

PF-127 Hydrogel-Delivered hUCMSC-Exosomes Attenuate Endometrial Injury by Inhibiting Epithelial–Mesenchymal Transition

Liwei Bao

Hunan Normal University

Li Li

Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University

Shengbin Tang

Hunan Shengbao Biotechnology Co., Ltd

Yezi Tang

Hunan Shengbao Biotechnology Co., Ltd

Zihan Wang

Hunan Normal University

Jiaxi Tan

Hunan Normal University

Rushi Liu

Hunan Normal University

Xia Zhang

zx717@hunnu.edu.cn

Hunan Normal University

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Abstract

Endometrial injury, often resulting from iatrogenic procedures or infections, can lead to fibrosis, adhesions, and infertility. Current therapies are inadequate for reversing fibrosis or restoring normal function. Mesenchymal stem cell-derived exosomes (MSC-exos) present therapeutic potential; however, their rapid clearance poses a challenge. In this study, a thermosensitive PF-127 hydrogel for sustained intrauterine delivery of human umbilical cord MSC-derived exosomes (PF/127-hUCMSC-exos) was developed, and its efficacy and underlying mechanisms were evaluated in a rat model of ethanol-induced endometrial injury. The PF-127 hydrogel extended the retention of exosomes at the injury site for up to 12 days. Compared with control treatment or hydrogel treatment alone, treatment with PF/127-hUCMSC-exos significantly enhanced endometrial morphology, increased thickness and gland number, reduced collagen deposition, and restored fertility. Mechanistically, hUCMSC-exos inhibited the epithelial–mesenchymal transition (EMT) process by upregulating E-cadherin and downregulating vimentin and β -catenin. Transcriptomic analysis of exosome-treated primary rat endometrial stromal cells revealed significant enrichment in EMT-related pathways and revealed early growth response 2 (EGR2) as a key downregulated target. hUCMSC-exos significantly decreased both the mRNA and protein levels of EGR2, a transcription factor known to promote fibrosis and EMT. In conclusion, the PF/127-hUCMSC-exos hydrogel effectively repaired endometrial injury and restored fertility by inhibiting EMT, potentially through the downregulation of EGR2, thereby providing a promising cell-free, targeted strategy for endometrial regeneration.

Introduction

The integrity of the endometrial basal layer is a physiological prerequisite for the regulation of the menstrual cycle and for embryo implantation. However, various iatrogenic procedures, including repeated intrauterine surgeries, as well as infections and inflammatory diseases, can damage the endometrial basal layer. This damage leads to impaired endometrial regeneration, abnormal fibrosis, and intrauterine adhesions. These conditions are significant contributors to female infertility, recurrent implantation failure, and pregnancy loss. Current treatments, such as hysteroscopic adhesiolysis combined with hormonal therapy, only partially restore the anatomical structure and do not effectively reverse fibrosis or recover functionality, resulting in high recurrence rates [1–3]. Therefore, novel regenerative strategies that can inhibit fibrosis and restore endometrial homeostasis are urgently needed.

In recent years, cell therapy, particularly mesenchymal stem cell (MSC) transplantation, has emerged as a prominent area of research. MSCs can be isolated from various tissues and administered via tail vein or intrauterine injection to promote endometrial thickening and gland and blood vessel formation and to enhance embryo receptivity [4–6]. However, the use of live cells carries risks of immune rejection and embolism, and large-scale cultivation may even diminish the therapeutic properties of MSCs[7]. The exosomes secreted from MSCs (MSC-exos), which mimic paracrine effects by transporting miRNAs and proteins to promote angiogenesis and suppress inflammatory fibrosis, have been validated in rat models

of ethanol-induced or mechanical endometrial injury [8, 9]. Nevertheless, exosomes are rapidly cleared after intrauterine administration, resulting in low delivery efficiency. PF127 thermosensitive hydrogel, known for its biocompatibility and injectability, can slowly release nanovesicles after gelation, thereby prolonging retention and providing a viable carrier for cell-free repair. Current research is focused on PF127 hydrogel loaded with stem cells or exosomes to promote wound healing [10, 11], while studies on its application in the noninvasive treatment of endometrial injury using MSC-exos are limited.

Alleviating fibrosis in damaged tissues is a key mechanism through which MSCs and MSC-exos facilitate tissue repair. Epithelial–mesenchymal transition (EMT) is often activated during inflammatory responses, wound healing, and fibrotic processes. In instances of mild injury, EMT promotes the transformation of epithelial cells into myofibroblasts, thereby enhancing tissue repair. However, under chronic inflammatory conditions, the abnormal and sustained activation of EMT results in excessive accumulation of myofibroblasts and extracellular matrix deposition, ultimately disrupting the structure and function of parenchymal organs [12]. The regulation of EMT involves multiple signaling pathways, with the transforming growth factor- β (TGF- β) family serving as the primary inducer [13, 14]. Additionally, the Notch signaling pathway [15, 16], the canonical Wnt pathway, and various inflammatory cytokines collectively contribute to the initiation and maintenance of EMT [17, 18]. Studies have demonstrated that MSCs can inhibit the EMT process and alleviate fibrosis by modulating signaling pathways such as Wnt/ β -catenin and TGF- β /Smad [19]. Furthermore, the reversal effect of MSC-exos on EMT is linked to miRNA delivery and transcriptional regulation. Specifically, miR-23a-3p and miR-182-5p carried by MSC-exos can inhibit *Ikbkb*, thereby downregulating the NF- κ B and Hedgehog signaling pathways and thus mitigating LPS-induced pulmonary fibrosis [20]. Similarly, miR-466f-3p derived from mouse MSC-exos can target c-MET and inhibit the AKT/GSK3 β pathway, thereby reversing the EMT process in radiation-induced lung injury [21]. These findings indicate that MSCs and their exosomes play crucial roles in suppressing fibrosis and promoting tissue regeneration by regulating EMT through multiple pathways and targets.

In this study, we prepared a PF/127 hydrogel for the sustained release of human umbilical cord MSC-derived exosomes (PF/127-hUCMSC-exos) and investigated its therapeutic potential for endometrial injury, its effects on fertility function, and its underlying mechanisms. We found that hUCMSC-exos improved endometrial fibrosis in injured rats by inhibiting EMT, potentially through the downregulation of the EMT-related molecule EGR2, thereby providing a translatable new approach for the clinical repair of moderate to severe endometrial damage.

Materials and Methods

Isolation of hUCMSC-exosomes

The supernatant from hUCMSCs cultured through passages 3 to 5 was collected. Following the removal of cells and debris via differential centrifugation (300 $\times$ g, 2000 $\times$ g, and 10,000 $\times$ g), the exosomes were pelleted by ultracentrifugation at 110,000 $\times$ g for 70 minutes at 4°C. The resulting exosome pellet was then resuspended in sterile PBS, aliquoted, and stored at -80°C.

Preparation of PF-127 hydrogel

PF-127 powder (Sigma) was added to pre-cooled PBS buffer at 4°C under sterile conditions to achieve the desired concentration (e.g., 30%, w/v). The mixture was placed on a magnetic stirrer at 4°C and stirred at low speed overnight until the powder was completely dissolved, resulting in a clear, transparent pre-gel solution. This solution remains liquid at low temperatures but transitions rapidly to a gel state at body temperature.

