

A pH-Responsive Nanocarrier Improves Astragaloside IV Bioavailability and Reverses Cardiac Dysfunction in Heart Failure

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Abstract

Background: Heart failure (HF) is a leading cause of morbidity and mortality worldwide, characterized by progressive myocardial remodeling, cardiomyocyte loss, and impaired cardiac function. Astragaloside IV (AS), a bioactive compound derived from *Astragalus membranaceus*, shows cardioprotective effects but is limited in clinical application due to poor solubility and low bioavailability. **Methods:** An acid-responsive metal-organic framework (MOF)-based nanoplatform was developed for targeted delivery of AS. The system was characterized using transmission electron microscopy, dynamic light scattering, drug loading analysis, and *in vitro* release profiling. *In vitro* efficacy was evaluated in Angiotensin II-induced H9c2 cardiomyocytes by assessing cell viability, hypertrophic markers (brain natriuretic peptide, atrial natriuretic peptide, β -myosin heavy chain), and cellular morphology. *In vivo* therapeutic effects were assessed in a transverse aortic constriction (TAC)-induced HF mouse model using echocardiography, serum biomarkers, and histological analysis. The involvement of apoptosis, angiogenesis, and PI3K/Akt signaling was evaluated by Western blotting. **Results:** The nanoplatform exhibited uniform nanoscale size, high drug loading, excellent colloidal stability, and pH-responsive release with enhanced drug liberation under acidic conditions. It significantly improved cardiomyocyte viability, suppressed hypertrophic gene expression, reduced myocardial injury and fibrosis, and improved cardiac function, including increased ejection fraction and fractional shortening. Mechanistically, it inhibited cardiomyocyte apoptosis, promoted angiogenesis, and activated the PI3K/Akt signaling pathway. Furthermore, the system also showed excellent hemocompatibility and negligible systemic toxicity. **Conclusions:** This acid-responsive nanoplatform significantly enhances the therapeutic performance of AS and provides a promising strategy for precision nanomedicine-based intervention in HF.

Keywords: ZIF-8, astragaloside IV, heart failure, pH-responsive, delivery system.

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Introduction

Heart failure (HF) is an increasingly grave global health concern, particularly among the elderly. With advancing age, the incidence of HF rises sharply [1]. It is estimated that more than 64 million people worldwide are living with HF, making it a major cause of hospitalization and mortality [2]. Due to its adverse prognosis, HF carries an overall mortality rate reaching 16.5% [3]. Although there have been some improvements in HF prognosis over the past few decades, approximately 20–30% of patients die within one year after hospitalization, and long-term mortality remains substantial [4]. Despite substantial progress in clinical and basic research on HF, there is still a shortage of ideal new drugs and therapeutic modalities.

For thousands of years, Traditional Chinese Medicine has been employed for the treatment of HF, displaying significant efficacy with advantages such as multi-target

actions and low cost. Among its constituents, Astragaloside IV (AS), the primary component of *Astragalus membranaceus*, has been shown to exert beneficial effects in both *in vivo* and *in vitro* HF models. These effects encompass myocardial ischemia protection, modulation of sarcoplasmic reticulum calcium ion pumps, promotion of angiogenesis, enhancement of myocardial energy metabolism, and inhibition of myocardial hypertrophy – collectively, these represent a plethora of “multi-target” actions [5,6]. These findings suggest that AS holds promise as a potential therapeutic agent for alleviating or treating HF. However, due to its chemical structure (**Supplementary Fig. 1A**), AS exhibits low solubility in biological fluids and poor bioavailability, which hinders its clinical application [7]. Thus, the construction of a novel drug delivery system capable of augmenting AS uptake by myocardial cells holds significant clinical relevance [8].

With the development of nanotechnology, nanomaterials have attracted considerable attention in the field of disease treatment [9,10]. Zeolitic imidazolate framework-8 (ZIF-8), a class of nanomaterials, have gained popularity in recent years, boasting excellent properties such as tunable pore size, structure, composition, size, versatile functionality, high drug-loading capacity, and good biocompatibility. ZIF-8 nanoparticles (ZIF-8 NPs) have demonstrated unique biological functions concerning biocompatibility and anti-tumor therapy. Moreover, *in vivo* studies have revealed unexpectedly high concentrations of ZIF-NPs in the lungs, possibly attributed to nanoparticle size and distribution [11,12]. Oxygenated blood from the lungs directly flows into the heart, facilitating the biological function of drugs once they enter the heart. This endows ZIF-NPs with potential applications in HF treatment [13]. However, the safety of ZIF-8 warrants attention. Intravenous administration of ZIF-8 at high concentrations (45 mg/kg) significantly disrupts red blood cell and platelet-related blood routine parameters and coagulation function indicators, likely due to its strong adsorption to cell membranes and plasma proteins [14]. Therefore, modifying and engineering ZIF-8 can mitigate the threats posed to the organism.

Polyethylene glycol (PEG) is a water-soluble polyether with excellent biocompatibility and immunogenicity. In recent years, PEG has been introduced as a hydrophilic component in polymer carriers, effectively enhancing drug solubility, increasing drug stability, and allowing nanoparticles to circulate stably in the bloodstream [15]. Surface modification of ZIF-8 with PEG, known as PEGylation, enhances the colloidal stability of ZIF-8 NPs and reduces their interaction with various components in the biological environment, such as proteins and immune cells [16]. Peng *et al.* [17] encapsulated baicalein in ZIF-8 and grafted it with PEG, achieving selective drug release in acidic microenvironments while maintaining the drug's anti-cancer activity.

