

Exosome-Encapsulated Photocurable Hydrogel Restores Erectile Function in Diabetic Rats via SIRT1-Mediated Inhibition of Ferroptosis

Xinda Chen

Renji Hospital

Mengbo Yang

Renji Hospital

Muhetaierjiang Mutailifu

Ruijin Hospital

Xiaolin Zhang

Renji Hospital

Ming Zhang

Renji Hospital

Yiliang Peng

Renji Hospital

Xiaoqi Wu

Renji Hospital

Weichun Liu

Renji Hospital

Mujun Lu

lumujun@163.com

Renji Hospital

Research Article

Keywords: Diabetic erectile dysfunction, Photocurable Injectable hydrogel, Sustained release hydrogel, Exosomes, Anti-ferroptosis

Posted Date: September 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10110909/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

Erectile dysfunction (ED) is a prevalent chronic condition, and diabetes mellitus (DM) is a major risk factor, making diabetes-induced ED (DMED) particularly difficult to cure due to complex pathology. Mesenchymal stem cell (MSC)-derived exosomes show therapeutic potential for DMED through signal regulation and damage repair. This study aimed to explore the therapeutic effect of MSC-exosomes on DMED and elucidate the underlying pathological mechanisms, and to develop a hyaluronate hydrogel (HAD) to overcome exosome delivery challenges.

Methods

A hyaluronate hydrogel (HAD) was developed to preserve and sustain the release of exosomes, it gels rapidly upon ultraviolet exposure, adheres to tissue, and forms a three-dimensional network for time-controlled exosome release. Diabetic rats were treated with exosome-encapsulated HAD hydrogel injection. After completing erectile function assessment, rat penile cavernous tissue was harvested, and fibrosis and ferroptosis indicators were evaluated by Masson staining, immunohistochemistry, and Western blot. In vitro cell experiments were performed to validate ferroptosis-related pathways.

Results

The exosome-encapsulated hydrogel treatment improved erectile function impaired by diabetes. It decreased cavernous smooth muscle fibrosis and inhibited diabetes-induced ferroptosis. Mechanistically, MSC-derived exosomes upregulated SIRT1 and promoted the downstream Nrf2/GPX4 anti-ferroptosis pathway.

Conclusions

MSC-derived exosomes can enhance erectile function in DMED by targeting SIRT1 and activating the Nrf2/GPX4 pathway to inhibit ferroptosis and fibrosis. The HAD hydrogel enables practical, sustained exosome delivery, supporting the potential of this approach as a viable therapy for DMED.

Introduction

Diabetes mellitus is a chronic metabolic disorder with a multifactorial etiology. Chronic hyperglycemia and metabolic dysregulation progressively impair the cardiovascular, renal, and nervous systems, as well as other tissues and organs^[1]. These complications pose serious health risks^[2]. Therefore, a comprehensive investigation into the pathogenesis of diabetic complications and the identification of novel therapeutic targets represent global public health priorities. Erectile dysfunction (ED) is a prevalent

complication of diabetes, and diabetic men exhibit a significantly higher risk of developing ED than non-diabetic men^[3]. In diabetic ED(DMED), hyperglycemia is associated with increased oxidative stress and the excessive production of reactive oxygen species (ROS)^[1]. This, in turn, causes damage to the vascular endothelium and smooth muscle^[4]. These pathological alterations contribute to the reduced efficacy of phosphodiesterase type 5 inhibitors(PDE5Is), the first-line treatment for ED, in managing DMED^[5]. Hence, further elucidating the pathophysiology of DMED and gaining deeper insight into the underlying molecular mechanisms are essential for developing more effective therapeutic strategies.

Ferroptosis is an iron-dependent, non-apoptotic form of regulated cell death driven by the accumulation of lipid peroxides^[6], and has recently been implicated in the progression of diabetic complications^[7]. The excessive production of ROS in diabetes also contributes to the initiation of ferroptosis^[8]. Moreover, diabetes-induced disruption of cellular iron metabolism is closely associated with the occurrence of ferroptosis^[7]. Studies have demonstrated that modulating ferroptosis can prevent and treat diabetic complications^[9, 10]. Therefore, targeting ferroptosis has emerged as a feasible therapeutic strategy for diabetes and its various complications.

Stem cells have been extensively investigated in recent years for the treatment of diabetic complications, owing to their immunomodulatory properties, tissue repair capacity, and multipotent differentiation potential^[11, 12]. However, several concerns limit the clinical application of stem cell therapy, including potential tumorigenicity, immune rejection of allogeneic transplants, and ethical restrictions^[13–16]. Furthermore, post-transplantation declines in cell viability and stemness contribute to suboptimal therapeutic outcomes. Extracellular vesicles (EVs) are lipid-bilayer-enclosed vesicles released by cells into the extracellular space. Based on their biogenesis, release pathways, size, cargo, and function, EVs can be classified into exosomes, microvesicles, and apoptotic bodies^[17]. Exosomes are the smallest among them, with a diameter usually ranging between 30–200 nm. The main components are primarily lipids, nucleic acids, and proteins^[18]. Their functions include intercellular communication and cellular vitality maintaining^[19]. Based on the role of exosomes in intercellular signaling communication, stem cell-derived exosomes may represent an excellent alternative to stem cell therapy. The use of exosomes could circumvent the risks of tumorigenicity, allogeneic immune rejection, and ethical concerns commonly associated with stem cell therapy. Moreover, exosomes offer advantages such as ease of storage and convenient application. Therefore, exosome-based therapy may constitute a more effective therapeutic strategy.

Currently, the injection of exosomes into the corpus cavernosum for the treatment of ED is limited by the poor local retention of EVs^[20]. This limitation reduces the therapeutic efficacy of exosomes and hinders clinical translation. Furthermore, while stem cells can be retained at the treatment site through cell–tissue adhesion, exosomes are more readily cleared by bodily fluids, particularly in highly perfused regions such as the cavernous sinus. To address this issue, a hyaluronic acid-modified injectable adhesive hydrogel (HAD hydrogel) was developed in this study. Compared with exosomes administered alone, the combination of HAD and exosomes significantly prolonged the release and increased the local

retention concentration of exosomes in rats. The HAD hydrogel is injectable and can rapidly fill the cavernous sinus, then adhere tightly to the sinus tissue upon ultraviolet (UV) irradiation. The encapsulated exosomes inhibited fibrosis and ferroptosis through the SIRT1 signaling pathway. This exosome-encapsulated HAD hydrogel strategy significantly improved erectile function in diabetic ED rats.

This study presents a photocurable injectable sustained-release hydrogel (HAD) loaded with exosomes derived from umbilical cord mesenchymal stem cells (uMSCs) that restored erectile function in diabetic rats. Exosomes were isolated from uMSCs, after which HAD was designed and synthesized to encapsulate these exosomes.

Diabetes mellitus was induced in rats by intraperitoneal injection of streptozotocin (STZ), and the animals were subsequently treated by intracavernosal injection of the exosome-encapsulated HAD hydrogel. The sustained release provided by the HAD hydrogel enabled the exosomes to remain effective for an extended period. After 4 weeks, assessment of erectile function demonstrated that the exosome-loaded HAD hydrogel significantly improved erectile function impaired by diabetes mellitus..

Materials and methods

2.1 Isolation of HUMSC-Exosomes

Umbilical cord-derived mesenchymal stem cells were isolated from discarded maternal placental and umbilical cord tissues. P2 generation umbilical cord MSCs were seeded into 10 cm cell culture dishes. The supernatant was collected at the time of fastest cell growth. After collecting the stem cell supernatant, it was transferred to a 50 ml centrifuge tube and centrifuged at 300 g for 10 min to precipitate the cells. Then, the supernatant was transferred to a new 50 ml centrifuge tube and centrifuged at 2000 g for 10 min to discard any cellular debris in the precipitate and collect the supernatant. The supernatant was transferred to a new centrifuge tube and centrifuged at 10000g for 30 minutes at 4°C. This step precipitated larger extracellular vesicles, such as apoptotic vesicles and microcysts, which were discarded. The remaining supernatant was then transferred to a new ultracentrifuge tube and ultracentrifuged at 100000g for 90 minutes at 4°C. The supernatant was discarded and the precipitate, which consisted of exosomes, was resuspended in PBS and stored at -80°C for long-term preservation^[21].