Cell culture

Rat renal epithelial cells (NRK-52E) were purchased from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) and 100 U penicillin/streptomycin (Gibco) at 37°C in a 5% CO₂ incubator. To generate primary rat endometrial stromal cells (rESCs), female SD rats were subcutaneously injected with 17 β -estradiol (3.5 mg/kg) for 3 consecutive days to synchronize their estrus. After euthanasia, uteri were excised, washed three times with HBSS, and minced into 0.5–1 mm³ pieces. The tissues were digested with 0.8 mg/mL collagenase I at 37°C for 60 min, terminated with DMEM/F12 (Gibco) supplemented with 10% FBS, filtered through a 400-mesh sieve, and centrifuged to collect the cells. Primary culture was performed until passage 3, with identification based on morphology and vimentin expression.

Transwell assay

The test cells, such as NRK-52E cells or rESCs, were resuspended in serum-free medium. The cell density was adjusted, and the cells were seeded in the upper chamber of the Transwell insert. Complete medium containing 10% FBS was added to the lower chamber as a chemoattractant. The culture plate was placed in a 37°C incubator with 5% CO₂ for 24 hours. The chamber was subsequently removed, and the cells that migrated to the membrane of the lower chamber were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Multiple random fields of view were then photographed and counted under a microscope, followed by statistical analysis of the cell migration count.

In Vivo Experimental Design

All animal protocols were approved by the Ethics Committee of Hunan Normal University (permit number: 2022431; approval date: October 2022). Eight-week-old female SD rats (Beijing Vital River) were synchronized using vaginal smears, and surgery was performed during estrus. Following intraperitoneal (i.p.) anesthesia, a midline laparotomy was conducted to expose both uterine horns. A microsyringe was used to infuse absolute ethanol into each lumen for five minutes to induce endometrial injury, followed by three saline washes. The sham operation group without ethanol injury served as the control group (n = 8). The injured animals (n = 24) were then randomly assigned to one of three groups: (1) injury-only (Model), (2) PF/127 hydrogel vehicle, or (3) PF/127-hUCMSC-exos. After in situ gelation, the uterus was

repositioned, and the abdomen was closed. Standard postoperative care was provided. At the third estrus postinjury, the rats were sacrificed, and the uteri were fixed in 4% paraformaldehyde for hematoxylin and eosin (H&E) and Masson's trichrome staining. Fertility was assessed on day 14 postintervention (n = 5/group), with each female paired 1:1 with a proven fertile male. The presence of a vaginal plug and a sperm-positive smear the following morning was designated as gestation day 0. On day 18 of pregnancy, the dams were euthanized, the uterine horns were opened, and implantation sites were counted to evaluate reproductive recovery.

Exosome labeling and in vivo tracking

hUCMSC-exosomes were labeled with PKH26 red fluorescent dye (Sigma, MINI26-1KT) following the manufacturer's instructions. After a rat endometrial injury model was established, a PF-127 hydrogel loaded with PKH26-labeled exosomes was injected into the uterine cavity. The animals were sacrificed at 0, 4, 6, 8, 10, and 12 days postinjection, after which the uterine tissues were collected. The overall fluorescence signals from the uterine tissues were captured using a small-animal in vivo imaging system (PerkinElmer) to semiquantitatively analyze the retention time of the exosomes at the injury site. Moreover, portions of fresh uterine tissue were embedded in optimal cutting temperature (OCT) compound and rapidly frozen in liquid nitrogen. Frozen sections were prepared, and after Hoechst 33342 nuclear staining was performed, the distribution and localization of the exosomes in the uterine tissue sections were observed using a laser confocal microscope (Leica).

Histological staining

Uterine tissues were fixed in 4% paraformaldehyde, dehydrated through a graded series of ethanol, cleared in xylene, embedded in paraffin, and sectioned at 4 μ m. For H&E staining, the sections were sequentially dewaxed in xylene, hydrated through graded ethanol, stained with hematoxylin, differentiated in hydrochloric acid–alcohol, blued in running water, counterstained with eosin, dehydrated, cleared, and mounted with neutral resin. For Masson's trichrome staining, dewaxed and hydrated sections were sequentially stained with Weigert's iron hematoxylin for nuclei and Ponceau-acid fuchsin for cytoplasm and muscle fibers, differentiated in a phosphomolybdic acid solution, stained with aniline blue for collagen fibers, and subsequently treated with a weak acid solution, dehydrated, cleared, and mounted. All stained sections were observed under an optical microscope, and images were captured. Quantitative analysis of endometrial thickness, gland number, and collagen fiber area percentage was performed using ImageJ software.

Immunofluorescence

Frozen sections of OCT-embedded uterine tissues were rewarmed to room temperature and rinsed with PBS to eliminate the embedding medium. The sections were then fixed in 4% paraformaldehyde for 10 minutes, followed by three washes with PBS. The sections were subsequently blocked using a solution

containing 5% bovine serum albumin and 0.3% Triton X-100 at room temperature for 1 hour. After the blocking solution was removed, a diluted primary antibody working solution was added, and the samples were incubated overnight at 4°C. The following day, the primary antibody was removed by washing with PBS, and species-specific fluorescently labeled secondary antibodies were added, followed by incubation at room temperature in the dark for 1 hour. After an additional PBS wash, the nuclei were counterstained with Hoechst 33342. Finally, anti-fade mounting medium was applied to seal the sections. Fluorescence images of specific target proteins were observed and captured using a laser confocal microscope.

RT–PCR

Total RNA was extracted from cells or ground uterine tissues using TRIzol reagent (Invitrogen), and its concentration and purity were measured with a microspectrophotometer. An equal amount of total RNA was used to synthesize first-strand cDNA according to the instructions provided with the reverse transcription kit (TAKARA). qPCR was performed on a real-time fluorescence quantitative PCR instrument using a SYBR Green premix system (TAKARA). The reaction program consisted of predenaturation at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 5 seconds and annealing/extension at 60°C for 30 seconds. All samples were run in triplicate, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used as the reference gene. The relative expression levels of target genes were calculated using the $2^{(-\Delta\Delta Ct)}$ method.

Western blot

Total protein was extracted from cells or tissues using RIPA lysis buffer supplemented with protease inhibitors, and the protein concentration was determined via the BCA method. Equal amounts of protein samples were separated using SDS–PAGE, after which the proteins were transferred to PVDF membranes by the wet transfer method. After blocking with 5% skim milk in TBST at room temperature for 1 hour, the membranes were incubated overnight at 4°C with diluted primary antibodies, including CD63 (Proteintech), TSG101 (Proteintech), calnexin (Proteintech), vimentin (ABclonal), β -catenin (ABclonal), and EGR2 (ABclonal). Following three washes with TBST, the membranes were incubated with the corresponding horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 hour. After thorough washing, the membranes were treated with enhanced chemiluminescence (ECL) detection reagents, and signals were captured using a chemiluminescence imaging system.