In our research, we encapsulated AS in ZIF-8 and grafted it with PEG for application in myocardial cells. This combination not only aggregates NPs loaded with AS (PEG/ZIF-8@AS NPs) around myocardial cells but also facilitates AS penetration. The preparation of PEG/ZIF-8@AS NPs involves the encapsulation of AS within ZIF-8, followed by PEGylation of ZIF-8. We meticulously examined the sustained release, stability, and impact on HF cell models of PEG/ZIF-8@AS NPs *in vitro*. Furthermore, we administered PEG/ZIF-8@AS NPs via intravenous injection to mice afflicted with HF, further investigating the *in vivo* therapeutic efficacy of PEG/ZIF-8@AS NPs in a mouse HF model. Lastly, we explored the biological safety of PEG/ZIF-8@AS NPs therapy.

Materials and Methods

Synthesis of PEG/ZIF-8@AS NPs

A solution was prepared by dissolving 10 mg of AS (dissolved in 100 μ L dimethyl sulfoxide as a co-solvent), 330 mg of 2-methylimidazole, and 480 mg of PEG-2000 in 9.9 mL of methanol. Simultaneously, 150 mg of zinc nitrate hexahydrate was dissolved in 5 mL of ultrapure water. At 20 °C, the 5 mL zinc-containing ultrapure water solution was slowly added to the 10 mL methanol solution while stirring, and the mixture was stirred for 20 minutes. The unreacted reagents were removed by centrifugation (12000 rpm, 15 min). The resulting product was then washed with methanol by repeating the centrifugation process three times, resulting in the formation of PEG/ZIF-8@AS NPs. ZIF-8 was prepared using the same method for comparison.

Characterization of PEG/ZIF-8@AS NPs

The morphology, microstructure, and composition of PEG/ZIF-8@AS NPs were characterized using transmission electron microscopy (TEM, FEI Talos F200X, USA). The crystalline structure of ZIF-8 and PEG/ZIF-8@AS NPs was examined using X-ray diffraction (XRD, Smart Lab, Japan), and their structural composition was analyzed via Fourier-transform infrared spectroscopy (FTIR, Tensor II, Germany). The hydrated particle size and ζ potential of ZIF-8 and PEG/ZIF-8@AS NPs were determined using a Zetasizer Nano. PEG/ZIF-8@AS NPs were dispersed in water, phosphate-buffered saline (PBS), and high-glucose Dulbecco's Modified Eagle's Medium (DMEM; containing 10% fetal bovine serum (FBS), Gibco) separately and incubated at 37 °C. The solutions were observed and photographed at 1, 3, and 7 days to record their appearance, and the particle size was measured.

Drug Loading Calculation

The drug loading content (DLC) of AS in PEG/ZIF-8@AS NPs was calculated using high-performance liquid chromatography (HPLC, Agilent 1200) with the following formula:

$$DLC = (AS \text{ content encapsulated in PEG/ZIF-8} / PEG/ZIF-8@AS \text{ content}) \times 100\% \quad (1)$$

The experimental conditions for HPLC detection of AS content were as follows: the HPLC system employed was Agilent 1260, using an Eclipse XDB-C18 column (5 μ m, 4.6 mm $\times$ 150 mm), both from Agilent Technologies (USA). The mobile phase consisted of acetonitrile and water in a 32:68 ratio, with a flow rate of 1.0 mL/min. UV detection was carried out at a wavelength of 201 nm, with an injection volume of 10 μ L.

Drug Release

The *in vitro* release of AS from PEG/ZIF-8@AS NPs was determined using an improved dynamic dialysis method based on published literature [18]. In brief, PEG/ZIF-8@AS NPs were adjusted to a concentration of 1 mg/mL in PBS. A dialysis bag containing 1 mg/mL PEG/ZIF-8@AS NPs was immersed in 10 mL of 10% FBS PBS with pH values of 5.5, 6.5, and 7.4, and stirred at 37 °C. At different time points (0, 1, 2, 4, 6, 8, 12, 18, 24, 36, 48 hours), aliquots were taken, and AS concentration in the release medium was detected by HPLC (Agilent 1260). After each interval of collecting 0.5 mL samples, an equal volume of release medium was immediately added, and the release curve was subsequently plotted.

Construction of an *In Vitro* HF Cell Model

H9c2 cells (iCell Biotechnology Co. Ltd., Shanghai, China) were cultured in DMEM containing 10% FBS and 100 mg/mL penicillin-streptomycin at 37 °C with a CO₂ concentration of 5%. Cells for RNA and total protein analysis were seeded at a density of 5×10^5 cells/3.5 cm culture dishes and treated with Angiotensin II (Ang-II) for 48 hours, binding to Ang-II receptors to simulate the myocardial cell environment of HF in mice.