2.2 Nanoparticle tracking analysis

The nanoparticle tracking analyser cover was assembled and rinsed with ddH₂O until the field of view was clean and free of impurities. Take 20µl of exosomes suspended in PBS and dilute it 30 times with PBS to obtain a 600µl solution. Slowly inject the diluted exosome PBS solution using a syringe. The exosome particles' scattered light was captured in the field of view, and the camera and counting were performed for 60 seconds each time. The syringe was then pushed to change the recording field of view

five times. Finally, the average particle size and density data of exosomes were obtained by calculating the particles' trajectory in the solution^[22].

2.3 Synthesis and characterization of the HAD hydrogel

For the synthesis of HAD polymer, hyaluronic acid was dissolved in deionized water at a concentration of 1%w/v. EDC (4 mmol), NHS (4 mmol), and AEMA (The above reagents were purchased from Sigma-Aldrich, Shanghai) were slowly added to the solution and stirred for 30 ~ 40 minutes. After that, dopamine hydrochloride (Sigma-Aldrich, Shanghai) was added to the mixture for 24 hours. All solutions were kept under nitrogen protection and stored in darkness at room temperature to prevent oxidation. The pH value was monitored and adjusted between 5 ~ 6 with 0.1 N hydrochloric acid and sodium hydroxide. After the reaction, the unreacted monomers were eliminated by dialysis (MWCO = 8000 ~ 12000 Da) under acidic conditions for 72 h and then lyophilized. The lyophilized extract(s) foam was stored in a closed container and kept from light till used. The mass ratios of HA: AEMA: DA were set as 1:3:0, 1:3:1, 1:3:2, and 1:4:2, and these polymers were named as H-3A, H-3A-1D, H-3A-2D, and H-4A-2D, respectively. To gelate the HAD hydrogel, the lyophilized HAD precursor was added to PBS with exosomes at a concentration of 3 wt%. Then, 0.1 wt% LAP was added as the photoinitiator. The solution was stored at 4°C in the dark until use. The HAD hydrogels were formed after photocrosslinking under 365 nm UV irradiation for 10 seconds.

2.4 Animal experiment

Male SD rats(8 weeks, 200-250g) were used for the animal experiment. STZ (streptozotocin Sigma-Aldrich, Shanghai) was intraperitoneally injected into all rats except the normal group at a dose of 30mg/d for 3 consecutive days. Rats with blood glucose levels exceeding 16.7 mmol/L, measured by glucometer 3 times within 1 week after STZ injection, were considered to have diabetes. Four weeks later, diabetic rats were injected subcutaneously with apomorphine (0.5 mg/kg; apomorphine 0.5 g/L dissolved in saline) at the back of their necks. The rats were then placed in a dark and quiet environment, and their penile congestion and erection were observed three times. Erectile function was determined based on the results, and rats with erectile dysfunction were screened out from the diabetic rats^[23]. The study involved dividing rats into four groups randomly: Normal, PBS, Exosome, and Exosomes + HAD (n = 8 in each group). The latter three groups received injections of 100µl of PBS, PBS solution of exosomes, and exosome-encapsulated HAD hydrogel, respectively. After four weeks of injection, the rats in all groups underwent an assessment of their erectile function. Four weeks after the injection, the rats in all groups underwent an assessment of their erectile function. Rats were anaesthetised with an intraperitoneal injection of sodium pentobarbital (40mg/kg). The depth of anaesthesia was monitored throughout the procedure by assessing the absence of pedal withdrawal reflex and a stable respiratory rate. After assessing erectile function, all rats were euthanised while still under deep pentobarbital anaesthesia by an intraperitoneal overdose of sodium pentobarbital (200 mg/kg). Death was confirmed by the irreversible cessation of respiration and heartbeat, followed by a secondary physical method (bilateral thoracotomy) in accordance with institutional and ARRIVE 2.0 guidelines. The corpus cavernosum were then removed from the penises and half were fixed in paraformaldehyde while the

other half were frozen in liquid nitrogen for further analysis. All experiments were approved by the Experimental Animal Ethics Committee of Renji Hospital, and animal welfare was ensured under their supervision.

2.5 In vivo tracing imaging

Exosomes were labelled with the near-infrared fluorescent dye DiR (Yeasen, Shanghai, China). After anaesthetising the rats, the tissue and mucosa surrounding the penis were bluntly separated. Subsequently, the cavernous body of the penis was exposed and injected with exosomes or hydrogel. The hydrogel group was irradiated with ultraviolet light for 15 seconds after injection to complete cross-linking of the hydrogel. Following the injection, the incision was sutured and the rats were returned to their rearing cage. At 1, 4, 7 and 14 days after injection, we observed the regions where DiR-labelled exosomes were located using a whole-body fluorescence imaging system and captured in vivo images.

2.6 Erectile function evaluation

After administering anaesthesia to the rats, the carotid artery on one side, the pedicle of the penis and the prostate were exposed. The cavernous nerves that emanate from the major pelvic ganglion (MPG) were found posterior to the prostate. Two catheters filled with heparin solution were inserted into the carotid artery and penile pedicle to record mean arterial pressure (MAP) and intracavernous pressure (ICP) simultaneously. The pressure transducers were connected to a data acquisition system (BL-420s, Chengdu Taimeng Software Co. Ltd., China). The cavernous nerves were stimulated using an electrode hook for 60 seconds each time, with the next stimulation occurring at 5-minute intervals after the intracavernous pressure returned to a steady state. The ICP and MAP of each stimulation were recorded, and the ratio of ICP to MAP was calculated to compare erectile function.

2.7 Masson trichrome staining

The middle section of the penis was harvested and cut to a thickness of approximately 4 mm for Masson trichrome staining. The tissue was fixed overnight in 4% paraformaldehyde, dehydrated in graded ethanol, and embedded in paraffin. The paraffin-embedded tissues were then sectioned into 5 μ m sections, stained using the Masson trichrome staining kit (Solarbio, United States), and observed under a microscope. The ImageJ software was used to compare collagen tissue stained blue with smooth muscle stained red in each section for statistical analysis.

2.8 Western blot

The cells or tissues were added to the lysis buffer to dissolve the protein. Next, loading buffer (Beyotime, Shanghai) was added to the protein samples and boiled at 95°C. The proteins were then separated from the samples using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes. Following the transfer, the membranes were immersed in 5% skimmed milk/tris buffered saline tween (TBST) for 1 hour at 25°C. The membranes were incubated with primary antibodies (Abcam, Shanghai) overnight at 4°. Following this, the membrane was washed 4–6 times with TBST buffer and the secondary antibody (Abcam, Shanghai) was incubated with the primary

antibody for 1 hour at room temperature. The protein bands were visualized using an ultrasensitive chemiluminescence detection kit (Yase, Shanghai, China) under a gel imager (Bio-Rad, USA). The signal intensity was measured using ImageJ software (National Institutes of Health, USA), and the protein bands were quantified.

2.9 Quantitative PCR

Total RNA was extracted using Trizol (Beyotime, Shanghai) following the RNA extraction method. First Strand cDNA Synthesis Kit (Beyotime, Shanghai) was used to synthesize cDNA. The cDNA, forward primer, reverse primer and SYBR Green qPCR Mix Kit (Beyotime, Shanghai) were added to a 384-well plate. The fluorescence real-time quantitative thermocycler (Roche, Shanghai) was used to perform the qPCR reaction. The relative expression of the corresponding genes in the samples was calculated based on the cycling threshold Ct value.

2.10 Exosomes sustained release curve

HAD hydrogel with encapsulated exosomes and HAD hydrogel without encapsulated exosomes were added to the upper chamber of a 24-well Transwell plate (Corning, Shanghai). Then, 200 μ L of PBS was added to the lower chamber. Subsequently, PBS from the lower chamber was collected on days 4, 7, 10, 14, and 18, and 200 μ L of fresh PBS was added. The amount of exosomes in the PBS was measured using the BCA Protein Assay Kit II (Abcam, Shanghai), and the percentage of exosomes released was calculated.

2.11 Cell viability

For cell viability assessment, CCSMCs were seeded into 96-well plates at a density of 5×10^5 cells/mL. The normal group was supplemented with 100 μ L of DMEM medium, the HAD group with the DMEM leachate of HAD hydrogel, and the EHAD group with the DMEM leachate of exosome-encapsulated HAD hydrogel. Cell viability was measured using the CCK8 kit (Beyotime, Shanghai), and the absorbance at 450 nm was recorded by an enzyme marker. Cell viability was also evaluated by fluorescence microscopy using a Live/Dead cell staining kit (Beyotime, Shanghai, China) after 24-48h of culture.