RNA sequencing

The rESCs were treated with exosomes (with PBS as the control) at a final concentration of 0.5 $\mu\text{g}/\text{mL}$ for 24 hours. Subsequently, total RNA was isolated using TRIzol agent, and mRNA was enriched using oligo(dT) magnetic beads and fragmented. The cDNA library was amplified by PCR with appropriate cycling parameters, quality controlled, and sequenced on the DNBSEQ platform (BGI). Raw sequencing data were processed using SOAPnuke (v2.2.1) for quality filtering. Subsequent bioinformatics analysis, visualization, and multiomics data mining were performed using the Dr. Tom integrated analysis

platform. The differentially expressed genes (DEGs) were defined as those whose $|\log_2\text{foldchange}|$ was ≥ 1.0 and whose FDR was < 0.05 .

Statistical analyses

Data from three independent experiments were analyzed using GraphPad Prism 10.0 software and are shown as the mean $\pm$ SEM. Statistical multigroup comparisons were performed by ANOVA, followed by a Student–Newman–Keuls post hoc test. Two-tailed unpaired Student's t tests were used to compare two groups. p values less than 0.05 were considered to indicate statistical significance.

Results

Characterization of PF/127-hUCMSC-exos

Exosomes were isolated using ultracentrifugation, and transmission electron microscopy revealed that the exosomes exhibited a round vesicle-like structure (Fig. 1A). Dynamic light scattering analysis revealed that the particle size distribution ranged from 100 to 1000 nm (Fig. 1B). Western blot analysis of exosomal surface protein markers revealed that both human umbilical cord mesenchymal stem cells and exosomes expressed CD63 and TSG101 (Fig. 1C), whereas calnexin was expressed only in human umbilical cord mesenchymal stem cells and not in exosomes. The quantified exosomes were incorporated into the PF127 hydrogel, which remained in a liquid state at 4°C and transformed into a solid state at 37°C (Fig. 1D). This hydrogel exhibits temperature sensitivity, injectability, and minimal flow after solidification.

In vivo and in vitro tracing of PF/127-hUCMSC-exos

We labeled exosomes with the red fluorescent PKH26 dye and traced them to renal tubular epithelial cells. Red exosome fluorescence signals were detected in the cells 6 hours after the direct addition of exosomes, with the fluorescence signals gradually intensifying over time. However, almost no exosome fluorescence signals were observed in the PF127-loaded exosome group at the 6-hour time point (Fig. 2A). These findings suggest that exosomes can be taken up by cells and that PF127 hydrogel-loaded exosomes can achieve sustained release.

We subsequently conducted in vivo exosome tracing in rats. The results demonstrated that exosome fluorescence signals were detected in the uterine cavity on day 0 post-injection and persisted until day 12 (Fig. 2B,C). This indicates that PF/127 hydrogel-loaded exosomes can be slowly released and preserved long term in the rat uterine cavity. Additionally, we obtained frozen sections of rat uterine tissue and collected fluorescence images using confocal laser scanning microscopy. Weak exosome fluorescence signals were observed in the uterine tissue on day 0; these signals initially increased but then decreased over time, which is consistent with previous results (Fig. 2D). These findings demonstrate that PF/127-loaded exosomes can achieve slow release and long-term preservation, while uterine tissue can take up these exosomes to exert sustained therapeutic effects.

PF/127-hUCMSC-exos can improve the function of damaged uterus

To investigate the effects of exosomes on endometrial injury, we established an endometrial injury model by injecting absolute ethanol into the uterine cavity of rats during the estrus phase, followed by treatment with PF/127-hUCMSC-exos. After 14 days of treatment, uterine tissues were collected for analysis (Fig. 3A). HE staining revealed a significant reduction in endometrial thickness and a decrease in the number of glands in both the model group and the PF127 hydrogel group. In contrast, the PF/127-hUCMSC-exos group exhibited a marked increase in endometrial thickness and restoration of gland numbers (Fig. 3B, C, D). Masson staining further revealed that the fibrotic area of the endometrium and collagen fiber deposition significantly increased in both the model and PF127 hydrogel groups, whereas the exosome-treated group demonstrated a significant reduction in collagen fiber area and alleviation of fibrosis (Fig. 3B, E).

We subsequently evaluated the fertility of the rats. Uterine injury modeling and PF127 hydrogel exosome therapy were conducted during the estrus phase. After 14 days of treatment, female rats were co-housed with male rats. Vaginal secretions were collected the following morning, and the presence of sperm observed under microscopy was recorded as gestational day 0. Uteri were harvested on gestational day 18, and the number of embryos was counted (Fig. 4A). The results indicated that rats with left uterine injuries induced by absolute ethanol and treated with PF127 hydrogel failed to achieve successful pregnancy, whereas those treated with exosomes successfully conceived (Fig. 4B, C).

hUCMSC-exos inhibit the EMT process

To elucidate the potential mechanisms through which hUCMSC-exos promote endometrial injury repair, we examined its effects on cell proliferation and migration. First, an anhydrous ethanol-induced renal tubular epithelial cell injury model was established, followed by exosome intervention. The results revealed no significant difference in cell proliferation activity between the exosome-treated group and the control group. Next, Transwell assays were conducted to assess the effects of exosomes on the migration capabilities of rat renal epithelial cells (NRK-52e) and primary endometrial stromal cells (ESCs). The findings revealed a significant decrease in the number of migrating cells in both cell types after exosome intervention (Fig. 5A, B). Based on these observations, we speculate that the reparative effect of hUCMSC-exos on the endometrium may be linked to the regulation of the EMT process.

To validate this hypothesis, Western blot and qPCR were used to detect changes in the expression of core EMT markers. The results demonstrated that, at both the mRNA and protein levels, the expression of vimentin and β -catenin was significantly downregulated in the exosome intervention group, whereas the expression of E-cadherin was markedly upregulated, suggesting that exosomes can inhibit the EMT process (Fig. 5C, D). Additionally, the expression of EMT marker proteins was evaluated by immunofluorescence staining of frozen sections. Compared with that in the sham surgery group, the fluorescence intensity of endometrial E-cadherin was significantly lower in both the model group and the

PF/127 group, whereas vimentin expression was higher. After treatment with PF/127-hUCMSC-exos, E-cadherin expression was significantly restored, and the vimentin fluorescence signal was markedly diminished (Fig. 5E). Collectively, these results indicate that hUCMSC-exos promote structural repair of the endometrium following injury by inhibiting the EMT process and maintaining the epithelial phenotype.