Determination of Material Concentration Using Cell Counting Kit-8 (CCK-8)

Ang-II-treated H9c2 cardiomyocytes were exposed to PEG/ZIF-8@AS NPs (0, 0.1, 1, 5, 10, 20, 50, 80, 100 µg/mL) for 4 hours and cultured in DMEM supplemented with 10% FBS. Cell viability was assessed using the CCK-8 assay kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions to select an appropriate concentration for modeling.

Analysis of Cardiac Hypertrophy, Apoptosis, Angiogenesis, and Akt Signaling Markers

Western blotting (WB) was employed to measure the expression of cardiac hypertrophy-, apoptosis-, angiogenesis-, and Akt signaling-related markers. Briefly, treated H9c2 cells were lysed with cell lysis buffer (Beyotime, Shanghai, China). The cell lysates were incubated on ice for 15 minutes and then centrifuged at 14000 g for 20 minutes at 4 °C. The protein concentration in the supernatant was determined using the BCA protein assay kit (Beyotime, Shanghai, China). Denatured proteins were separated by 10% SDS-PAGE under reducing conditions and transferred onto PVDF membranes (Merck-Millipore). After overnight incubation with primary antibodies at 4 °C, membranes were washed with TBST and subsequently incubated with secondary antibodies conjugated with horseradish peroxidase, followed by signal detection using an ECL assay kit. Primary antibodies used were as follows: rabbit anti-BNP (1:1000, ab19645, Abcam), rabbit anti-β-MHC (1:2000, ab37484,

Abcam), rabbit anti-ANP (1:10000, ab181242, Abcam), rabbit anti-Bax (1:2000, 50599-2-Ig, Proteintech), rabbit anti-Bcl-2 (1:2000, 26593-1-AP, Proteintech), rabbit anti-Cleaved Caspase-3 (1:5000, RMX00003, Proteintech), rabbit anti-VEGF (1:5000, 19003-1-AP, Proteintech), rabbit anti-VEGFR2 (1:5000, 26415-1-AP, Proteintech), rabbit anti-HIF-1α (1:5000, 20960-1-AP, Proteintech), rabbit anti-p-Akt (1:5000, 28731-1-AP, Proteintech), rabbit anti-Akt (1:5000, 10176-2-AP, Proteintech), mouse anti-β-actin monoclonal antibody (1:1000, ab8227, Abcam), and mouse anti-GAPDH monoclonal antibody (1:5000, ab8245, Abcam).

Real-time quantitative polymerase chain reaction (RT-qPCR) was also employed to measure the cardiac markers BNP, β-MHC, and ANP. The primer sequences are provided in **Supplementary Table 1**.

Immunofluorescence Staining

After treatment with the respective drugs for 4 hours, cells were fixed in 4% paraformaldehyde for 30 minutes. Phalloidin-TRITC (R415, Invitrogen) was used for 1-hour staining to observe the cell cytoskeleton composed of filamentous F-actin. Cell nuclei were incubated with 4',6-diamidino-2-phenylindole (DAPI) for 5 minutes in the dark. Images were captured using a microscope, and cell areas were quantified.

Construction of the Mouse HF Model

Twenty-five male C57BL/6 mice (6–8 weeks, 20–23 g) were purchased from Sipeifu Biotechnology Co., Ltd. All animals were housed under specific pathogen-free conditions with controlled temperature (22 ± 2 °C), relative humidity (50–60%), and a 12 h light/dark cycle. Among the mice, 20 underwent chronic HF model construction via transverse aortic constriction (TAC), while the remaining 5 were used as the sham surgery group (abdominal aorta isolation without ligation). All surgical procedures were performed under 2% isoflurane anesthesia to minimize pain and distress. After the successful establishment of the model, the mice were randomly divided into five groups using a computer-generated random number sequence: sham surgery group (control), model group (TAC), AS group, ZIF-8 group, and PEG/ZIF-8@AS group, with five mice in each group. The experimental unit was a single mouse. Administration method: model group (iv 0.9% saline 2 mL/d), sham surgery group (iv 0.9% saline 2 mL/d), and treatment groups (AS, ZIF-8, and PEG/ZIF-8@AS). After modeling, penicillin was injected for three consecutive days to prevent infection. Mice were fed a normal diet and treated with AS and PEG/ZIF-8@AS NPs via tail vein injection on days 7, 14, and 21. All drug-containing formulations were administered at an AS-equivalent dose of 2 mg/kg. The ZIF-8 group received the corresponding amount of blank NPs equivalent to that present in the PEG/ZIF-8@AS NPs. To avoid the accelerated blood clearance phenomenon in-

duced by PEGylated NPs, the dosing interval was set at 7 days [19]. Treatment was administered for three consecutive weeks. At the end of the experiment, mice were euthanized by overdose of isoflurane anesthesia. Predefined humane exclusion criteria were established a priori. All animals met the inclusion criteria, and no animals or data points were excluded from the final analysis. Then, the echocardiography was performed to assess cardiac structure and function, and hematoxylin and eosin (H&E) and Masson's staining were used to observe myocardial tissue and structural pathological changes. Outcome assessment and data analysis were performed in a blinded manner using coded samples. The animal experiments were approved by the Experimental Animal Ethics Committee of Wenzhou Medical University (wydw2025-0262).