2.12 Measurement of iron concentration

The corpus cavernous samples were washed twice with pre-cooled PBS buffer. Then, the tissue was cut into pieces on ice and 10 times the volume of iron detection buffer (reagents purchased from Sigma-Aldrich, Shanghai) was added. The tissue was homogenised on ice using a tissue homogeniser. After completing the homogenisation, the tissue was centrifuged at 4°C, 12000g for 10 minutes. The precipitate was discarded, and the supernatant was collected and placed on ice. In a 96-well plate, 100 μ L of the supernatant was added to each well. For the wells measuring Fe³⁺, 5 μ L of iron reductant was added, while for the other wells, 5 μ L of iron detection buffer was added. The plate was then incubated at 37°C for 30 minutes. Next, 100 μ L of iron probe was added to each well, and the plate was incubated at 37°C for 60 minutes, protected from light. Finally, the plate was placed on a microplate reader to detect the absorbance at 593nm. Finally, to calculate the concentration, we obtained the iron concentration of

the measured samples by comparing the concentration curve of the standards based on the absorbance.

2.13 Measurement of lipid ROS

CCSMC cells were cultured in serum-free DMEM for 24 hours. Subsequently, the cells were inoculated into six-well plates at a density of 105 cells per well. After 24 hours of incubation, the lipid peroxidation specific dye BODIPY™ 581/591 C11 (Thermo Fisher, Shanghai, China) was added at a concentration of 5 $\mu\text{mol/L}$. The cells were incubated in dark conditions at 37°C for 30 minutes, washed twice with PBS buffer, and digested with 0.25% trypsin without EDTA for 1 minute. The cells were then collected in centrifuge tubes and centrifuged at 1200 g for 3 minutes at room temperature. After centrifugation, the cells were resuspended in 300 μl of PBS buffer. Finally, flow cytometry was performed using a Beckman CytoFLEX flow cytometer, and the FACS data were analysed using FlowJo V10.

2.14 Measurement of MDA

Malondialdehyde was detected using a malondialdehyde assay kit from Biyun Tian in Shanghai, China. The cells were inoculated under various conditions in six-well plates and cultured for 24 hours. Afterward, the cells were lysed, centrifuged, and the supernatants were collected. 200 μl of the configured MDA working solution was added to 100 μl of the supernatant in EP tubes. The tubes were incubated at 100°C in a metal bath for 15 minutes, then cooled to room temperature in a water bath. After centrifugation at 1000g for 10 minutes at room temperature, 200 μl of the resulting supernatant was added to 96-well plates. The absorbance at 532 nm was detected using a microplate reader.

2.15 Statistical analysis

The results are presented as mean $\pm$ standard deviation. Statistical differences were determined using one-way or two-way analyses of variance (ANOVA) with GraphPad Prism 9.0.0 (GraphPad Software, San Diego, CA, USA). Details on the statistics used can be found in the figure legends. Differences were considered statistically significant at $p < 0.05$.

Results

3.1 Preparation and characterization of exosomes-encapsulated hydrogel

Exosomes isolated from umbilical cord mesenchymal stem cells by ultra-fast differential centrifugation had typical lipid bilayer-enclosed cup-shaped morphology, a 50–200 nm size distribution (mean $\sim$ 100 nm), and exosomal markers (Fig. 1a–c).

To improve exosome delivery, exosomes were encapsulated in a hyaluronic acid–dopamine (HAD) hydrogel prepared by adding AEMA for UV-crosslinkable network formation, dopamine hydrochloride for tissue adhesion, and LAP as a photoinitiator. The precursor exhibited good injectability and rapidly

polymerized under 365 nm UV after injection, forming a stable, adhesive gel that stayed at the site(Fig.S1). Dopamine moieties underwent Michael addition with tissue amino groups upon contact, ensuring robust adhesion (Fig. 1d). Hydrogels with HA:AEMA:DA mass ratios of 1:3:0, 1:3:1, 1:3:2, and 1:4:2 (designated H3A, H3A1D, H3A2D, and H4A2D) exhibited similar rheological properties(Fig.S2). Among them, H3A1D demonstrated optimal sustained exosome release (Fig. 1f), and all groups maintained satisfactory injectability. Therefore, H3A1D was selected as the carrier. Zeta potential analysis confirmed that the exosomes were successfully encapsulated within the HAD hydrogel((Fig. 1g). Post-gelation, the AEMA network markedly increased mechanical strength and Young's modulus and generated a porous mesh architecture (Fig.S3). To evaluate the biosafety of the hydrogel, CCSMCs were cultured in medium conditioned with the HAD hydrogel. Live/dead staining (Fig. 2a) and CCK-8 assays (Fig. 2b) demonstrated that the hydrogel did not significantly compromise cell viability and exhibited no appreciable cytotoxicity.

3.2 Characterization of exosome-encapsulated HAD hydrogel

For in vivo tracking, exosomes were labeled with the near-infrared fluorescent dye DiR. Rats were divided into two groups and administered intracavernosal injections of either exosomes suspended in PBS or exosomes encapsulated within the HAD hydrogel. Whole-body fluorescence imaging was performed on days 1, 4, 7, and 14 post-injection to monitor the distribution of the DiR-labeled exosomes at the injection site (Fig. 4c). Imaging analysis revealed that directly injected exosomes underwent rapid local clearance, whereas hydrogel-mediated delivery substantially prolonged exosome retention in the corpus cavernosum (Fig. 4d).

3.3 Exosome-encapsulated HAD hydrogel restore erectile function in diabetic rats

To evaluate the therapeutic efficacy of the exosome-encapsulated HAD hydrogel, diabetic rats were generated by intraperitoneal injection of streptozotocin (STZ). Rats that developed diabetic erectile dysfunction (DMED) were screened and randomly assigned to three groups: Exosome, Exosome + HAD, and PBS. These groups received intracavernosal injections of exosome-loaded HAD hydrogel, exosomes suspended in PBS, and an equivalent volume of PBS, respectively. Four weeks after treatment, erectile function was assessed by measuring the intracavernous pressure (ICP) and mean arterial pressure (MAP) (Fig. 3B). The ICP/MAP ratio revealed that diabetes caused a marked impairment of erectile function. Both exosome-treated groups demonstrated improved erectile function relative to the PBS group, with the Exosome + HAD group exhibiting the most substantial recovery. Notably, erectile function in the Exosome + HAD group was restored to near-normal levels (Fig. 3C).

3.4 Exosome-encapsulated HAD hydrogel inhibit fibrosis

To elucidate the pathophysiological mechanisms, cavernous tissues were collected after manometric assessment and stained with Masson's trichrome (Fig. 3D). Compared with normal rats, diabetic rats

showed significantly reduced smooth muscle content and marked accumulation of collagen fibers, indicating cavernous fibrosis. Both Exosome and Exosome + HAD groups exhibited attenuated fibrosis compared to untreated diabetic rats, with the Exosome + HAD group showing a significantly greater reduction.

Previous studies have demonstrated that exosomes can mitigate fibrosis by suppressing TGF- β signaling, a canonical profibrotic pathway^[25]. To verify this, cavernous tissue proteins were analyzed by Western blot, which revealed upregulation of TGF- β and its downstream mediators Smad2/3 in DMED rats (Fig. 3F). These findings indicate that a major contributor to diabetes-induced erectile dysfunction is cavernous smooth muscle damage and consequent fibrosis driven by the hyperglycemic environment.

3.5 Downregulation of SIRT1 and upregulation of ferroptosis in hyperglycemia group

To investigate how diabetes induces injury in the penile corpus cavernosum and the specific pathological mechanisms involved, we performed transcriptome sequencing on normal and high-glucose-treated rat corpus cavernosum smooth muscle cells (CCSMCs). Differentially expressed genes (DEGs) were screened using the criteria $|\log_2(\text{FoldChange})| > 1$ and $p < 0.05$. The results identified 106 up-regulated and 99 down-regulated genes (Fig. 4A and B). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were subsequently conducted. In the GO enrichment analysis, several significantly enriched terms were associated with “lipid binding” and “lipid metabolism,” indicating altered lipid metabolic processes in the cells (Fig. 4C). Consistently, KEGG enrichment analysis also pointed to dysregulation of lipid metabolism (Fig. 4D). Given that among the DEGs, the expression of Gpx4 and Slc7a11, genes critical for lipid peroxide metabolism, was down-regulated in the diabetic group, we postulate that under hyperglycemic conditions, the disruption of lipid metabolism and the impaired capacity to counteract lipid peroxidation lead to ferroptosis, a form of lipid peroxide-driven cell death in the diabetic group.