Transcriptomic analysis of differentially expressed genes in ESCs cocultured with hUCMSC-exos

To investigate the transcriptomic characteristics of primary ESCs following hUCMSC-exos intervention, we employed high-throughput RNA sequencing. The results revealed 1,173 transcripts whose expression was significantly altered, including 527 upregulated genes and 646 downregulated genes. Gene Ontology (GO) analysis revealed significant enrichment in biological processes related to “cell migration,” “endothelial cell migration,” and “epithelial to mesenchymal transition (EMT)” (Fig. 6A). KEGG pathway classification further revealed that the DEGs were enriched primarily in categories such as “TGF-beta signaling pathway”, “Wnt signaling pathway”, and “Notch signaling pathway”, which are closely related to EMT (Fig. 6B). Based on expression magnitude and functional relevance, we identified key candidate genes, such as *NOG*, *NPR3*, *MMP10*, and *EGR2*. The results of quantitative real-time PCR (qPCR) validation were highly consistent with the sequencing data: *NOG* and *NPR3* mRNA expression was significantly upregulated ($P < 0.01$), whereas *MMP10* and *EGR2* mRNA expression was significantly downregulated ($P < 0.01$) (Fig. 6C, D).

hUCMSC-exos inhibit cell migration by downregulating EGR2

To further elucidate the downstream targets of hUCMSC-exos, we performed Western blot analysis to assess the protein levels of the abovementioned genes. The results revealed a significant decrease in *EGR2* protein expression, which aligned with the transcriptome sequencing data, further confirming that hUCMSC-exos exert a negative regulatory effect on *EGR2* expression (Fig. 6E). To investigate the role of *EGR2* in cell migration, we overexpressed *EGR2* in rat renal epithelial cells using plasmid transfection technology. The Western blot results demonstrated a notable increase in *EGR2* protein levels in the overexpression group (Fig. 6F). Transwell migration assays revealed a marked increase in the number of infiltrating cells in the *EGR2*-overexpressing group, indicating that *EGR2* promotes cell migration. However, following exosome treatment, these pro-migration phenotypes were reversed. In conclusion, the findings of this study confirm that hUCMSC-exos can inhibit cell migration by downregulating *EGR2* expression (Fig. 6G).

Discussion

This study demonstrated that a PF/127 thermosensitive hydrogel loaded with hUCMSC-exos effectively delivered and prolonged exosome retention in the injured uterus. By inhibiting the EMT process, the

hydrogel significantly improved ethanol-induced endometrial damage in rats, promoted tissue morphological repair, reduced fibrosis, and restored fertility. The underlying molecular mechanism may be associated with the downregulation of the key transcription factor EGR2 by exosomes.

First, our results confirm the superiority of PF/127 hydrogel as an exosome delivery vehicle. The hydrogel undergoes rapid gelation at body temperature, achieving both localization and sustained exosome release (up to 12 days) at the injury site, while demonstrating excellent biocompatibility without eliciting significant foreign body reactions. These findings provide a viable strategy to overcome the application bottlenecks of the short in vivo half-life and poor targeting of exosomes, aligning with recent research trends that utilize biomaterials to enhance the delivery of cytokines or exosomes for improved tissue regeneration effects [22]. Previous studies have employed PF/127-loaded mesenchymal stem cells for intrauterine injection therapy in rat endometrial injury models [23, 24]. Other studies have utilized collagen scaffolds or HA gel-loaded MSC-exos to promote endometrial regeneration [25, 26]. For the first time, we have adopted PF/127-loaded MSC-exos for intrauterine injection, achieving excellent sustained-release effects.

In terms of therapeutic effects, PF/127-hUCMSC-exo treatment significantly increased endometrial thickness and the number of glands, reduced collagen deposition, and ultimately improved the embryo implantation rate in damaged uteri. These findings align with those of previous reports indicating that various sources of MSCs, including bone marrow MSCs (BMMSCs) [27], menstrual blood-derived MSCs (MenSCs) [28], UC-MSCs [29], and adipose tissue-derived MSCs (AD-MSCs) [30], have potential efficacy in endometrial regeneration therapy. Furthermore, exosomes derived from BMMSCs can significantly increase the number of endometrial glands and markedly reduce the area of endometrial fibrosis. The underlying mechanism may involve the suppression of EMT through the activation of the Wnt/ β -catenin signaling pathway [31, 32]. A collagen scaffold structure loaded with UCMSC-exos promotes endometrial regeneration and restores fertility by inducing CD163 + M2 macrophage polarization [25]. In summary, MSCs and their exosomes primarily facilitate the repair of endometrial damage through mechanisms such as promoting endometrial cell proliferation and regeneration, suppressing inflammation and fibrosis, and modulating immune responses.

This study further investigated the repair mechanism through the regulation of EMT. We found that hUCMSC-exos can upregulate the expression of the epithelial marker E-cadherin while downregulating the expression of the mesenchymal markers vimentin and β -catenin both in vivo and in vitro, thereby inhibiting the migration ability of endometrial cells. These findings strongly suggest that exosomes exert their antifibrotic effects by reversing or suppressing the abnormal EMT induced by injury. Transcriptomic analysis revealed that differentially expressed genes were significantly enriched in EMT- and related-pathway genes after exosome intervention. Studies have demonstrated that exosomal miR-466f-3p derived from mouse MSCs reverses the EMT process by inhibiting the AKT/GSK3 β pathway via c-MET in radiation-induced lung injury [21]. Additionally, the delivery of lncRNAGAS5 by hUCMSC-exos alleviates EMT through competitive binding to miR-21, thereby restoring PTEN expression [33]. In an epithelial–mesenchymal transition cell model of the human peritoneal mesothelial cell line (HMrSV5), lnc-CDHR

contained in hUCMSC-exos modulates the inhibition of the target gene PTEN by competitively binding to miR-3149, thus attenuating EMT in HMrSV5 through the AKT/FOXO pathway [34]. Therefore, we believe that the repair of endometrial injury by hUCMSC-exos is primarily associated with the inhibition of EMT progression. To this end, we further identified the enriched differentially expressed genes and validated EGR2 (early growth response 2) as a key downstream target of the effects of hUCMSC-exos.

EGR2 is a zinc finger transcription factor that belongs to the early growth response protein family. It is typically rapidly induced by growth factors, stress signals, or differentiation signals and participates in the regulation of various pathological processes, including inflammation, fibrosis, and cell migration [35, 36]. Its mechanism is significantly associated with EMT. In liver fibrosis models, EGR2 expression was significantly upregulated during TGF- β 1-induced EMT, and genetic silencing of EGR2 markedly suppressed the expression of EMT-related markers, suggesting a positive regulatory role in fibrosis progression [37]. Another study on liver fibrosis revealed that reduced EGR2 expression could inhibit the transition from benign hepatic steatosis to liver fibrosis [38]. In renal fibrosis patient samples, EGR2 expression was increased. Overexpression of EGR2 promoted renal fibrosis in unilateral ureteral obstruction mice, while EGR2 gene deletion alleviated obstructive nephropathy and reduced the occurrence of EMT, which was associated with the p-STAT3-EGR2-p-Smad3 axis [39]. Studies have revealed that EGR2 can form a transcriptional complex with the histone acetyltransferase p300/CBP, increasing chromatin accessibility and activating the transcription of downstream target genes (such as SNAI2) [40]. This epigenetic regulatory mechanism is particularly critical during EMT, as SNAI2, a core EMT transcription factor, can directly suppress E-cadherin expression and activate mesenchymal markers such as vimentin [41]. Therefore, under pathological conditions such as chronic inflammation and cancer, EGR2 is often abnormally induced and acts as a driver of EMT, promoting invasion and fibrosis.