Cardiac M-Mode Echocardiography

Following the completion of HF modeling, mice were subjected to transthoracic echocardiography using a small animal ultrasound imaging system (VINNO6) to assess cardiac function. Mice were anesthetized with a 3% isoflurane/oxygen mixture and placed in a supine position on a 37 °C temperature-controlled heating platform with their chest fur removed. M-mode echocardiography was recorded for each group of mice using the imaging platform. Parameters such as left ventricular internal dimension in diastole (LVIDd), left ventricular internal dimension in systole (LVIDs), ejection fraction (EF), and fractional shortening (FS) were recorded based on the system calculations.

Serum Cardiac Injury Marker Levels

After the experiment, mice were euthanized, and serum was collected and left to stand at room temperature for approximately 2 hours. After layering, the serum was centrifuged at room temperature at $2000 \times g$ for 20 minutes. The supernatant was then transferred to 1.5 mL centrifuge tubes. Serum was mixed with sterile $1 \times$ PBS solution at a 1:3 ratio, and levels of cardiac injury-related biomarkers, including creatine kinase (CK), hydroxybutyrate dehydrogenase (HBDH), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST), were measured using an automated biochemical analyzer (Model 3100, Hitachi, Tokyo, Japan).

Heart-to-Body Weight Ratio

After the experiment, mice were weighed, euthanized, and their hearts were photographed and weighed. The heart-to-body weight ratio was calculated as the ratio of heart weight to body weight.

H&E Staining and Masson Staining

After euthanasia, the hearts of the mice were removed, photographed, and weighed. The hearts were cut horizontally, and the middle portion was placed in 4% paraformaldehyde solution and immersed for at least 24

hours at 4 °C. The remaining part was stored at –80 °C for further use. Fixed heart tissues were removed, placed in a tissue embedding box, and dehydrated in 75% ethanol. H&E staining and Masson staining were performed for observation, followed by photography.

In Vivo Imaging and Tissue Distribution

To investigate the *in vivo* biodistribution of AS, a small animal live imaging study was performed using a fluorescently labeled probe (Cy5-AS). The labeling was achieved by covalently conjugating Cy5-NHS ester to AS via its hydroxyl groups. HF model mice were randomly assigned to two groups ($n = 3$): the free drug group (Free AS^{Cy5}) and the nanoparticle formulation group (PEG/ZIF-8@AS^{Cy5}). Both groups received intravenous administration via the tail vein. The administered dose was calculated based on the drug loading of Cy5-AS (5 mg/kg). At 3, 6, and 24 hours post-injection, mice were anesthetized with isoflurane, and *in vivo* fluorescence images were acquired using an imaging system. After the final imaging time point, mice were euthanized, and major organs (heart, liver, spleen, lungs, and kidneys) were harvested. Tissues were rinsed with pre-cooled saline, blotted dry, and weighed. Subsequently, tissues were minced and homogenized in saline using a tissue homogenizer. Finally, the concentration of AS in tissue homogenates was quantified using High-Performance Liquid Chromatography–Mass Spectrometry (HPLC–MS) analysis.

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) and Immunohistochemistry (IHC) Staining

The fixed heart tissue was deparaffinized, and 20 µg/mL of proteinase K free from DNase was added, followed by incubation at 37 °C for 30 minutes. After washing with PBS, 50 µL of TUNEL detection solution (C1086, Beyotime, China) was applied, and the tissue was incubated at 37 °C in the dark for 60 minutes. Finally, the slides were mounted with anti-fade mounting medium and observed under a fluorescence microscope. To assess angiogenesis, CD31 IHC staining was performed. The tissue sections were incubated with anti-CD31 antibody (11265-1-AP, Proteintech, USA), followed by incubation with SignalStain®Boost IHC Detection Reagent (#8114, Cell Signaling Technology, USA). Color was developed using the DAB peroxidase substrate kit (#8059, Cell Signaling Technology, USA).

Mouse Erythrocyte Hemolysis

The mouse orbital blood (1 mL) was added to 10 mL of PBS, and centrifuged at $10,000 \times g$ for 5 minutes to remove the supernatant. This process was repeated three times until the supernatant became clear, and the blood cells were collected. The blood cells were resuspended in 10 mL of PBS, and 200 µL of blood cells were added to 800 µL of

different concentrations of PEG/ZIF-8@AS NPs (in PBS) and incubated at 37 °C for 1 hour. Simultaneously, 800 μ L of PBS was used as a negative control, and 800 μ L of H₂O was used as a positive control. After centrifugation at $10,000 \times g$ for 5 minutes, photographs were taken to record any hemolysis, and the absorbance at 570 nm (OD570) was measured using an enzyme reader. The hemolysis rate was calculated as follows:

$$\text{Hemolysis rate(\%)} = (\text{Sample absorbance} - \text{Negative control absorbance}) / (\text{Positive control absorbance} - \text{Negative control absorbance}) \times 100\% \quad (2)$$

Tissue Staining

After the treatment of HF in mice, the mice were euthanized, and heart, liver, spleen, lung, and kidney tissues were collected. After processing the samples using the same method as described for heart tissue in the aforementioned H&E procedure, both H&E staining and Masson staining were performed on these tissues.

