then fixed in 4% paraformaldehyde for 10 minutes and stained with crystal violet for 10 minutes. Following staining, the membranes were washed with PBS to remove any excess dye and allowed to air-dry. Each insert was mounted onto a glass slide, and 12 independent fields of view were selected to capture images and count the number of cells that had migrated through the membrane. The average cell count across fields was calculated for comparative analysis.

2.10 Real-time quantitative polymerase chain reaction (RT-qPCR)

Cells were cultured in 6 cm dishes, and upon reaching 50% confluence, the medium was replaced with DMEM containing the specified concentration of hUCMSC-Exos for an additional 24 hours. RNA was extracted using TRIzol reagent (Thermo, USA), and complementary DNA (cDNA) was synthesized with the PrimeScript™ RT Master Mix (Takara, Japan). Quantitative PCR (qPCR) was conducted using TB Green® Premix Ex Taq™ II (Takara, Japan), with relative expression levels of target genes calculated by the 2-ΔΔCt method, using RPL13A as the internal control. The amplification primers were as follows:

Table 1
Primers used in research.

Gene		Primer(5'-3')
Collagen I	Forward primer	cacagaggtttcagtggtttgg
	Reverse primer	gcaccagtagcaccatcatttc
Fibronectin	Forward primer	aagattggagagaagtgggacc
	Reverse primer	gagcaaatggcaccgagata
Elastin	Forward primer	gggttgtgtcaccagaagca
	Reverse primer	caaccccgtaagtaggaatgc
RPL13A	Forward primer	gcacgaccttgagggcagcc
	Reverse primer	catcgtggctaaacaggtactg

2.11 WB analysis

Total protein was extracted from HFF-1 cells or hUCMSC-Exos using RIPA lysis buffer (Absin, China), and protein concentration was determined using a BCA assay kit (Thermo, USA). After electrophoresis, proteins were transferred from the gel to a PVDF membrane. The membrane was blocked at room temperature for 2 hours with TBST containing 5% non-fat dry milk (Solarbio, China), then incubated overnight at 4°C with primary antibodies against CD9, CD63, TSG101, Smad2/3, P-Smad2/3, Smad4, TGFβ1, SP1 (Abcam, UK), and TGFβR, GAPDH (CST, USA). Following three 10-minute washes with TBST, the membrane was incubated with a secondary antibody (CST, USA) at room temperature for 1 hour. Data analysis was conducted using ImageJ software.

2.12 ChIP-qPCR assays

Cells were cultured until reaching 50% confluence, after which DMEM containing the specified concentrations of Plicamycin and hUCMSC-Exos was added for an additional 24 hours prior to the ChIP-qPCR experiment. The ChIP assay was conducted using the Pierce™ Magnetic ChIP-qPCR Kit (Thermo, USA) with an SP1 antibody (Abcam, UK). qPCR was performed using TB Green® Premix Ex Taq™ II (Takara, Japan), and the fold enrichment of target genes was calculated using the Fold Enrichment method. The primers for the Elastin promoter region were as follows: Forward primer (5'-3'): CTCCCTTCTGCTTCCTCTCC, and Reverse primer (5'-3'): TCTCTTTCCCGCTTTCCCTC.

2.13 Statistical analysis

Continuous variables are presented as means ± standard deviation (SD). Data were analyzed using one-way ANOVA in GraphPad Prism (GraphPad, La Jolla, USA), with a p-value of ≤ 0.05 considered indicative of statistically significant differences.

3. Results

3.1 Identification of hUCMSC-Exos and Their Uptake by HFF-1 Cells

Nanoparticle tracking analysis indicated that the extracted extracellular vesicles (EVs) exhibited a uniform diameter of 63.8 ± 14.6 nm and a concentration of 1.76×10^9 particles/mL. Electron microscopy confirmed their characteristic cup-shaped morphology and expression of surface markers CD9 and CD63, as well as the internal marker TSG101, consistent with established EV characteristics (Fig. 1A, 1B and 1C).

To investigate the interaction between hUCMSC-Exos and HFF-1 cells, EV membranes were labeled with PKH-26 dye, resulting in red fluorescence, while HFF-1 cell nuclei were stained with DAPI, producing blue fluorescence. After co-culturing for 24 and 48 hours, fluorescence microscopy showed localization of hUCMSC-Exos around the nuclei of HFF-1 cells, indicating successful internalization. The red fluorescence intensity increased over time, demonstrating that HFF-1 cells can continuously absorb hUCMSC-Exos (Fig. 1D).

These findings underscore the capacity of hUCMSC-Exos for cellular communication and their potential for therapeutic applications.

3.2 hUCMSC-Exos Enhance Proliferation and Migration of HFF-1 Cells

To assess the impact of hUCMSC-Exos on HFF-1 cell proliferation, a concentration gradient from 1×10^5 to 1×10^9 particles/mL in DMEM was applied, with a control group (Con) at 0 concentration. After 24, 48, and 72 hours of incubation, results from the CCK-8 assay indicated that 1×10^9 particles/mL significantly enhanced HFF-1 proliferation (Fig. 2A). Based on these findings, the effective concentrations selected for subsequent experiments were designated as Exos1: 1×10^8 and Exos2: 1×10^9.

Cell cycle analysis showed that following 24 hours of stimulation with Exos1 and Exos2, the proportion of cells in the G0/G1 phase decreased, while the S phase increased, leading to a higher proliferation index (PI). These changes were statistically significant (Fig. 2B).