This study is the first to demonstrate that hUCMSC-exos can significantly reduce both the mRNA and protein levels of EGR2 and that EGR2 overexpression promotes EMT. We speculate that exosomes may inhibit the expression of EGR2 through their specific miRNA or protein components, thereby alleviating its activation of a series of pro-EMT/fibrotic genes and ultimately maintaining the homeostasis of endometrial epithelial cells. This discovery provides new insights into the precise molecular mechanisms through which MSC-derived exosomes inhibit fibrosis. However, this study has certain limitations. First, while PF/127 hydrogel enhances retention time, its specific in vivo degradation kinetics and long-term effects on the uterine cavity microenvironment require further detailed evaluation. Second, the specific active components in exosomes (such as the particular miRNA or protein) responsible for downregulating EGR2 have not yet been identified, which represents a key direction for future functional research. Future research should focus on identifying the key functional molecules in exosomes responsible for therapeutic effects and optimizing the physicochemical properties of the hydrogel to advance this strategy toward clinical application. Finally, although the rat endometrial injury and repair model can simulate some pathological features of humans, there are differences in etiology and pathological progression compared with the complex intrauterine adhesions observed in humans. Therefore, clinical translation of the research findings should be approached with caution.

Declarations

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Consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and material The datasets generated and/or analyzed in this study will be made available by the corresponding author upon reasonable request.

Code availability Not applicable.

Clinical Trial Number Not applicable.

Author Contributions: Liwei Bao, Li Li, Shengbin Tang and Yezi Tang performed the experiments; Zihan Wang, Jiaxi Tan and Rushi Liu analyzed the data; Liwei Bao, Xia Zhang and Rushi Liu wrote the manuscript, Xia Zhang and Li Li obtained the funding. All authors have read and agreed to the published version of the manuscript.

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Figures

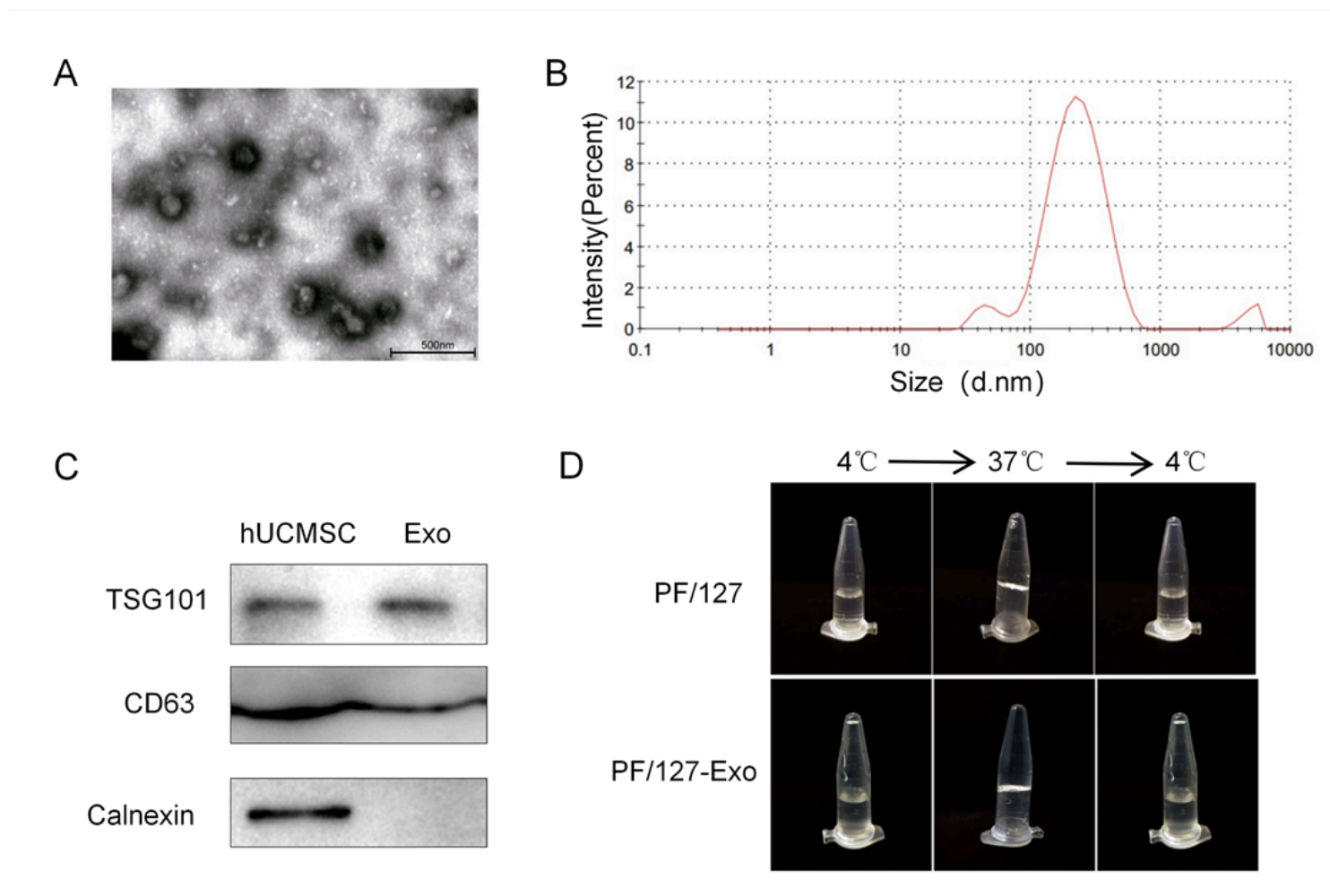


Figure 1

Characterisation of hUCMSC-Exo and Preparation of Thermosensitive PF/127 Hydrogel. (A) Representative transmission electron microscopy (TEM) image of isolated exosomes. (B) Size distribution profile of exosomes as determined by dynamic light scattering (DLS). (C) Western blot analysis of exosomal and cellular markers, including TSG101, CD63 and Calnexin. (D) Schematic illustration of the reversible sol-gel transition behaviour of PF/127 hydrogel.