Statistical Analysis

All statistical analyses were conducted using commercially available software (PRISM version 5.01, GraphPad Inc., San Diego, CA, USA). Data are presented as mean $\pm$ standard deviation. Significant differences between the two groups were determined by Student's t-test, while one-way analysis of variance (ANOVA) was used to determine significant differences among multiple groups. A p -value < 0.05 was considered statistically significant.

Results

Characterization of PEG/ZIF-8@AS NPs

PEG/ZIF-8@AS NPs were prepared using a one-step synthesis method. The DLC of AS in PEG/ZIF-8@AS NPs was determined to be 24.6%. The PDI of PEG/ZIF-8@AS NPs was less than 0.2, indicating a narrow size distribution. (Table 1). TEM images demonstrated that ZIF-8 NPs exhibited a uniform polyhedral morphology, whereas PEG/ZIF-8@AS nanoparticles showed a relatively rounded appearance (Fig. 1A). Dynamic light scattering measurements revealed the average particle sizes of ZIF-8 and PEG/ZIF-8@AS NPs to be 64.58 ± 0.54 nm and 79.8 ± 0.64 nm, respectively (Fig. 1B). The stability of PEG/ZIF-8@AS NPs was evaluated in water, PBS, and cell culture medium for seven days. No visible aggregation or sedimentation was observed, and particle size remained stable throughout the observation period (Fig. 1C and **Supplementary Fig. 1B,C**). Further characterization of the crystalline structures of ZIF-8 and PEG/ZIF-8@AS NPs was conducted using XRD. ZIF-8 displayed characteristic peaks, confirming the successful synthesis of ZIF-8. Importantly, the XRD peaks

of PEG/ZIF-8@AS NPs were nearly identical to those of ZIF-8, suggesting that the loading of PEG and AS did not alter the structure of ZIF-8 (Fig. 1D). The infrared analysis results are shown in Fig. 1E. The absorption peak at 421 cm^{-1} corresponds to the stretching vibration of Zn-N bonds in ZIF-8. Additionally, peaks at 2875 cm^{-1} , 1645 cm^{-1} , and 3400 cm^{-1} are attributed to the stretching vibrations of C-H, bending vibrations of C-O-H, and stretching vibrations of O-H in PEG, respectively. The peak at 1735 cm^{-1} is attributed to the carbonyl stretching vibration of AS. These results indicate the successful synthesis of relatively uniform and stable ZIF-8 and PEG/ZIF-8@AS NPs. What's more, the ζ potentials of the synthesized nanoparticles were characterized (Fig. 1F), with ZIF-8 and PEG/ZIF-8@AS NPs having average potentials of -23.92 ± 0.58 mV and -26.10 ± 0.12 mV, respectively. In comparison to ZIF-8, PEG/ZIF-8@AS NPs exhibit a reduced ζ potential, likely due to the loading of AS, which carries a negative charge and thus contributes to the decrease in potential.

Studies have shown that in ZIF-8 structures, the coordination bonds between 2-methylimidazole and Zn^{2+} can degrade in an acidic environment, leading to the release of the encapsulated drug, achieving a responsive drug release effect [20]. AS release from PEG/ZIF-8@AS NPs was tested at pH 5.5, 6.5, and 7.4. The release of AS was significantly higher under acidic conditions compared to neutral conditions. At pH 5.5, 76.8% of AS was released within 8 hours, while 65.1% was released at pH 6.5, and only 36.3% was released at pH 7.4. Moreover, AS release under acidic conditions continued for more than 12 hours, while it significantly slowed down after 1 hour under neutral conditions (Fig. 1G).

PEG/ZIF-8@AS NPs Ameliorate Myocardial Cell Damage

After successfully obtaining pH-responsive PEG/ZIF-8@AS NPs, we proceeded to evaluate their impact on HF cells *in vitro*. The results from the CCK8 assay showed that 50 $\mu\text{g/mL}$ of PEG/ZIF-8@AS NPs increased the cell viability of Ang-II-activated H9c2 cells by 33.6% (Fig. 2A). The lack of further increase in cell viability at PEG/ZIF-8@AS NPs concentrations above 50 $\mu\text{g/mL}$ may be attributed to either the excessive release of Zn^{2+} , inducing cytotoxicity, or the aggregation of high concentration NPs, which reduces cellular uptake. Therefore, we used 50 $\mu\text{g/mL}$ as the applied concentration for subsequent *in vitro* experiments. Compared to the model group, treatment with PEG/ZIF-8@AS NPs significantly increased the cell viability of HF cells (Fig. 2B, $p < 0.001$). Furthermore, Western blot analysis of cardiac injury markers β -MHC, ANP, and BNP in H9c2 cells showed that treatment with PEG/ZIF-8@AS NPs significantly reduced the levels of these three cardiac markers compared to cells treated with Ang-II alone ($p = 0.0102$, $p < 0.001$, $p < 0.001$). Treatment with free AS or ZIF-8 alone did not achieve such a significant effect (Fig. 2C,D). Additionally, similar results were obtained through

Table 1. Characterization of ZIF-8 and PEG/ZIF-8@AS NPs (n = 3).