To evaluate the effect of hUCMSC-Exos on HFF-1 cell migration, scratch and Transwell assays were performed. In the scratch assay, treatment with hUCMSC-Exos at both concentrations significantly enhanced cell migration at 12 hours, with Exos2 showing marked promotion at 24 hours (Fig. 2C and 2E).

Transwell assay results demonstrated that after 12 hours of stimulation, hUCMSC-Exos significantly promoted HFF-1 cell migration to the lower chamber (Fig. 2D and 2F).

These findings indicate that hUCMSC-Exos enhance both the proliferation and migration capabilities of HFF-1 cells.

3.3 hUCMSC-Exos Enhance Gene Expression of Collagen I, Fibronectin, and Elastin in HFF-1 Cells

Collagen I, Fibronectin, and Elastin are essential components of the ECM, providing its structural framework. Collagen I plays a critical role in preventing skin dehydration, maintaining tissue firmness, and imparting skin tension. Fibronectin aids in cell adhesion to the ECM and is integral to cell migration, while Elastin contributes to the resilience and elasticity of skin tissue through the elastic fiber network.

To elucidate the mechanism by which hUCMSC-derived exosomes (hUCMSC-Exos) facilitate skin wound healing, we performed qRT-PCR analysis to evaluate the gene expression levels of Collagen I, Fibronectin, and Elastin. The results showed that hUCMSC-Exos significantly upregulated RNA levels of these ECM components in HFF-1 cells, with Collagen I and Fibronectin levels increasing by 1-2 times and Elastin levels rising by 3-10 times (Fig. 3A).

Based on these findings, further experiments will focus on the specific enhancement of Elastin expression by hUCMSC-Exos.

3.4 hUCMSC-Exos Promote Expression of TGFβ1-Smad Pathway Proteins in HFF-1 Cells

To investigate how hUCMSC-Exos enhance Elastin expression in HFF-1 cells, we used the STRING database to identify proteins associated with Elastin expression. Literature indicates that this effect is linked to Transforming Growth Factor Beta 1 (TGFβ1), which is abundant in hUCMSC-Exos. TGFβ1 enters cells and activates the TGFβ1-Smad signaling pathway, influencing the binding of the transcription factor SP1 to the GC box in the Elastin gene promoter, thereby regulating its transcription.

Upon activation, TGFβ1 binds to TGFβRII, which recruits TGFβRI to form a receptor complex. TGFβRII phosphorylates TGFβRI, which subsequently phosphorylates Smad2 and Smad3. These receptor-regulated Smads (R-Smads) then form oligomers with Smad4, translocating to the nucleus to regulate target gene expression. Although Smad3 and Smad4 have limited DNA-binding activity, they are likely to interact with SP1 to modulate Elastin gene expression.

Western blot analysis confirmed that hUCMSC-Exos significantly upregulated the expression levels of TGFβ1, Smad2/3, phosphorylated Smad2/3 (P-Smad2/3), TGFβRII, and SP1 in HFF-1 cells at the Exos2 concentration, with statistically significant differences observed (Fig. 3B and 3C).

These results suggest that hUCMSC-Exos promote Elastin expression in HFF-1 cells through activation of the TGFβ1-Smad signaling pathway.

3.5 Reversal of Plicamycin-Induced Inhibition of Elastin Gene Expression by hUCMSC-Exos

To further investigate whether hUCMSC-Exos influence Elastin gene transcription through the TGFβ1-Smad pathway by promoting SP1 binding to the GC box, we used Plicamycin, a specific SP1 inhibitor that binds to the GC box to suppress SP1 activity.

qRT-PCR analysis was performed to assess the impact of various Plicamycin concentrations (10 nM to 200 nM) on Elastin gene transcription in HFF-1 cells. Results indicated a concentration-dependent inhibition of Elastin expression, with significant effects observed at 200 nM (Fig. 3D).

We also evaluated the combined effect of 200 nM Plicamycin and hUCMSC-Exos on Elastin transcription. qRT-PCR results revealed that hUCMSC-Exos significantly mitigated the inhibitory effect of Plicamycin on Elastin RNA levels (Fig. 3E), suggesting that while Plicamycin inhibits SP1 binding to the GC box, hUCMSC-Exos promote SP1-GC box interaction, thereby enhancing Elastin gene transcription.

Our findings indicate that hUCMSC-Exos can reverse the inhibitory effects of Plicamycin on Elastin gene expression in HFF-1 cells by modulating the TGFβ1-Smad pathway, subsequently promoting SP1 interaction with the GC box in the Elastin promoter region.

To confirm that these effects are mediated through SP1 binding to the GC box, we conducted ChIP-qPCR experiments following treatment of HFF-1 cells with hUCMSC-Exos and Plicamycin. The results (Fig. 3F) showed that Plicamycin significantly reduced the enrichment of the GC box region in the Elastin gene promoter. However, treatment with Exos1 (1×10^8 exosomes/mL) restored the enrichment to control levels, while Exos2 (1×10^9 exosomes/mL) significantly enhanced enrichment by approximately threefold.

These findings confirm that hUCMSC-Exos promote SP1 binding to the Elastin promoter, effectively counteracting the inhibitory effects of Plicamycin.