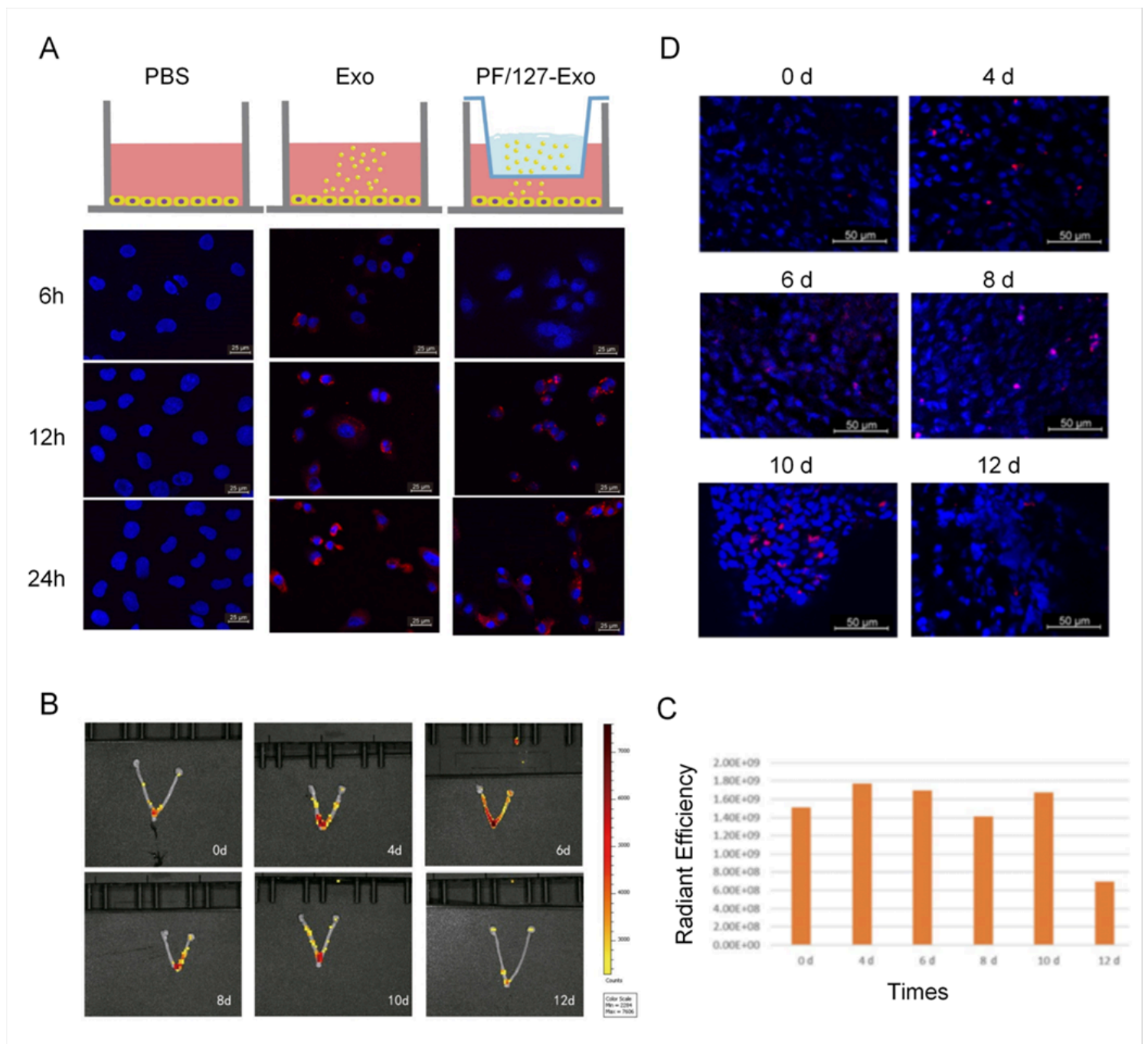


Figure 2

In Vitro and In Vivo Tracking of PKH26-Labelled Exosomes (PKH26-Exo). (A) Representative immunofluorescence images show PKH26 (red) signal in cells treated with PKH26-Exo with or without PF-127 hydrogel encapsulation. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 50 μ m. (B) In vivo fluorescence imaging of the rat uterine cavity following intrauterine injection of PF/127-PKH26-Exo hydrogel. Images were acquired at the indicated time points (days 0, 2, 4, 6, 8, 10, and 12) using a small animal in vivo imaging system. (C) Quantification of the fluorescence intensity in the uterine cavity from the images shown in (B) over the 12-day period. (D) Representative fluorescence images of uterine tissues harvested at the indicated time points, demonstrating the retention and distribution of PKH26-Exo. Nuclei were stained with Hoechst 33342 (blue). Scale bar, 100 μ m.

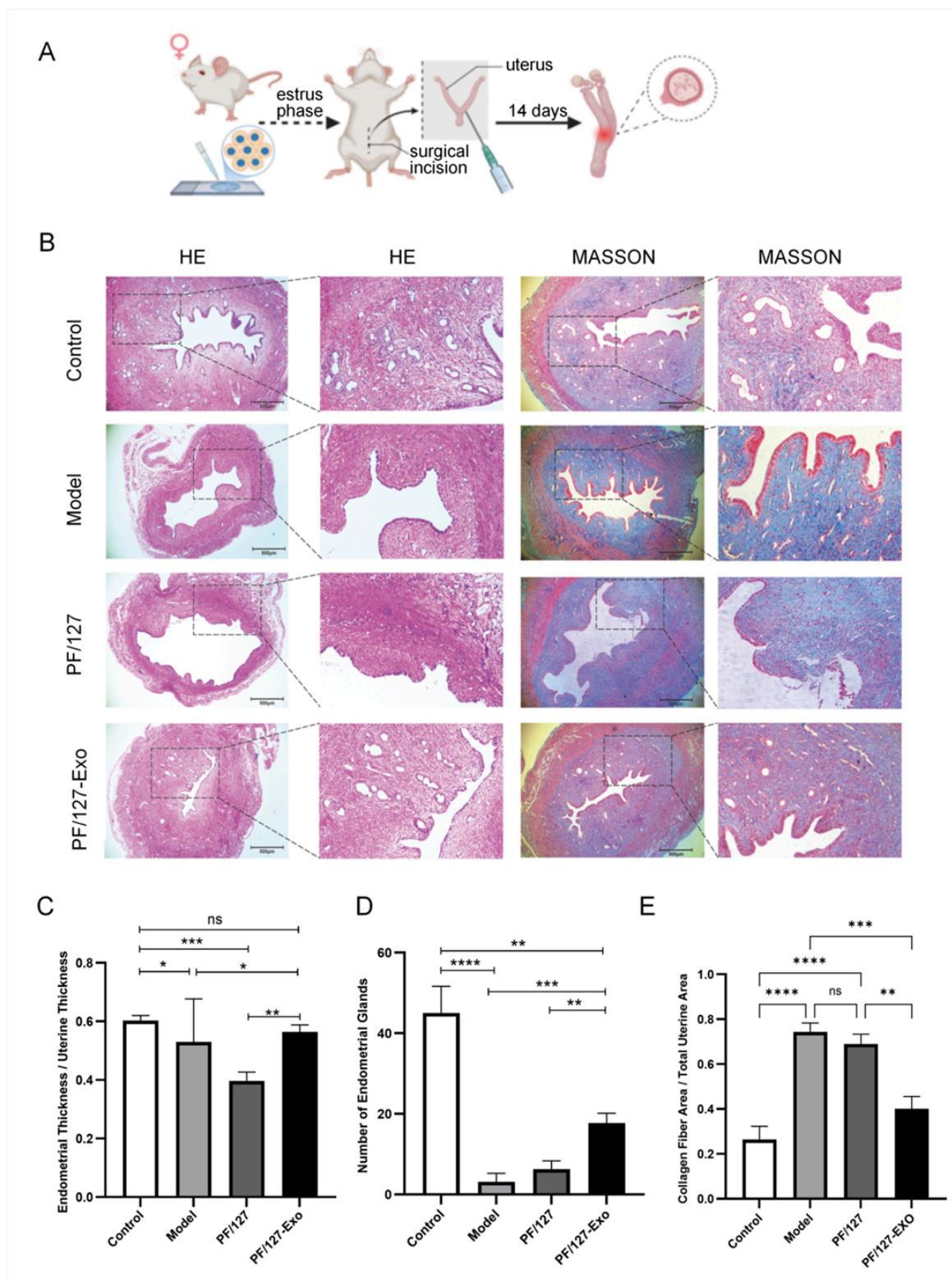


Figure 3

Therapeutic Efficacy of PF/127-hUCMSC-Exo in a Rat Model of Absolute Ethanol-Induced Uterine Injury. (A) Schematic diagram of the experimental timeline for establishing the uterine injury model and subsequent PF-127-Exo treatment. (B) Representative images of HE staining showing uterine tissue histoarchitecture and Masson's trichrome staining indicating collagen deposition (blue) in fibrotic areas. Scale bars, 200 μ m (H&E) and 100 μ m (Masson). (C–E) Quantitative analysis of histological parameters.