Characteristics	ZIF-8	PEG/ZIF-8@AS
Particles size (nm)	64.58 ± 0.54	79.8 ± 0.64
Zeta ζ potential (mV)	−23.92 ± 0.58	−26.10 ± 0.12
DLC (%)	/	24.60 ± 1.84
PDI	0.146 ± 0.01	0.131 ± 0.01

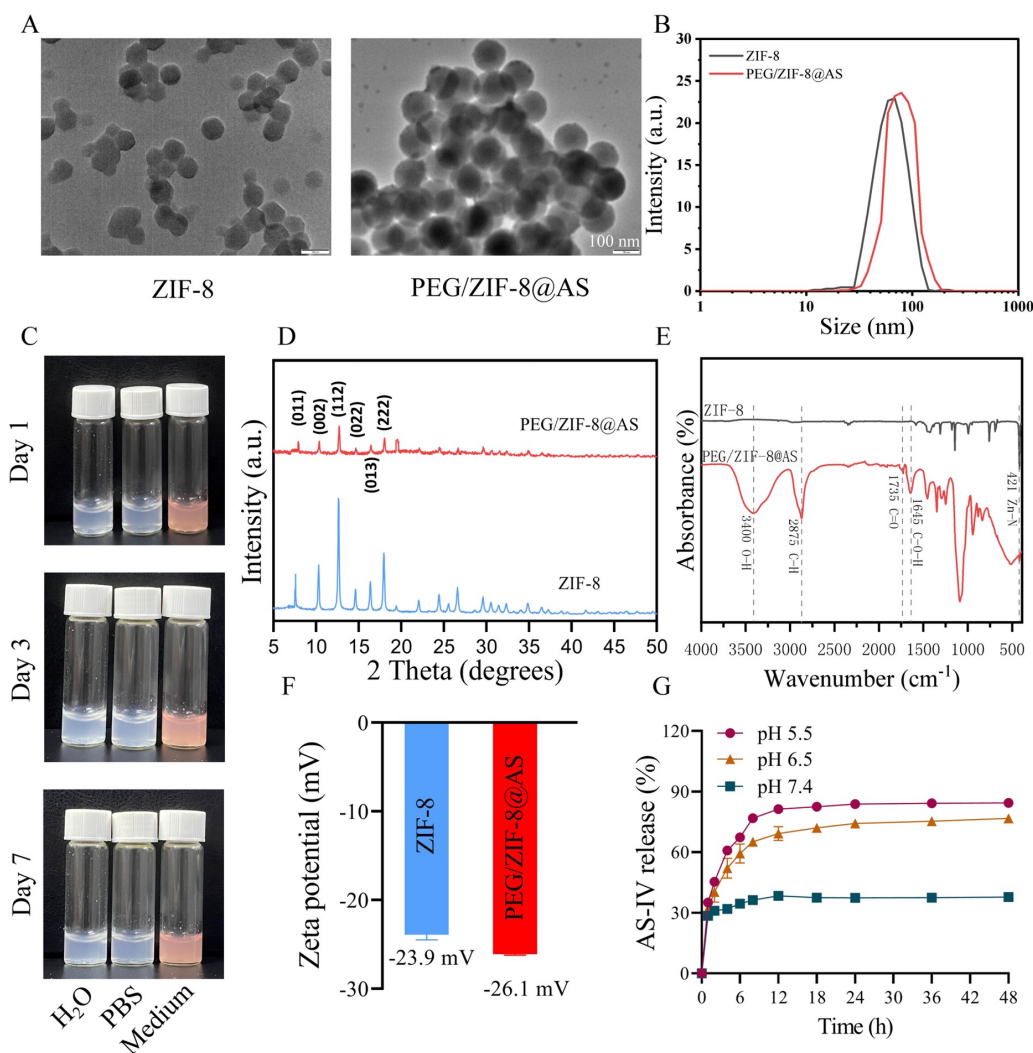


Fig. 1. Characterization of ZIF-8 and PEG/ZIF-8@AS NPs. (A) TEM images. Scale bar = 100 nm. (B) Size distributions. (C) The state of PEG/ZIF-8@AS NPs dispersed in water, PBS, and high-glucose DMEM medium for seven days. (D) Crystal structure of the ZIF-8 and PEG/ZIF-8@AS NPs. (E) Absorption peaks of ZIF-8 and PEG/ZIF-8@AS NPs using infrared analysis. (F) ζ potential of ZIF-8 and PEG/ZIF-8@AS NPs. (G) Evaluation of the release behavior of AS at pH 5.5, 6.5, and 7.4 using dynamic dialysis. Graphs were generated using GraphPad Prism 8.0, and figure assembly was performed using Microsoft PowerPoint.

qPCR analysis (Fig. 2E). Our study further confirms that PEG/ZIF-8@AS NPs effectively reduce the severity of HF induced by Ang-II. Moreover, we induced cell hypertrophy in H9c2 cells using Ang-II and confirmed the impact of PEG/ZIF-8@AS NPs on cell hypertrophy by examining changes in cell size. The results showed that Ang-II induced a significant increase in the surface area of H9c2 cells ($p < 0.001$), while PEG/ZIF-8@AS NPs effectively reduced

cell surface area ($p < 0.001$) and improved cell hypertrophy (Fig. 2F and **Supplementary Fig. 2**).