3.6 hUCMSC-Exos combined with hydrogel promotes skin wound healing in mice and promotes extracellular matrix remodeling

An 8 mm full-thickness skin wound model was established in female Kunming mice. Photographs were taken on days 0, 3, 6, 9, 12, and 14 post-surgery to calculate wound healing rates. Results showed that both the exosome group and the exosome + hydrogel group significantly accelerated wound healing compared to the control group, with the hydrogel group exhibiting a slightly faster healing rate than the control (Fig. 4A).

Histological analysis with H&E staining revealed defects in the epidermis and dermis in the control group, whereas the exosome and exosome + hydrogel groups demonstrated nearly complete wound closure with tightly connected tissues and intact structures (Fig. 4B). Inflammatory cell infiltration was more pronounced in the control and exosome groups, while the exosome + hydrogel group showed significantly reduced inflammatory cells and milder hyperkeratosis.

Masson staining further highlighted differences in tissue composition (Fig. 4C). The control group displayed sparse collagen fibers, while the exosome + hydrogel group exhibited a well-organized structure with abundant, aligned collagen fibers, suggesting that both exosomes and their combination with hydrogel contribute to reduced scarring during recovery.

Elastic fibers, evaluated through the modified Gomori's aldehyde fuchsin method, showed that the PBS group contained few, thin elastic fibers (Fig. 4D). In contrast, the exosome group displayed thicker fibers, and the exosome + hydrogel group showed robust, well-aligned fibers parallel to the skin surface. This suggests that exosomes enhance the formation and crosslinking of elastic fibers during wound healing, with hydrogel further improving fiber organization, potentially facilitating scar-free healing.

Immunohistochemical staining for the angiogenic marker CD31 indicated substantial vascular formation within the wounds. As shown in Figure 4E and 4F, both the exosome and exosome + hydrogel groups exhibited a significant increase in blood vessel formation, indicating enhanced angiogenesis.

Collectively, these findings underscore the potential of hUCMSC-derived exosomes in promoting effective wound healing and tissue regeneration.

4. Discussion

The skin, as the body's largest organ, serves as the primary barrier against external stimuli. Wound healing is a complex, continuous process and a focus of clinical research due to its susceptibility to various environmental and physiological factors. Despite the availability of numerous treatments aimed at promoting wound healing, their effectiveness and scope are often limited, necessitating more adaptable solutions for complex wounds. Recent studies have highlighted the potential of exosomes in enhancing wound healing, reducing scarring, and improving skin restoration. However, intravenous administration of exosomes may reduce their efficacy, and localized injections can lead to secondary damage or uneven distribution. This study explores the combination of hUCMSC-Exos with hydrogel to elucidate their molecular mechanisms in promoting wound healing, providing insights for potential clinical applications.

In the early stages of wound healing, including the hemostasis and inflammation phases, processes such as fibroblast proliferation, ECM deposition, angiogenesis, and re-epithelialization are essential for effective wound closure[30]. Our study confirmed that hUCMSC-Exos significantly enhanced HFF-1 cell proliferation, migration, and ECM deposition, notably upregulating the expression of Elastin, Collagen I, and Fibronectin. Remarkably, Elastin expression increased by 3 to 10 times, contributing to reduced scar formation by enhancing tissue elasticity and cell communication, both of which are critical for optimal wound healing.

To investigate the mechanism of Elastin regulation, we examined the role of the transcription factor SP1, which binds to the GC box in the Elastin promoter and is associated with the TGFβ1-Smad signaling pathway[31]. The SP1 inhibitor Plicamycin reduced Elastin expression in a dose-dependent manner; however, this effect was reversed with the introduction of hUCMSC-Exos, suggesting that hUCMSC-Exos modulate Elastin expression via the TGFβ1-Smad-SP1 pathway.

Hydrogels, known for their biocompatibility and moisture-retention properties, are widely used in wound healing. Studies indicate that hydrogels facilitate the controlled release of exosomes, thereby enhancing wound healing outcomes[32][33][34]. In our animal experiments, both hUCMSC-Exos alone and in combination with hydrogel accelerated wound healing. Histological analysis revealed that the combined treatment led to more organized collagen fiber deposition, reduced inflammation, and increased elastic fiber content, indicating a potential reduction in scar formation. Additionally, hUCMSC-Exos promoted angiogenesis, further contributing to enhanced wound repair.However, this study has several limitations, including potential biases due to the experimental design, the use of an animal model that may not fully replicate human wound healing, and the inherent variability in treatment administration and outcome measurements. These factors may affect the accuracy and generalizability of the results.

In summary, hydrogels augment the therapeutic effects of hUCMSC-Exos, presenting a promising strategy for improving wound healing outcomes in clinical applications.

5. Conclusions

hUCMSC-exos promote skin wound healing by enhancing SP1 binding to the Elastin gene promoter, upregulating Elastin expression, and aiding extracellular matrix remodeling. This suggests a potential therapeutic role for hUCMSC-exos in clinical wound healing applications.

Declarations

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Competing interests

The authors declare that they have no competing interests

Authors' contributions

Yi Zi was responsible for experimental design, conducting cell experiments, animal experiments, data analysis, article writing, etc.; Jie Li assisted in completing cell experiments, animal experiments and part of the data analysis; Xinping Qian and Jian Li assisted in completing animal experiments and CHIP experiments and data analysis; Yan Jin and Zibo Zhang assisted in completing part of the experiments and related data analysis; Yanhua Jin guided the experimental design and data analysis.The first draft was written by Yi Zi, all authors commented on the manuscript, and all authors read and approved the final manuscript.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The animal experiments were approved by the Yanbian University Experimental Animal Welfare Ethics Committee with the ethics approval number: YD20240510008.