3.6 Exosome-encapsulated HAD hydrogel resist ferroptosis in CCSMC

To verify whether exosome treatment improves erectile function in diabetic rats by inhibiting ferroptosis, we examined the expression of ferroptosis-related proteins in the corpus cavernosum tissues of each group by Western blot. Among the differentially expressed genes (DEGs), SIRT1 was found to be downregulated in the diabetic group (Fig. 4B). As SIRT1 is a potential upstream regulator of ferroptosis that modulates the activity of Nrf2, a key ferroptosis suppressor^[26, 27], we also assessed the expression of these two proteins. The results showed that SIRT1 expression was significantly reduced in the diabetic group, accompanied by downregulation of its downstream target Nrf2. This process was reversed in the exosome-treated groups. GPX4, the central negative regulator of ferroptosis, directly eliminates lipid peroxides to block the execution phase of ferroptosis, a process in which SLC7A11 also participates. Both GPX4 and SLC7A11 were downregulated in the diabetic group and exhibited higher expression levels in the two exosome-treated groups. Immunohistochemical staining and quantitative

PCR (qPCR) analyses yielded consistent results. We additionally examined the expression of transferrin receptor 1 (TFRC). TFRC was upregulated in the corpus cavernosum tissues of diabetic rats, suggesting potential iron overload. We therefore measured the tissue iron concentration. The results revealed that diabetic rats exhibited markedly elevated iron levels in the corpus cavernosum compared with normal rats. In contrast, both the exosome-treated groups showed significantly reduced iron content, indicating that exosomes may attenuate pathological iron accumulation and thereby contribute to the inhibition of ferroptosis in diabetes. Furthermore, the results indicate that the therapeutic effect of the exosomes-encapsulated HAD hydrogel was marginally superior to that of exosomes injected directly. This may be attributed to the extended time of controlled release facilitated by the hydrogel.

3.7 Exosomes up-regulate SIRT1 to inhibit diabetic ferroptosis

To further validate that exosomes ameliorate ferroptosis by modulating SIRT1, rat corpus cavernosum smooth muscle cells (CCSMCs) were cultured in vitro. For mechanistic analysis, CCSMCs were treated with the SIRT1 agonist SRT1720 or the SIRT1 inhibitor EX527. Exosomes were subsequently added following EX527 treatment, and changes in the expression of SIRT1 and its downstream proteins were evaluated. Western blot analysis revealed that SIRT1 is intimately linked to ferroptosis resistance. Upregulation of SIRT1 led to a concomitant increase in the expression of Nrf2, a downstream effector, whereas inhibition of SIRT1 suppressed the Nrf2/GPX4 anti-ferroptotic axis. Importantly, exosomes upregulated SIRT1 expression even in the presence of EX527 and restored the activity of anti-ferroptosis-related proteins (Fig. 6A and B).

To elucidate the mechanisms by which a high-glucose environment triggers smooth muscle injury, CCSMCs were exposed to high-glucose medium. The impaired viability of CCSMCs under hyperglycemic conditions was partially rescued by the ferroptosis inhibitor Fer-1 and by exosomes (Fig. 6D). Exosome administration markedly reduced the excessive accumulation of lipid reactive oxygen species (ROS) in high-glucose-cultured CCSMCs (Fig. 6E). Furthermore, malondialdehyde (MDA) content was elevated in CCSMCs under high-glucose stress but was significantly decreased upon exosome treatment (Fig. 6C). These results suggest that hyperglycemia is a potent driver of ferroptosis in CCSMCs. We subsequently assessed SIRT1 expression in CCSMCs cultured in high-glucose medium and found that SIRT1 was suppressed. Notably, exosome-mediated SIRT1 upregulation counteracted this downregulation and prevented the onset of ferroptosis (Fig. 6F and G).

Discussion

The first-line treatment for erectile dysfunction is currently PDE5 inhibitors. However, their efficacy in diabetic ED remains suboptimal^[4]. This limitation is largely attributed to diabetes-associated damage to the endothelium and corpus cavernosum smooth muscle^[28–30]. Physiologically, erection is achieved through relaxation of smooth muscle cells in the cavernous arterioles and sinusoids, which allows blood to fill the corpora cavernosa^[31]. In diabetic ED, injury to the cavernous smooth muscle leads to

insufficient penile blood flow and failure to maintain rigidity, and the functional capacity of the damaged smooth muscle cannot be restored by PDE5 inhibitors^[29]. In recent years, stem cells and their derivatives have increasingly become a new therapeutic tool and have been consistently proven in animal models and clinics^[20, 32, 33]. Previous studies have demonstrated the potential of adipose-derived stem cell injection into the corpus cavernosum for the recovery of erectile function in rats with diabetic ED^[23]. This approach leverages the tissue regeneration and repair capabilities of stem cells. Nonetheless, the clinical translation of stem cell-based therapies is constrained by ethical considerations and the risk of allogeneic immune rejection. These limitations have prompted the search for alternative therapeutic strategies. Exosomes are nanoscale extracellular vesicles that carry a diverse cargo of signaling proteins, lipids, nucleic acids, and other bioactive molecules^[18]. They serve multiple roles, such as intercellular communication, and can be taken up and endocytosed by distant target cells, which can alter the biological behaviors of the target cells^[17, 34]. Compared with stem cells, exosomes derived from stem cells exhibit substantially lower risks of allogeneic rejection and fewer ethical concerns, while also offering distinct practical advantages including ease of storage and convenient administration. These features make exosomes a particularly attractive modality for the treatment of erectile dysfunction^[21].

However, direct exosome injection is associated with a critical limitation. Unlike stem cells, exosomes tend to rapidly disperse in body fluids after administration, lack intrinsic adhesion to cells or the extracellular matrix, and are cleared from the injection site within a short period, making it difficult to sustain local therapeutic concentrations^[35]. Therefore, a time-controlled release strategy is required to avoid rapid depletion and to maintain prolonged bioactivity. Previous studies have largely relied on direct bolus delivery of exosomes, resulting in inefficient utilization and loss of therapeutic potential^[36, 37]. To overcome these challenges and improve the bioavailability of exosomes, we have developed HAD hydrogel. The hydrogel is based on hyaluronic acid, which possesses excellent biocompatibility, and its three-dimensional polymeric network enables efficient encapsulation and sustained release of exosomes. Unlike typical hydrogel applications in wound healing or osteochondral repair, the corpus cavernosum presents a unique anatomical environment. The tissue is highly vascularized with constant blood flow, yet the organ is structurally delicate, demanding both sufficient mechanical integrity and high injectability. Accordingly, the synthesis ratio was carefully optimized to balance fluidity for injection with post-gelation mechanical strength. Photocrosslinking allows the hydrogel precursor to rapidly gel in situ following a brief ultraviolet exposure of approximately 10 seconds, without inducing detectable cytotoxicity or physical damage. After crosslinking the hydrogen bonds would be formed between the dopamine groups of the HAD hydrogel and the amino groups on the exosome surface. This allows for a slow release of exosomes and adhesion to tissues, ensuring retention of the hydrogel at the treatment site.

After constructing the exosome-encapsulated hydrogel, we treated diabetic ED rats with cavernous injection to investigate the effect of exosomes on improving erectile function. We concentrated on whether exosomes could inhibit or reverse smooth muscle injury. Diabetes mellitus is known to promote fibrosis in multiple tissues and organs^[38–40], including cavernous smooth muscle^[41], which represents a

major contributor to DMED. Our results demonstrate that exosome treatment can rescue this fibrotic process. One finding of this process is that exosomes regulate the TGF- β /Smads signalling pathway. In response to exosomes, SIRT1 expression is upregulated, inhibiting TGF- β , a significant fibrotic factor^[42]. Thus, in terms of the gross specimen, we observed inhibition of corpus cavernous fibrosis.