(C) Endometrial thickness relative to total uterine wall thickness. (D) Gland count per uterine cross-section. (E) Fibrotic area percentage relative to total tissue area. Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

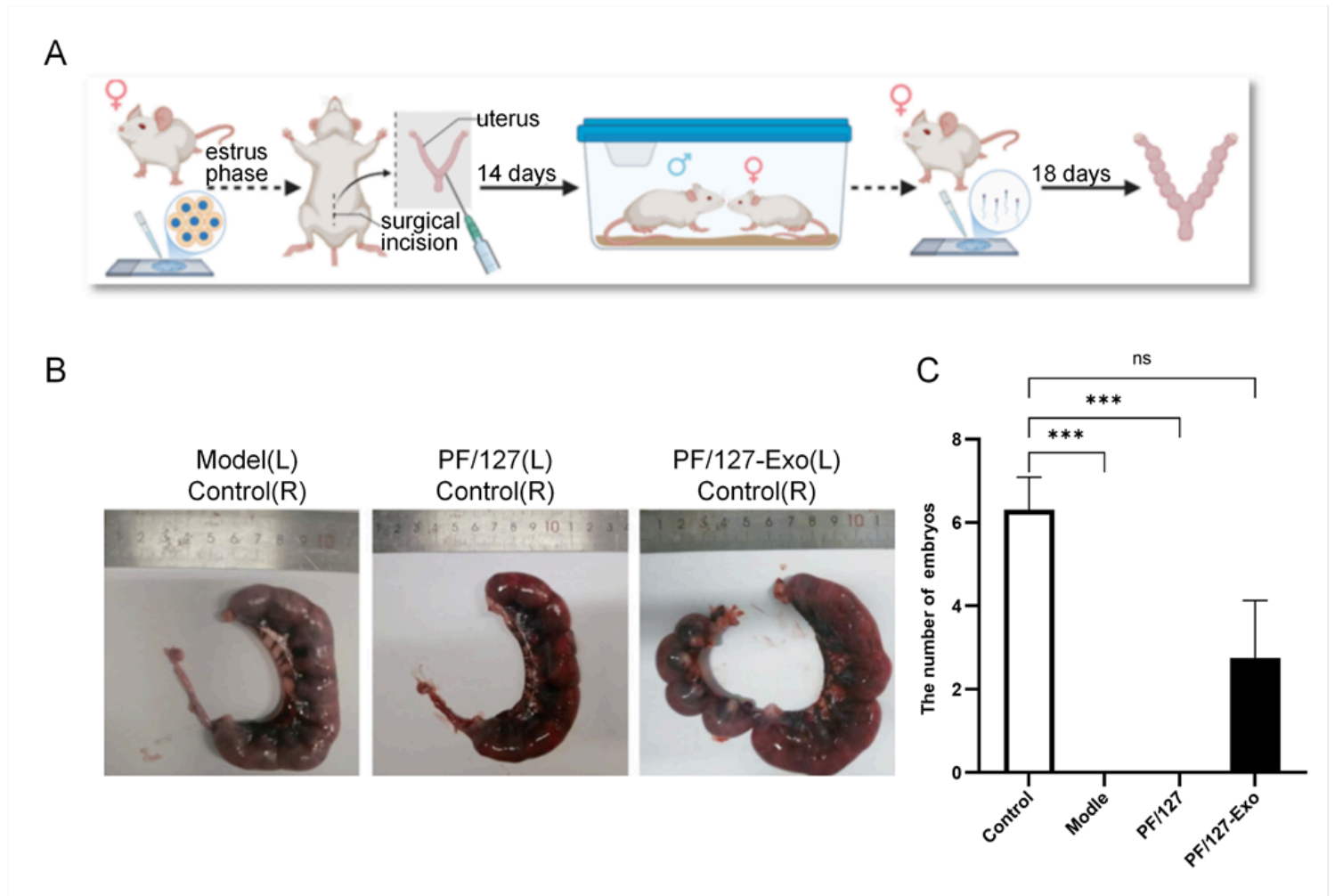


Figure 4

Restoration of Fertility in Injured Rats Following PF/127-hUCMSC-Exo Treatment. (A) Schematic of the mating experiment to assess fertility recovery after uterine injury and treatment. (B) Representative photographs of uteri excised at embryonic day 18 post-mating. Embryos are visible in the control and PF/127-hUCMSC-Exo-treated groups, but absent in the model and PF/127-only groups. (C) Statistical comparison of the number of implanted embryos per uterus across different groups. Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$.