PEG/ZIF-8@AS NPs Improve Cardiac Function in HF Mice

The experiment design is illustrated in Fig. 3A, where C57BL/6 mice were subjected to TAC for modeling. They were provided with a normal diet and treated with 2 mg/kg

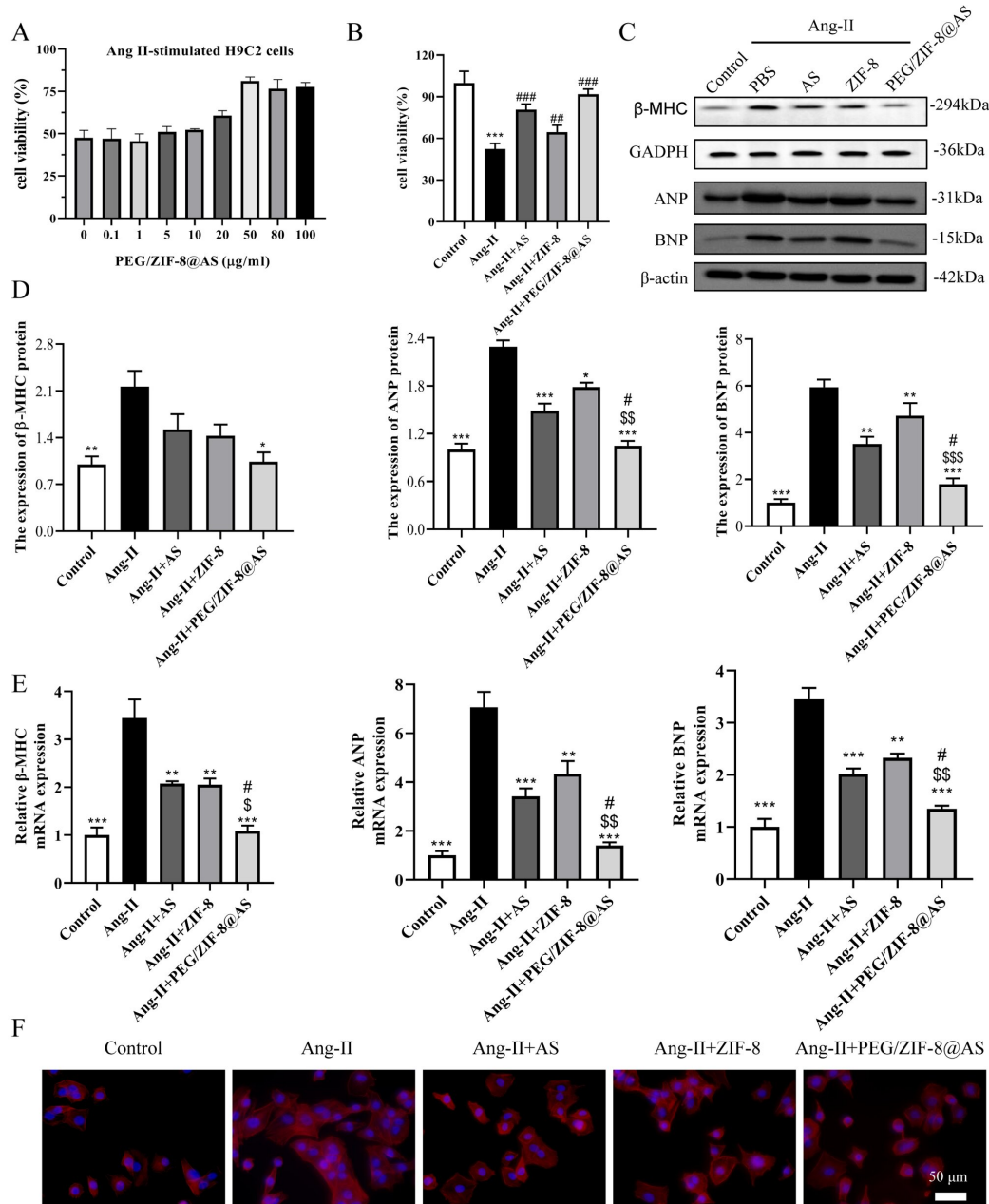


Fig. 2. PEG/ZIF-8@AS NPs' impact on Ang-II-induced HF cells *in vitro*. (A) Assessment of the appropriate concentration of PEG/ZIF-8@AS NPs for improving the viability of HF cell models using CCK-8. (B) Effects of PEG/ZIF-8@AS NPs (50 $\mu\text{g/mL}$) on the viability of HF cell models measured using CCK-8. (C, D, and E) Influence of PEG/ZIF-8@AS NPs on cardiac markers in HF cell models detected through WB and RT-qPCR. (F) Assessment of the effect of PEG/ZIF-8@AS NPs on cell area using phalloidin-TRITC staining (red represents the cell skeleton). Scale bar = 50 μm . # $p < 0.05$, vs. Ang-II + AS; \$ $p < 0.05$, \$\$ $p < 0.01$, vs. Ang-II + ZIF-8; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, vs. Ang-II. $n = 3$. Graphs were generated using GraphPad Prism 8.0, and figure assembly was performed using Microsoft PowerPoint.