SIRT1 is a NAD-dependent deacetylase that regulates cellular functions such as resistance to oxidative stress and regulation of apoptosis by deacetylating key proteins^[43, 44]. SIRT1 controls the deacetylation of Nrf2, a crucial transcription factor that regulates cellular antioxidant activity and is essential for maintaining cellular redox homeostasis^[45, 46]. It was found that exosomes can regulate the expression of SIRT1^[47]. In combination with the ferroptosis that occurred in the corpus cavernosum of diabetic rats, we believed that exosomes may be able to inhibit ferroptosis in diabetic ED by regulating SIRT1. The SIRT1/Nrf2 pathway may be important for the therapeutic role of exosomes. The experimental results confirmed the hypothesis that exosomes can act on SIRT1 and regulate this signalling pathway. The elevation of SIRT1 expression can inhibit the process of ferroptosis, as verified in our in vitro experiments. When cultured with high glucose in vitro, CCSMC showed an increase in the expression of important antioxidant system proteins, such as GPX4 and SLC7A11, as well as a decrease in the ferroptosis index after the addition of exosome treatment, which supports the conclusion that SIRT1 promotes Nrf2 expression to enhance cell antioxidant capacity.

Conclusions

In this study, we presented a photocurable injectable sustained-release hydrogel (HAD) encapsulating umbilical cord mesenchymal stem cell-derived exosomes, which restored erectile function in diabetic rats. The HAD hydrogel exhibited excellent injectability along with rapid photocuring and remarkable mechanical strength upon brief UV exposure after injection, making it highly user-friendly. Moreover, the hydrogel displayed outstanding adhesive properties, ensuring stable retention at the injection site. The sustained-release profile of the HAD hydrogel further enabled exosomes to remain bioactive over an extended period.

We next validated the therapeutic efficacy of the exosome-loaded HAD hydrogel in a diabetic rat model. This innovative strategy effectively ameliorated erectile dysfunction induced by diabetes mellitus. Mechanistically, exosome treatment upregulated the expression of SIRT1, a critical regulatory target, which protected corpus cavernosum smooth muscle against both fibrosis and ferroptosis. Concomitantly, exosomes enhanced the expression of Nrf2 and GPX4, key components of the anti-ferroptotic machinery, thereby exerting a protective effect against diabetic ferroptosis. These findings suggest that targeting ferroptosis in corpus cavernosum smooth muscle may represent a promising therapeutic avenue for diabetic erectile dysfunction.

In summary, the exosome-encapsulated HAD hydrogel we developed in the study hold the potential to overcome current translational barriers in the field of exosome therapy for diabetic erectile dysfunction.

Declarations

Ethics approval and consent to participate

The donors of hUCMSCs provided written informed consent for participation in the study (Supplementary 2, pp. 1–4). This study was approved by the Ethics Committee of Renji Hospital, Shanghai Jiao Tong University School of Medicine, under the project title ‘Experimental Study of Human Umbilical Cord Mesenchymal Stem Cells for the Treatment of CNI-Erectile Dysfunction’ (Approval No. KY2022-180-B; date of approval: October 26, 2022). This ethics approval also applies to our animal study (Supplementary 2, pp. 5–6).

Consent for publication

Not applicable

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Information

This work was supported by the National Natural Science Foundation of China (No. 82171611, No. 82371631, No. 82401887) and National Key Research and Development Program of China (2024YFA1108100).

Author Contribution

ML and XW conceived this project and supervised all the experiments. XC, MY and MM designed and performed most of the experiments. MM and XW designed and synthesised the hydrogel. XZ, MZ and YP helped with the analysis of the data. WL acquired the stem cells. ML and XW revised the paper. MY and ML required funding.

Acknowledgments

The authors declare that they have not use AI-generated work in this manuscript.

Data availability

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

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Figures

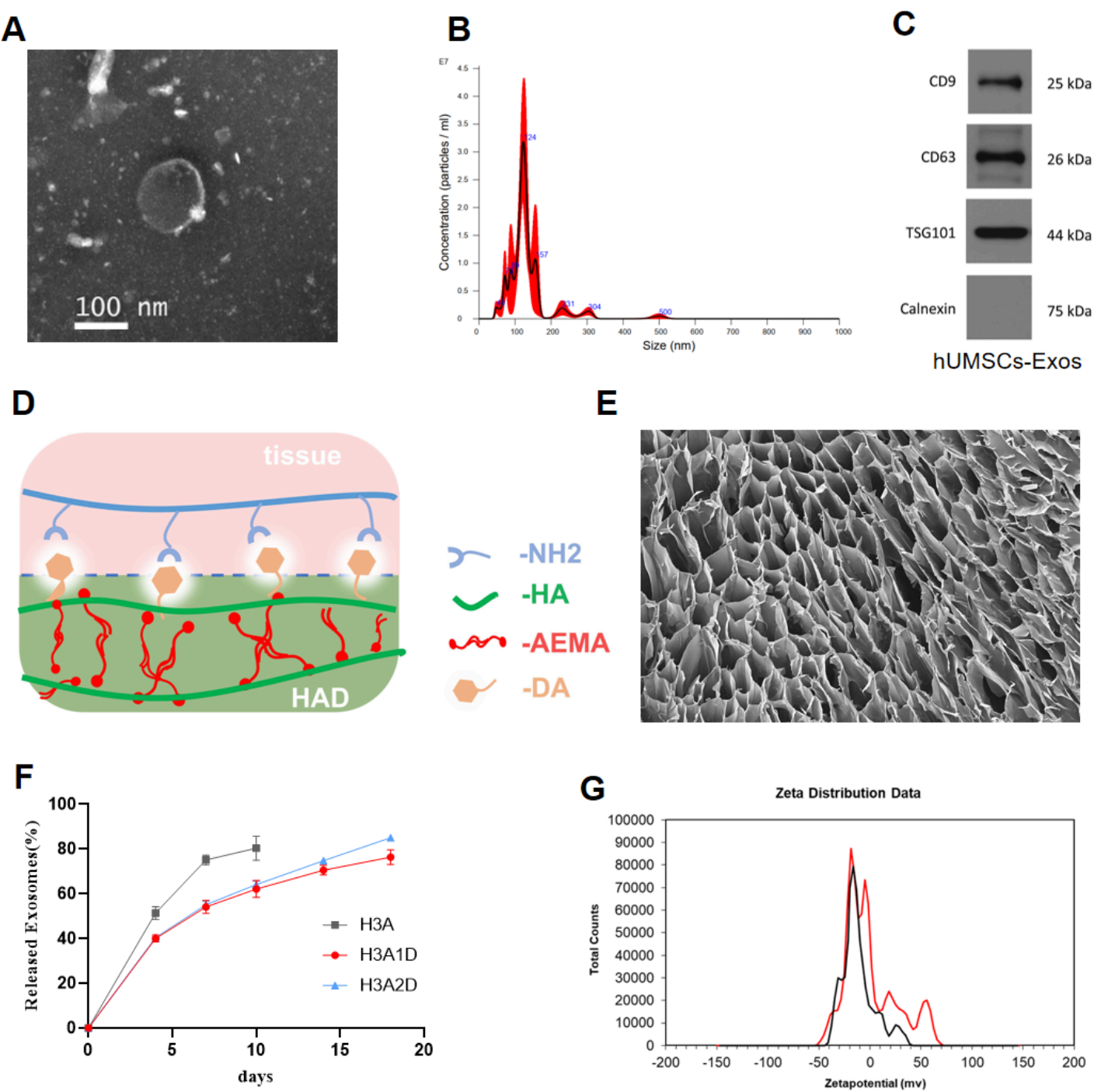


Figure 1

Characterization of exsomes and properties of exosome-encapsulated HAD hydrogel

A) SEM of typical exosome nanoparticle. B) Nanoparticle tracking analysis of UMSCs-Exosomes. C) Biomarkers of exosome by western blotting. D) Adhesion of HAD hydrogel with the amino groups on the tissue surfaces. (E) Three-dimensional network structure of HAD hydrogel under electron microscope scanning. F) HSustained release of exosomes in HAD hydrogels. G) Zeta distribution of HAD hydrogel with or without exosomes.