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Consent for publication

Not applicable.

References

1. Lee DE, Ayoub N, Agrawal DK. Mesenchymal stem cells and cutaneous wound healing: novel methods to increase cell delivery and therapeutic efficacy. Stem Cell Res Ther. 2016;7:37. Published 2016 Mar 9. doi:10.1186/s13287-016-0303-6
2. Kim WS, Park BS, Sung JH, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J Dermatol Sci. 2007;48(1):15-24. doi:10.1016/j.jdermsci.2007.05.018
3. Liu L, Yu Y, Hou Y, et al. Human umbilical cord mesenchymal stem cells transplantation promotes cutaneous wound healing of severe burned rats. PLoS One. 2014;9(2):e88348. Published 2014 Feb 20. doi:10.1371/journal.pone.0088348
4. oon BS, Moon JH, Jun EK, et al. Secretory profiles and wound healing effects of human amniotic fluid-derived mesenchymal stem cells. Stem Cells Dev. 2010;19(6):887-902. doi:10.1089/scd.2009.0138
5. Zhao Bin, Wu Gaofeng, Zhang Yijie, et al. Effects of human amniotic epithelial stem cells-derived exosomes on healing of wound with full-thickness skin defect in rats[J]. Chin j Burns, 2017, 33(1): 18-23. Doi: 10.3760/cma.j.issn.1009-2587.2017.01.005
6. Heldring N, Mäger I, Wood MJ, Le Blanc K, Andaloussi SE. Therapeutic Potential of Multipotent Mesenchymal Stromal Cells and Their Extracellular Vesicles. Hum Gene Ther. 2015;26(8):506-517. doi:10.1089/hum.2015.072
7. Maas SLN, Breakefield XO, Weaver AM. Extracellular Vesicles: Unique Intercellular Delivery Vehicles. Trends Cell Biol. 2017;27(3):172-188. doi:10.1016/j.tcb.2016.11.003
8. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018;19(4):213-228. doi:10.1038/nrm.2017.125
9. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200(4):373-383. doi:10.1083/jcb.201211138

10. Ma L, Li Y, Peng J, et al. Discovery of the migrasome, an organelle mediating release of cytoplasmic contents during cell migration. Cell Res. 2015;25(1):24-38. doi:10.1038/cr.2014.135
11. Nikfarjam S, Rezaie J, Zolbanin NM, Jafari R. Mesenchymal stem cell derived-exosomes: a modern approach in translational medicine. J Transl Med. 2020;18(1):449. Published 2020 Nov 27. doi:10.1186/s12967-020-02622-3
12. Trohatou O, Roubelakis MG. Mesenchymal Stem/Stromal Cells in Regenerative Medicine: Past, Present, and Future. Cell Reprogram. 2017;19(4):217-224. doi:10.1089/cell.2016.0062
13. Hosseini M, Dalley AJ, Shafiee A. Convergence of Biofabrication Technologies and Cell Therapies for Wound Healing. Pharmaceutics. 2022;14(12):2749. Published 2022 Dec 8. doi:10.3390/pharmaceutics14122749
14. Han C, Sun X, Liu L, et al. Exosomes and Their Therapeutic Potentials of Stem Cells. Stem Cells Int. 2016;2016:7653489. doi:10.1155/2016/7653489
15. Martin P, Nunan R. Cellular and molecular mechanisms of repair in acute and chronic wound healing. Br J Dermatol. 2015;173(2):370-378. doi:10.1111/bjd.13954
16. Sangha MS, Deroide F, Meys R. Wound healing, scarring and management. Clin Exp Dermatol. 2024;49(4):325-336. doi:10.1093/ced/llad410
17. ZHANG Siyu, MA Shiqi, WANG Mengci, et al. Research Progress of Skin Tissue Engineering Scaffolds and Their Materials in Wound Repair[J]. Medical Journal of Peking Union Medical College Hospital, 2023, 14(3): 603-610. DOI: 10.12290/xhyxzz.2022-0648
18. Ling Huajun, Cui Ruiwen, Wang Qiyou. 3D extracellular matrix hydrogel loaded with exosomes promotes wound repair[J]. Chinese Journal of Tissue Engineering Research, 2023, 27(12): 1900-1905.
19. Zhou Y, Zhao B, Zhang XL, et al. Combined topical and systemic administration with human adipose-derived mesenchymal stem cells (hADSC) and hADSC-derived exosomes markedly promoted cutaneous wound healing and regeneration. Stem Cell Res Ther. 2021;12(1):257. Published 2021 May 1. doi:10.1186/s13287-021-02287-9
20. Gardeazabal L, Izeta A. Elastin and collagen fibres in cutaneous wound healing. Exp Dermatol. 2024;33(3):e15052. doi:10.1111/exd.15052
21. Naylor EC, Watson RE, Sherratt MJ. Molecular aspects of skin ageing. Maturitas. 2011;69(3):249-256. doi:10.1016/j.maturitas.2011.04.011
22. Sorrell JM, Caplan AI. Fibroblasts-a diverse population at the center of it all. Int Rev Cell Mol Biol. 2009;276:161-214. doi:10.1016/S1937-6448(09)76004-6
23. Shaw TJ, Martin P. Wound repair at a glance. J Cell Sci. 2009;122(Pt 18):3209-3213. doi:10.1242/jcs.031187
24. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med. 2014;6(265):265sr6. doi:10.1126/scitranslmed.3009337