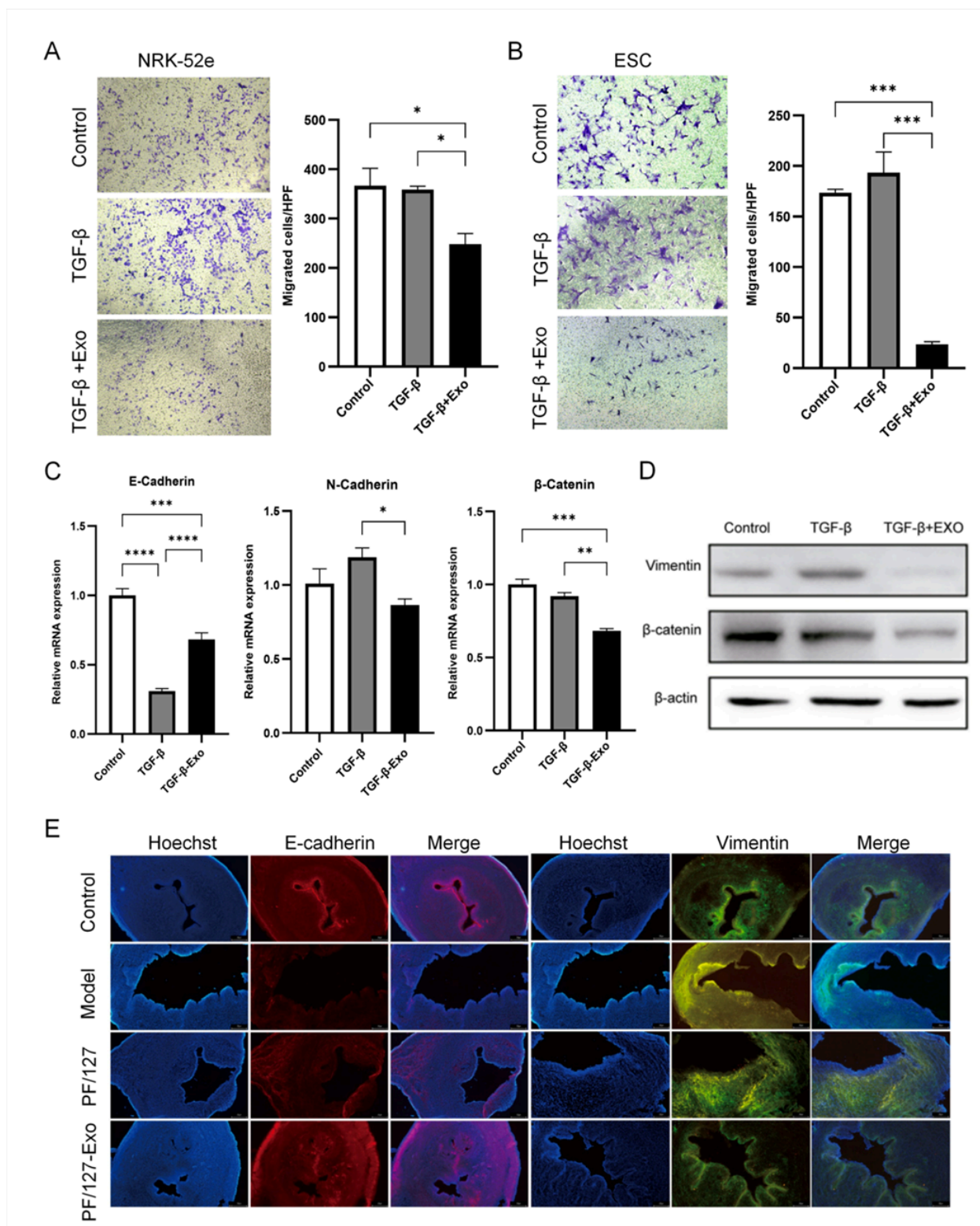


Figure 5

hUCMSC- Exo Inhibit EMT In Vitro and In Vivo. (A, B) (Left) Representative images of Transwell migration assays (crystal violet staining) for (A) NRK-52e and (B) ESCs. (Right) Quantification of migrated cells per field. (C) qPCR analysis of mRNA expression levels for the EMT-related genes E-Cadherin, N-Cadherin, and β -Catenin in NRK-52e cells. (D) Western blot analysis of protein expression levels for the EMT markers Vimentin and β -Catenin in NRK-52e cells. (E) Representative immunofluorescence images of

uterine tissue sections stained for E-Cadherin (red) and Vimentin (green). Nuclei were counterstained with DAPI (blue). Scale bar, 50 μ m. Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

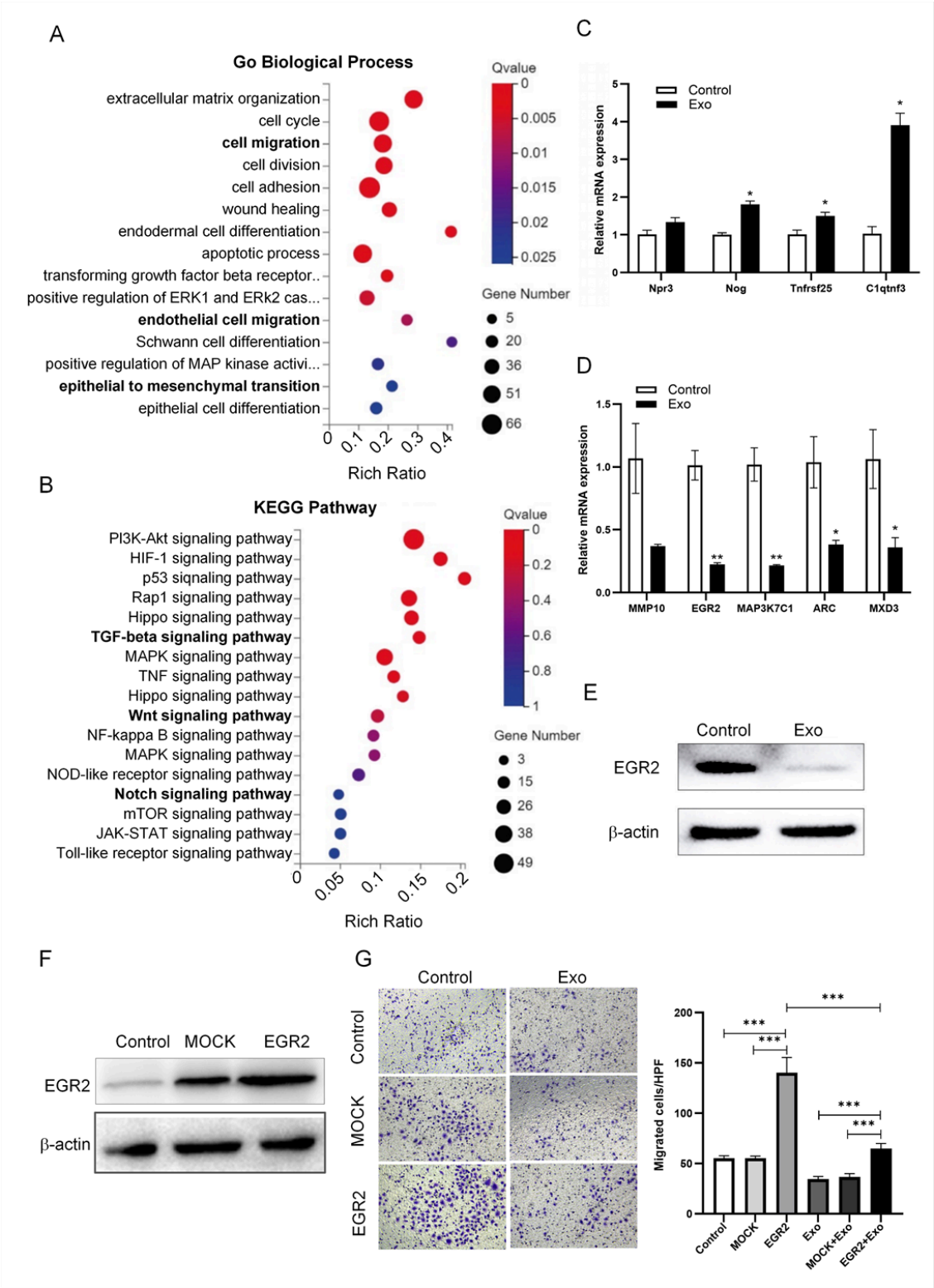


Figure 6

Transcriptomic Analysis and Functional Validation of EGR2 in hUCMSC- Exo-Mediated Therapy. (A, B) Enrichment analysis of differentially expressed genes in primary rat endometrial cells after exosome

treatment. (A) Gene Ontology (GO) terms and (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were significantly enriched. (C,D) The mRNA expression of upregulated genes (C) and downregulated genes (D) in primary rat endometrial cells after exosome treatment. (E) EGR2 protein expression in NRK-52e cells after exosome treatment was detected by Western blot. (F) EGR2 protein expression in NRK-52e cells transfected with an EGR2 plasmid versus an empty vector was detected by Western blot. (G) (Left) Transwell migration assay (crystal violet staining) of NRK-52e cells overexpressing EGR2 or empty vector, with or without exosome treatment. (Right) Quantification of migrated cells. Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Files

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