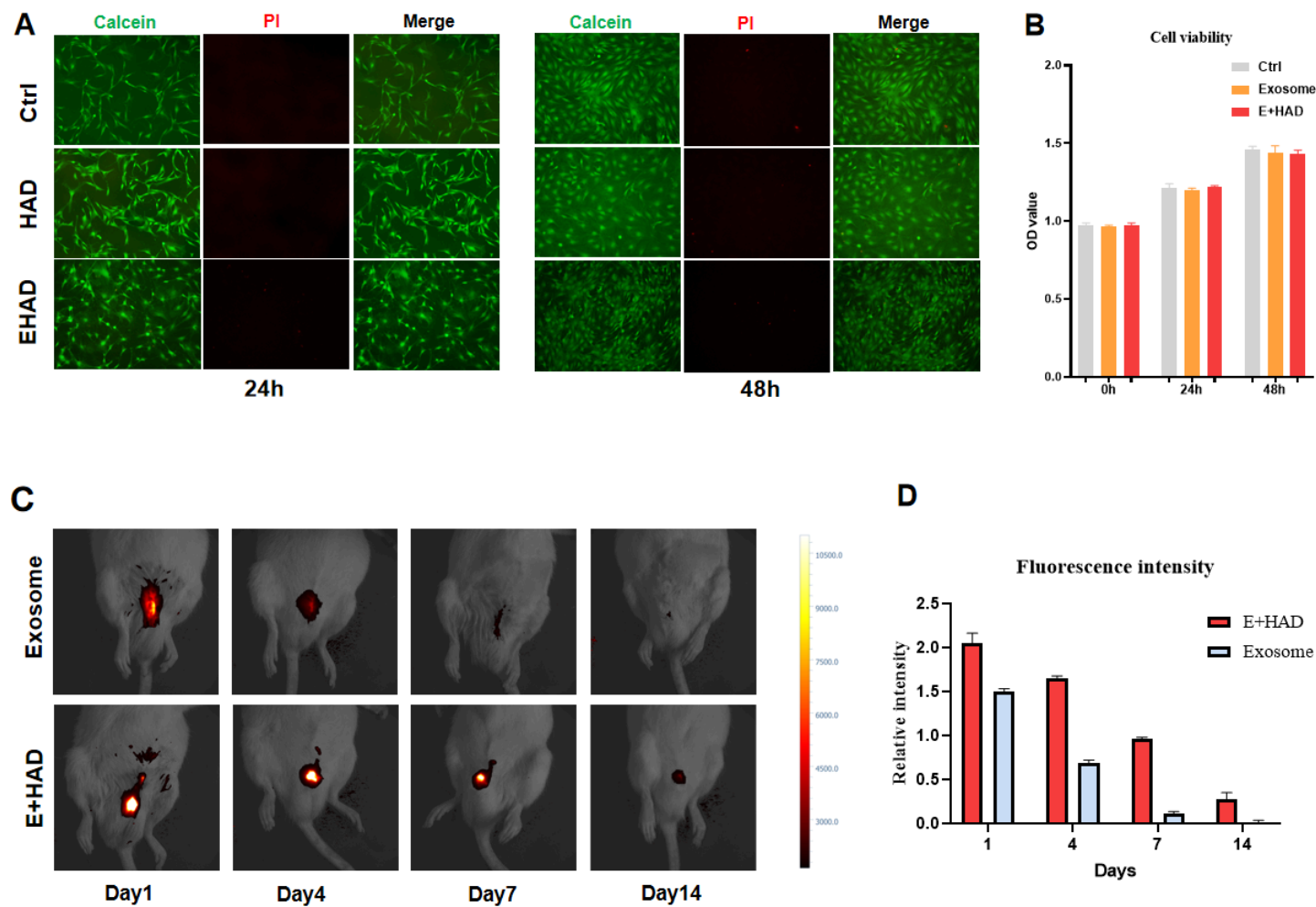


Figure 2

Biocompatibility of HAD hydrogel and in vivo tracing image of labelled exosomes

A) Live-dead cell staining of CCSMC cultured with normal or leachate of HAD hydrogel DMED medium. B) CCSMC cell viability measured by CCK8. C and D) Near-infrared fluorescence in vivo imaging of labelled exosomes1, 4, 7 and 14 days after cavernous injection

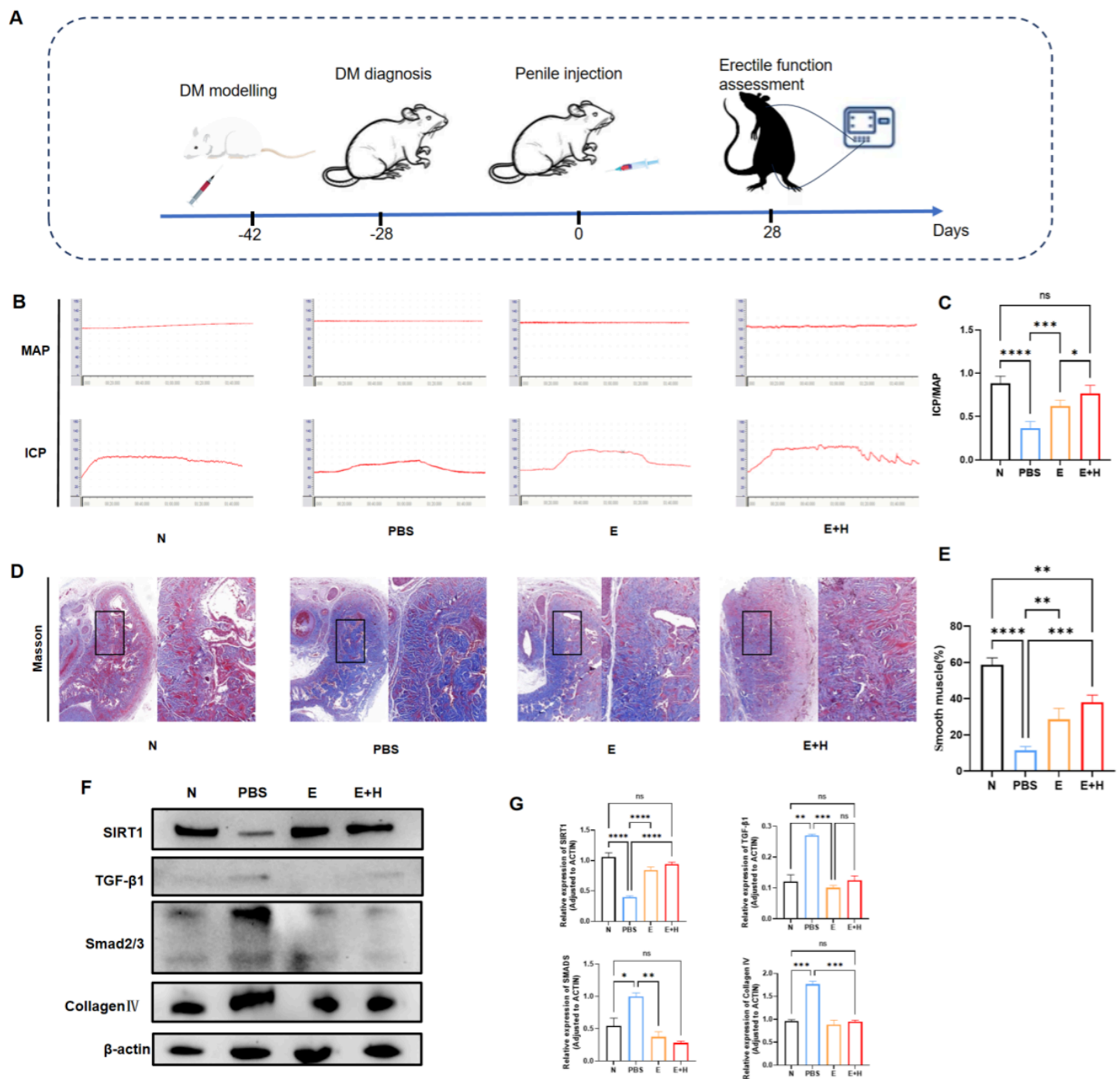


Figure 3

Result of animal experiment

A) Timeline of animal modeling, treatment, and erectile function assessment. B and C) Assessment of erectile function by measuring ICP and MAP, the ratio of ICP to MAP indicates the level of erectile function. D and E) Masson trichrome staining of corpus cavernosus for each group. F) Western blotting of SIRT1, TGF-β, Smad2/3 and Collagen IV of cavernous tissue in different groups of rats.