25. Rippa AL, Kalabusheva EP, Vorotelyak EA. Regeneration of Dermis: Scarring and Cells Involved. Cells. 2019;8(6):607. Published 2019 Jun 18. doi:10.3390/cells8060607
26. Wen Q, Mithieux SM, Weiss AS. Elastin Biomaterials in Dermal Repair. Trends Biotechnol. 2020;38(3):280-291. doi:10.1016/j.tibtech.2019.08.005
27. Van Doren SR. Matrix metalloproteinase interactions with collagen and elastin. Matrix Biol. 2015;44-46:224-231. doi:10.1016/j.matbio.2015.01.005
28. Almine JF, Wise SG, Hiob M, et al. Elastin sequences trigger transient proinflammatory responses by human dermal fibroblasts. FASEB J. 2013;27(9):3455-3465. doi:10.1096/fj.13-231787
29. Rnjak J, Wise SG, Mithieux SM, Weiss AS. Severe burn injuries and the role of elastin in the design of dermal substitutes. Tissue Eng Part B Rev. 2011;17(2):81-91. doi:10.1089/ten.TEB.2010.0452
30. Martin P, Nunan R. Cellular and molecular mechanisms of repair in acute and chronic wound healing. Br J Dermatol. 2015;173(2):370-378. doi:10.1111/bjd.13954
31. Procknow SS, Kozel BA. Emerging mechanisms of elastin transcriptional regulation. Am J Physiol Cell Physiol. 2022;323(3):C666-C677. doi:10.1152/ajpcell.00228.2022
32. Zhang A, Liu Y, Qin D, Sun M, Wang T, Chen X. Research status of self-healing hydrogel for wound management: A review. Int J Biol Macromol. 2020;164:2108-2123. doi:10.1016/j.ijbiomac.2020.08.109
33. Chen Y, Wang X, Tao S, et al. Research advances in smart responsive-hydrogel dressings with potential clinical diabetic wound healing properties. Mil Med Res. 2023;10(1):37. Published 2023 Aug 23. doi:10.1186/s40779-023-00473-9
34. Zhao C, Zhou L, Chiao M, Yang W. Antibacterial hydrogel coating: Strategies in surface chemistry. Adv Colloid Interface Sci. 2020;285:102280. doi:10.1016/j.cis.2020.102280