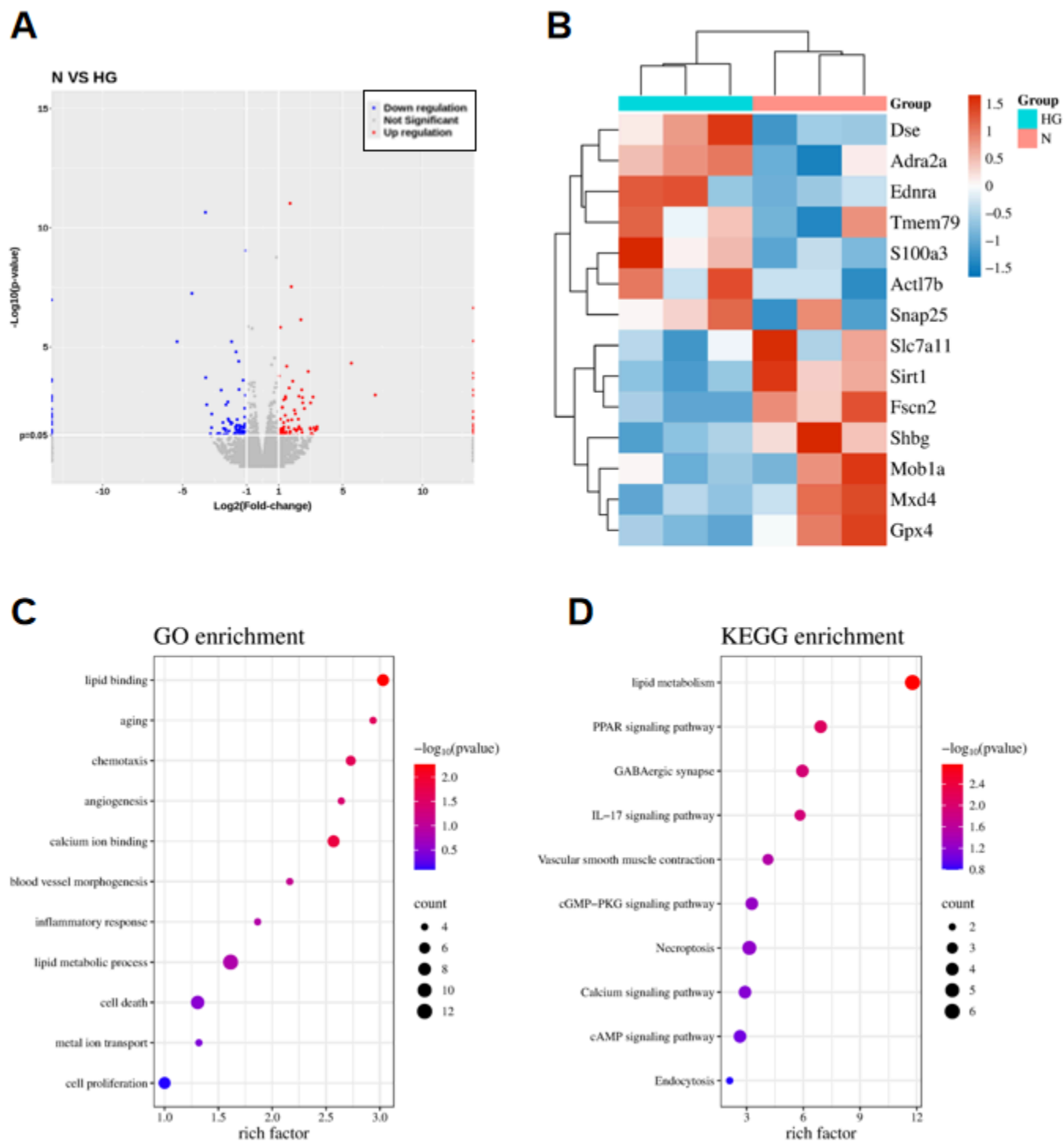


Figure 4

Differences in RNA expression between normal and hyperglycemia group

A) Volcano plots showing the differentially expressed genes. B) Heatmap showing the differential expression levels of genes between two groups. C-D) Significant enrichment of target genes in GO analysis (C) and KEGG signaling pathways(D).

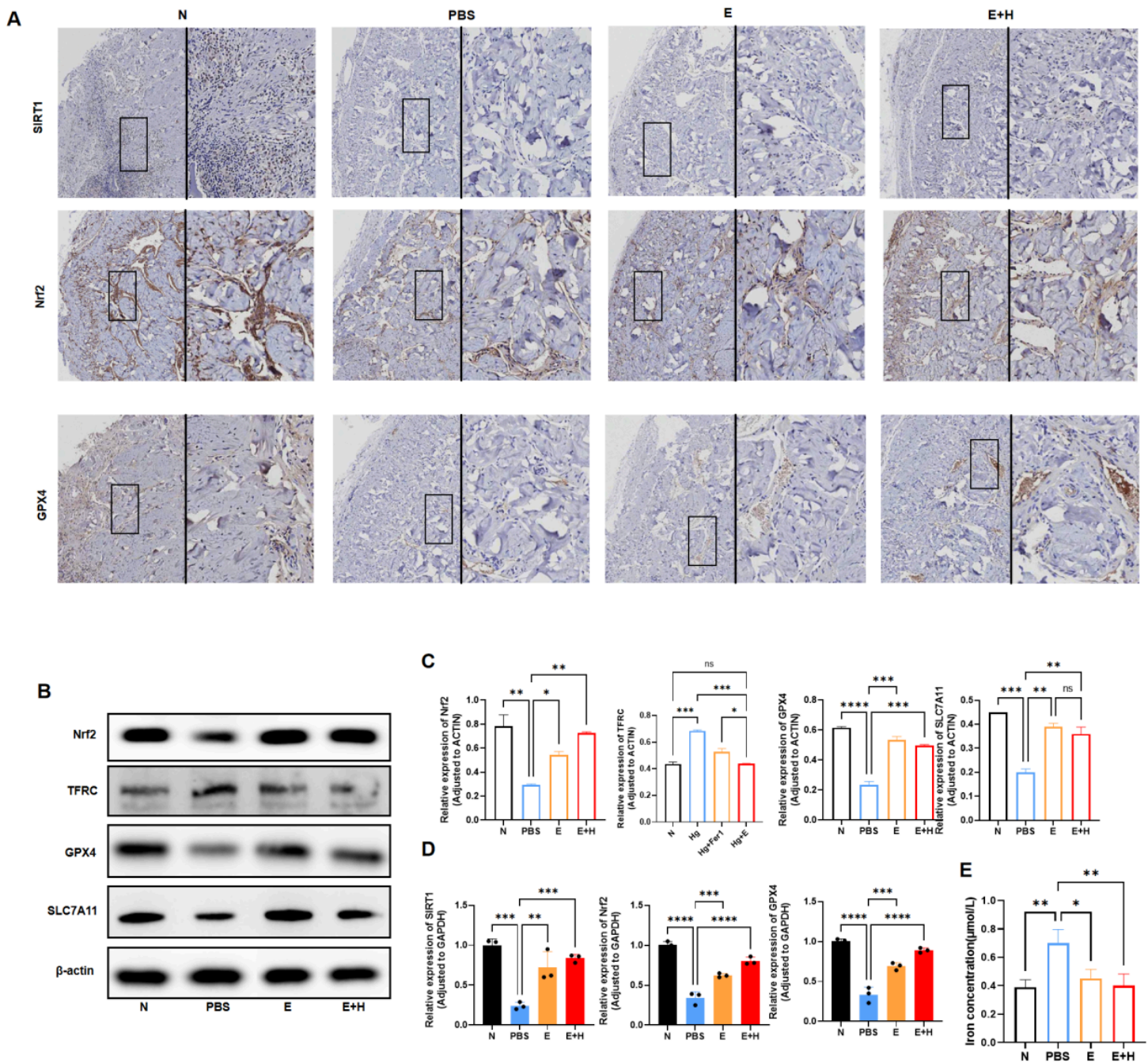


Figure 5

Exosome-encapsulated HAD hydrogel protect penile tissue from ferroptosis

A) Immunohistochemical staining of SIRT1, Nrf2, and GPX4 in corpus cavernosum. B and C) Western blotting of Nrf2, TFRC, GPX4, and SLC7A11 of cavernous tissue, which indicates the ferroptosis level in rat penis. D) The expression of SIRT1, Nrf2, and GPX4 was detected by qPCR. E) Iron concentration in rat penile tissue.