Figures

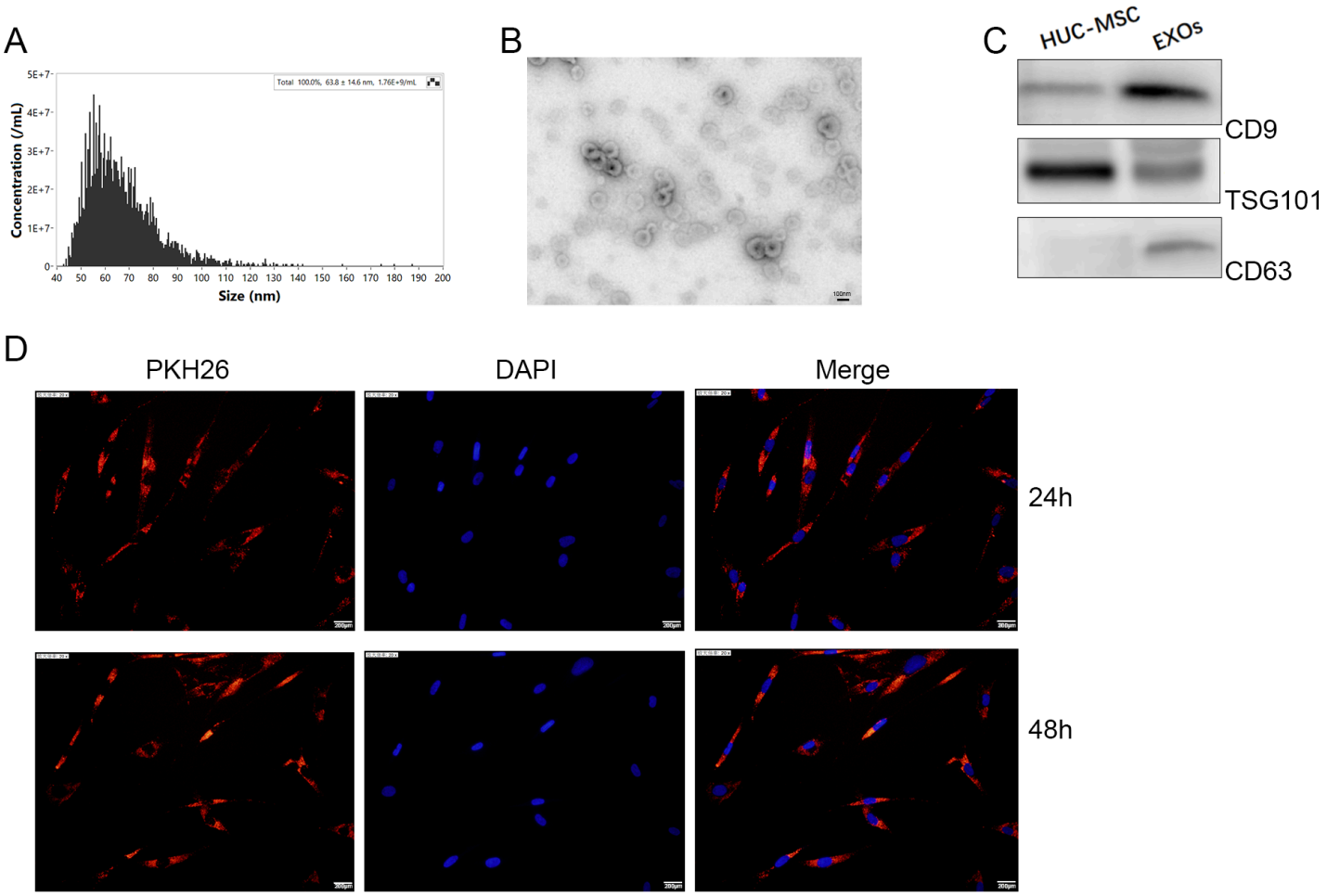


Figure 1

Identification results of hUCMSC-Exos **A**: Results of hUCMSC-Exos identification by nano-flow cytometry; **B**: Results of hUCMSC-Exos identification by electron microscopy; **C**: WB identification of hUCMSC-Exos markers CD9, TSG101 and CD63; **D**: Uptake of PKH-26-labeled hUCMSC-Exos by HFF-1, scale bar = 20μm

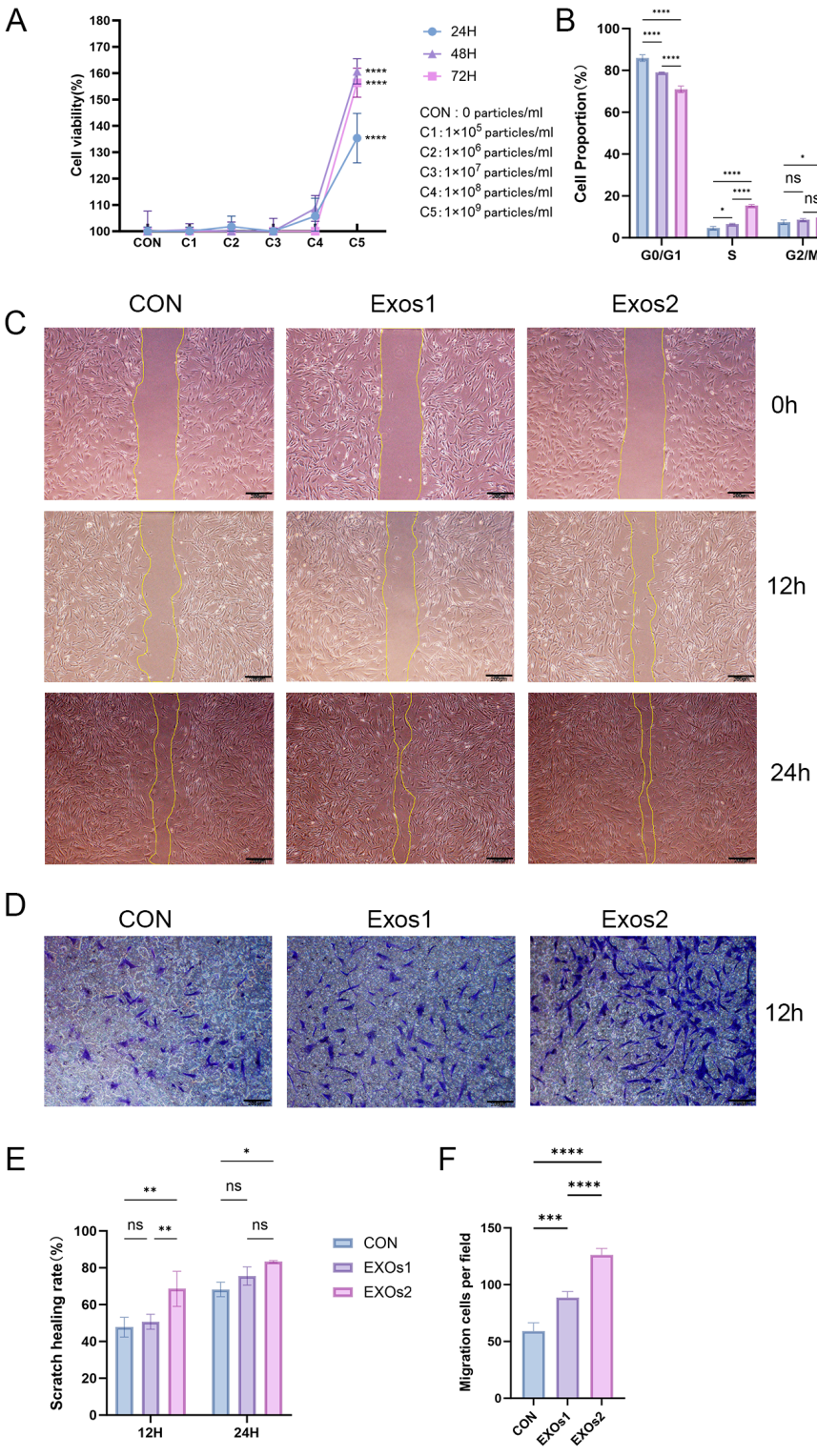


Figure 2

hUCMSC-Exos Enhance Proliferation and Migration of HFF-1 Cells. **A**: CCK-8 showed the effect of different concentrations of hUCMSC-Exos on the proliferation of HFF-1 cells. 1×10^9/ml hUCMSC-Exos significantly promoted the proliferation of HFF-1 cells; **B**: Cell cycle results showed that the proportion of cells in the G0/G1 phase decreased, the proportion of cells in the S phase increased, and PI increased; **C,E**: Cell scratch experiments showed that within 24 hours, the concentration of Exos2 significantly