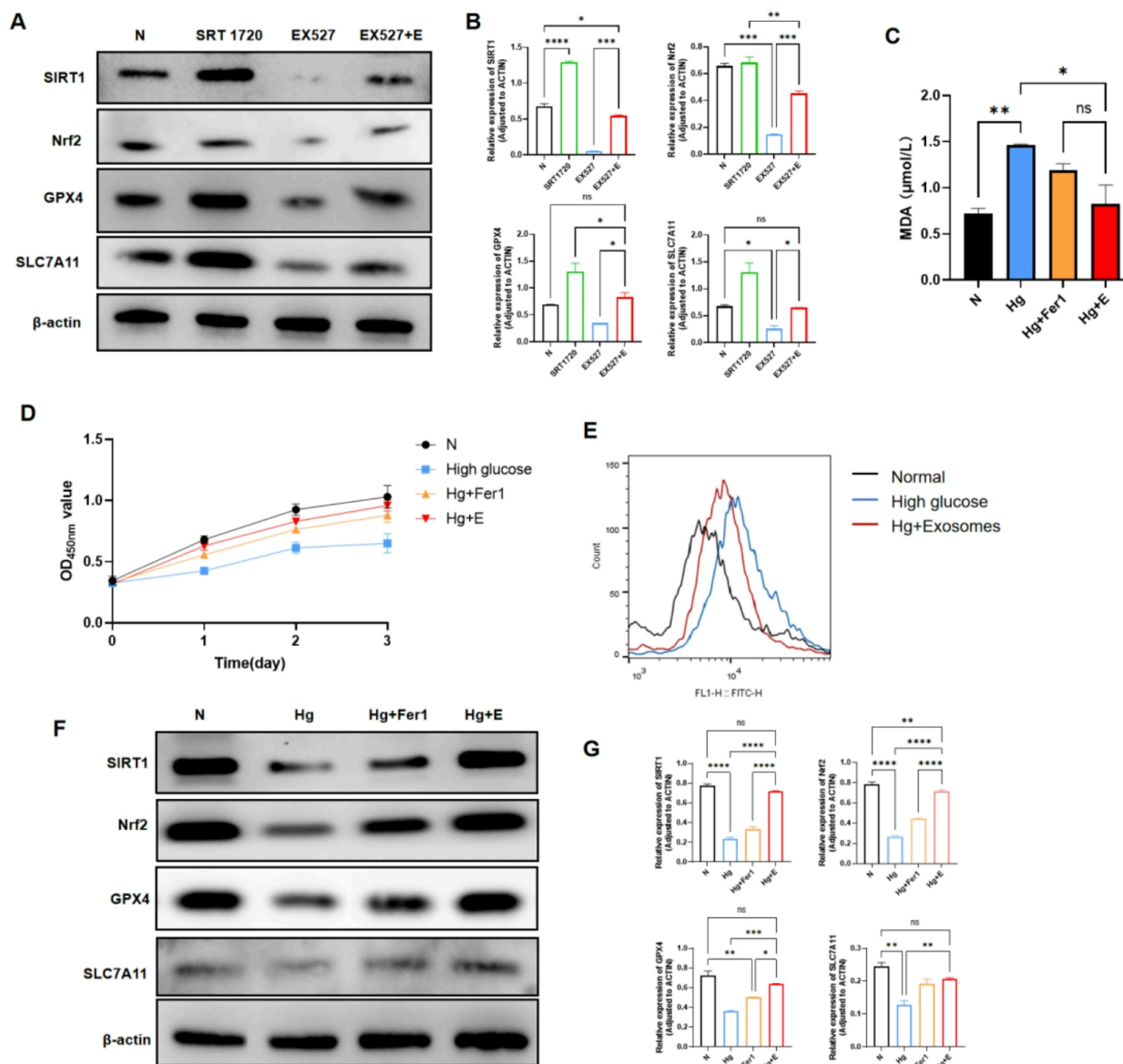


Figure 6

Exosomes inhibit ferroptosis by targeting SIRT1 to restore CCSMCs function

A and B) Western blot analysis of SIRT1, Nrf2, GPX4, and SLC7A11 protein levels in CCSMCs following treatment with SRT1720, EX527, or exosomes. C) MDA content in CCSMCs cultured in high-glucose DMEM supplemented with Fer-1 or exosomes. D) Viability of CCSMCs under the indicated high-glucose conditions measured by CCK-8 assay. E) Lipid ROS levels in CCSMCs cultured in high-glucose DMEM in the presence or absence of exosomes, assessed using the C11-BODIPY 581/591 probe. F and G) The expression of SIRT1, Nrf2, GPX4 and SLC7A11, TFRC was detected by western blotting.

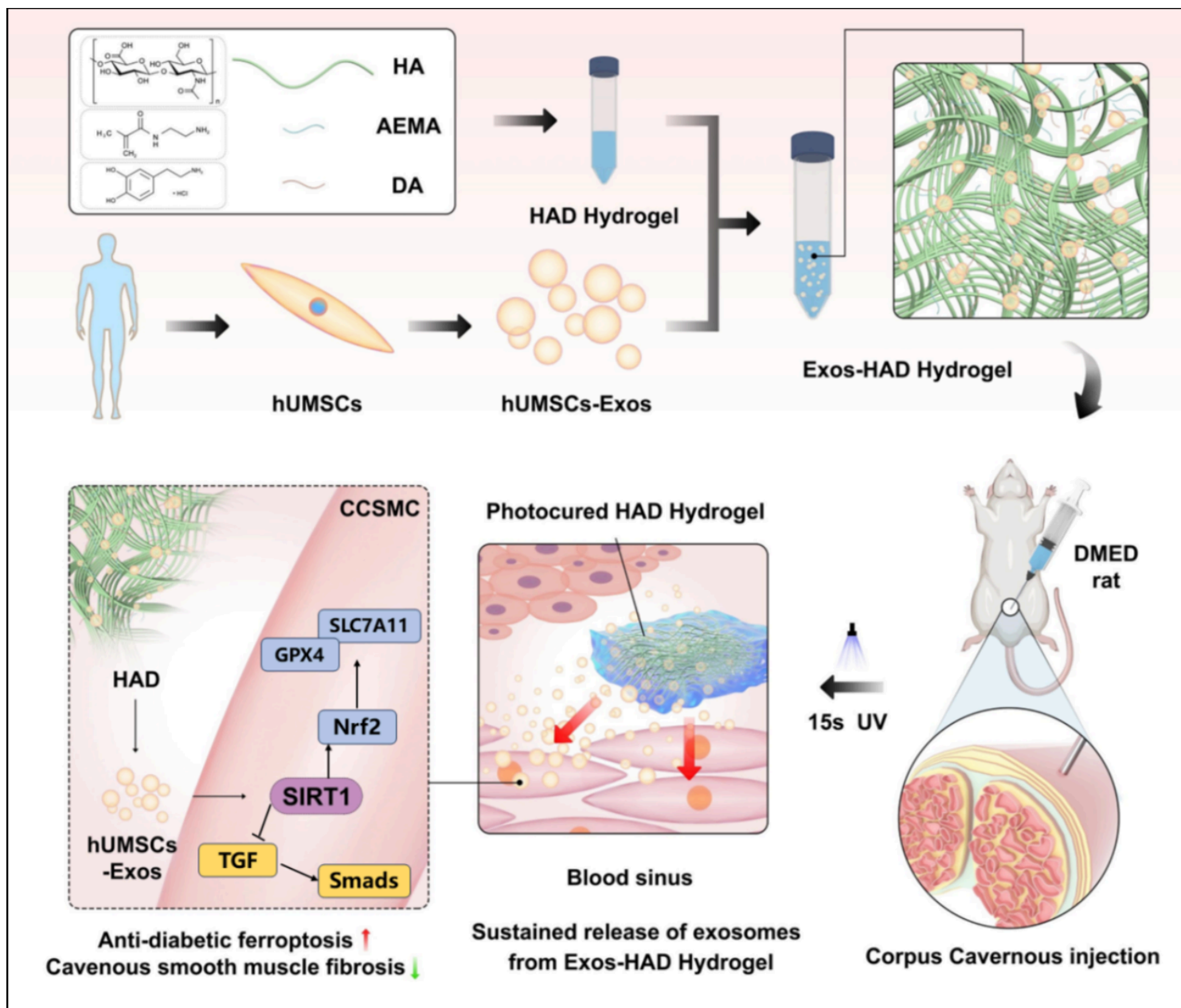


Figure 7

Exosomes modulate SIRT1 to suppress ferroptosis and fibrosis in CCSMCs, thereby restoring erectile function in diabetic rat.

Supplementary Files

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