promoted the migration ability of HFF-1 cells; **D,F**: Transwell experimental results showed that hUCMSC-Exos at two concentrations of Exos1 and Exos2 significantly promoted the migration ability of HFF-1 cells, scale bar = 200μm. ****p＜0.0001, ***P＜0.001,**P＜0.01,*P＜0.1, ^{ns}P＞0.5, n=3）

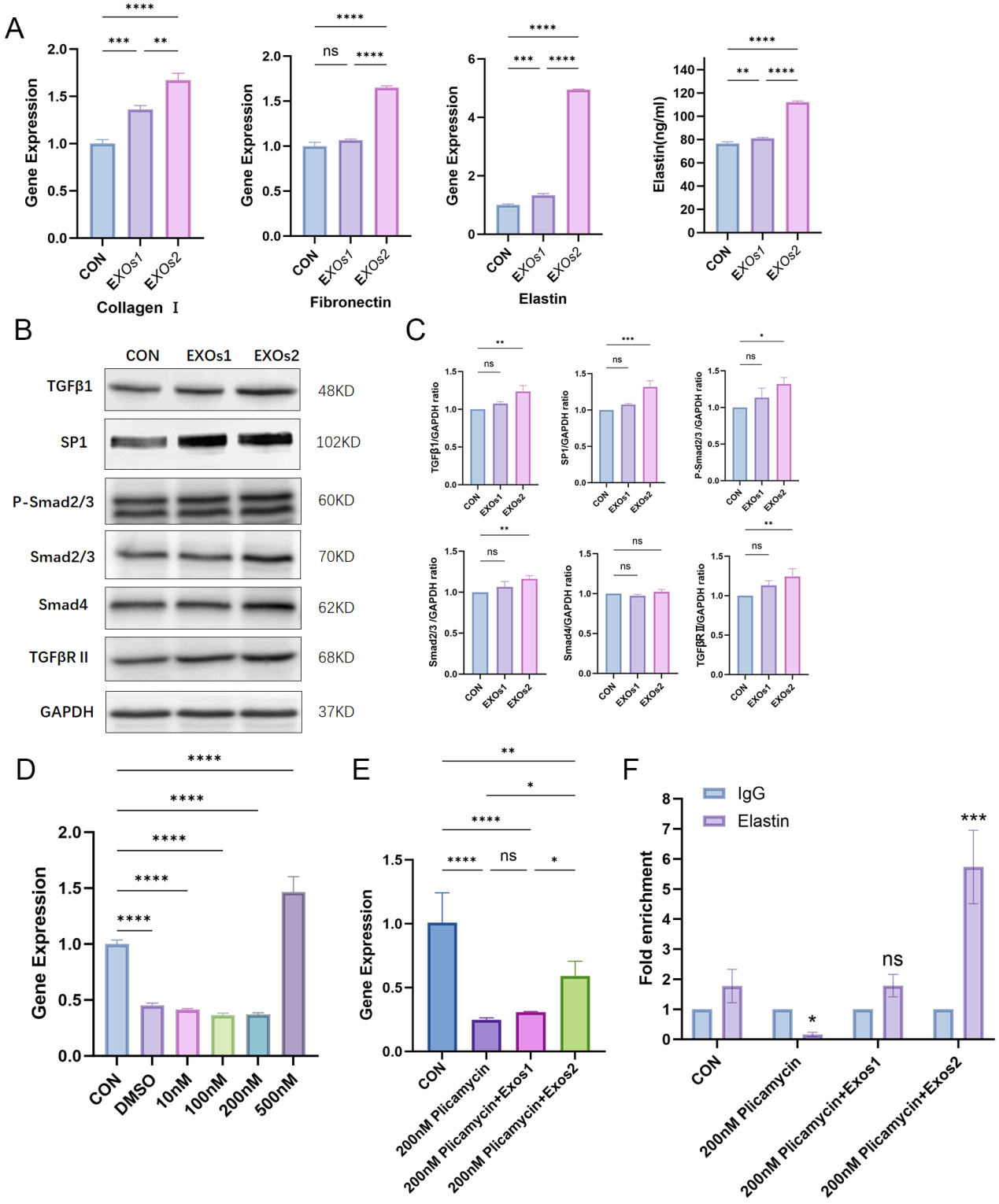


Figure 3

hUCMSC-Exos affect Elastin transcription in HFF-1 cells via TGFβ1-Smad pathway. **A**: hUCMSC-Exos significantly increase the gene expression levels of Collagen I, Fibronectin and Elastin in HFF-1 cells; **B,C**:

The results of WB experiments showed that at the concentration of Exos2, the expression levels of TGFβ1, Smad2/3, P-Smad2/3, TGFβR□ and SP1 increased, and the differences were statistically significant; **D**: The effect of gradient concentrations of Plicamycin on the transcription of Elastin gene in HFF-1 cells is within the concentration range of 10nM-200nM. As the concentration increases, the inhibitory effect becomes more obvious; **E**:The results of qRT-PCR experiments showed that hUCMSC-Exos could reverse the effect of Plicamycin on the expression of Elastin gene in HFF-1 cells; **F**:ChIP-qPCR measurement of the effects of Plicamycin and hUCMSC-Exos on HFF-1: Plicamycin significantly reduced the enrichment of genes in the GC box region of the Elastin gene promoter. As the concentration of hUCMSC-Exos increased, genes in this region Increased enrichment

****p□0.0001, ***P□0.001,**P□0.01,*P□0.1, ^{ns}P□0.5, n=3

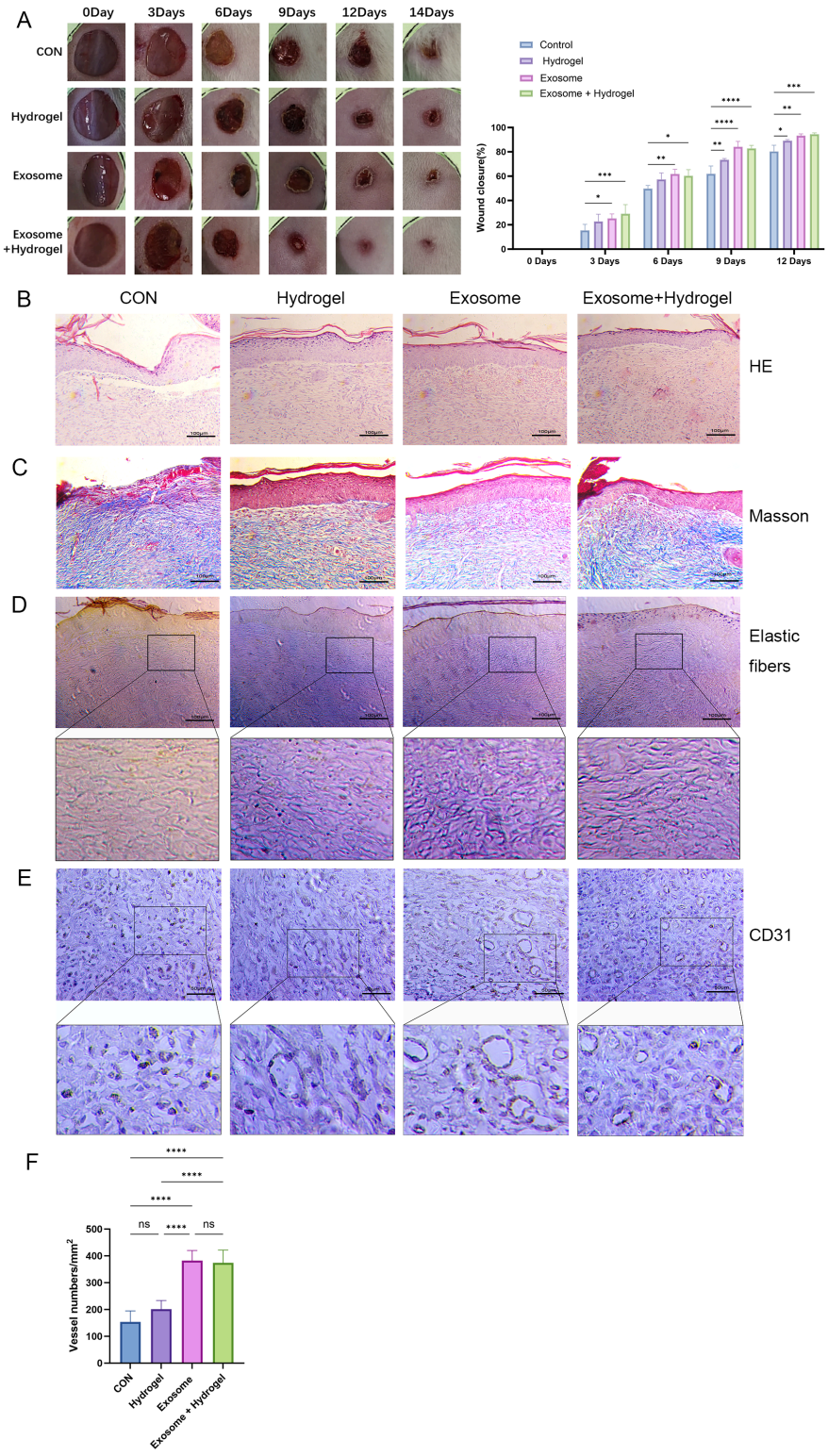


Figure 4

hUCMSC-Exos and hydrogels combined to accelerate skin wound healing in mice. **A**: Analysis of wound healing and wound healing rate of four groups of mice on days 0, 3, 6, 9, 12, and 14 days. hUCMSC-Exos can significantly improve the wound healing rate when used alone or in combination with hydrogel.(n=5); **B**: HE staining of wound tissue,scale bar = 100μm; **C**: Masson staining of wound tissue,scale bar =

100μm; **D**: Elastic fiber staining of wound tissue, scale bar = 100μm; **E,F**: CD31 staining shows angiogenesis, scale bar = 50μm.****p≤0.0001, ***P≤0.001,**P≤0.01,*P≤0.1, n=10