of AS, ZIF-8, and PEG/ZIF-8@AS NPs by tail vein injection on days 7, 14, and 21 after modeling. On the 22nd day, the mice's cardiac function was assessed. Initially, we used a small animal ultrasound system to examine the mice's cardiac function. The echocardiography results showed that after TAC surgery, the cardiac function of the mice was significantly impaired, with a notable decrease in cardiac contractility and diastolic capacity. Treatment with PEG/ZIF-

8@AS NPs resulted in enhanced cardiac contractility and diastolic function, leading to a significant improvement in cardiac function (Fig. 3B). Furthermore, based on the echocardiographic images, we calculated LVIDd, LVIDs, EF, and FS values, and the statistical results showed that TAC significantly disrupted heart function, manifested as increased LVIDs ($p < 0.001$) and decreased EF ($p < 0.001$) and FS ($p < 0.001$) values. PEG/ZIF-8@AS treatment sig-

nificantly improved abnormalities in these parameters (Fig. 3C). At the end of the experiment, the values of heart mass to body mass ratio were measured to assess cardiac hypertrophy. From Fig. 3D,E, it can be observed that TAC led to a significant increase in heart mass to body mass ratio in the TAC group ($p < 0.001$). However, after treatment with PEG/ZIF-8@AS NPs, the heart mass to body mass ratio significantly decreased ($p < 0.001$), even returning to normal levels. These results collectively indicate that PEG/ZIF-8@AS NPs have the ability to improve cardiac function in HF mice.

Myocardial Injury and Fibrosis

In the assessment of cardiac function in mice, we observed a significant improvement in cardiac function in all groups after drug administration. CK, HBDH, LDH, AST, and other markers are classic biomarkers that reflect cardiac injury [21]. To more accurately measure the therapeutic effect of the drugs on cardiac injury in mice, we performed marker detection. The statistical results for each group are shown in Fig. 4A. Compared to the control group, the TAC group of mice had significantly increased levels of CK ($p < 0.001$), HBDH ($p < 0.001$), LDH ($p = 0.004$), and AST ($p < 0.001$) in the serum, indicating significant cardiac damage in the mice after surgery. After treatment with the drugs in various groups, the levels of these markers all showed significant decreases.

To further observe the protective effect of PEG/ZIF-8@AS NPs on the heart, we performed H&E staining on paraffin sections of heart tissue from each group of mice. The H&E staining results are shown in Fig. 4B. In the control group, myocardial cells and their nuclei exhibited normal morphology, size, and orderly arrangement. The TAC group showed myocardial cell swelling, reduced nuclear size, and the presence of vacuoles. The area of myocardial fibrosis was significantly increased (Supplementary Fig. 3, $p < 0.001$). Additionally, the cells were loosely arranged with significant gaps between them. After treatment with PEG/ZIF-8@AS NPs, the myocardial cell morphology and structure returned to normal, and the area of myocardial fibrosis was notably reduced ($p < 0.001$). This suggests that PEG/ZIF-8@AS NPs can significantly reduce cardiac muscle damage compared to treatment with AS alone and have a protective effect on the heart. We further observed the fibrosis of the heart after different treatments using Masson's staining. The results showed that the use of PEG/ZIF-8@AS NPs led to the disappearance of collagen fibers, indicating its strong antagonistic effect against myocardial fibrosis.

The *in vivo* biodistribution of AS was investigated using small animal fluorescence imaging. At 3 h post-injection, the Cy5-AS exhibited notable accumulation in the heart, and the fluorescence intensity further increased at 6 h, indicating sustained cardiac enrichment. In contrast, free Cy5-AS was predominantly distributed to the

liver for metabolic clearance, with minimal accumulation observed in the heart. At 24 h, a strong fluorescence signal remained detectable in the hearts of mice treated with PEG/ZIF-8@AS NPs, whereas the signal from free Cy5-AS had almost completely disappeared (Fig. 4C). *Ex vivo* fluorescence imaging of major organs further confirmed these findings, revealing that the concentration of Cy5-AS in the heart was more than fivefold higher in the PEG/ZIF-8@AS group than in the free AS^{CY5} group (Fig. 4D).

PEG/ZIF-8@AS NPs Inhibit Cardiomyocyte Apoptosis and Promote Angiogenesis

To elucidate the mechanism by which the PEG/ZIF-8@AS nanodelivery system alleviates HF, TUNEL assays were conducted to assess cell apoptosis in myocardial tissue, while immunohistochemistry was performed to detect the expression of the angiogenesis marker CD31. As shown in Fig. 5A, the green fluorescence in the TAC group was most prominent, with a noticeable alleviation observed following AS treatment. Notably, PEG/ZIF-8@AS NPs exhibited a more significant suppression of cardiomyocyte apoptosis than AS alone. Research further revealed that AS promotes angiogenesis following myocardial infarction. As shown in Fig. 5B, the levels of CD31 in the hearts of TAC + AS group and TAC + PEG/ZIF-8@AS group mice were significantly higher than in the TAC group, with the PEG/ZIF-8@AS group showing even higher levels compared to the TAC + AS group.

To further investigate the involvement of apoptosis, angiogenesis, and the PI3K/Akt signaling pathway, the expression of related proteins was analyzed by WB. As shown in Fig. 5C and Supplementary Fig. 4, Ang-II stimulation markedly increased the expression levels of Bax and cleaved caspase-3 while significantly decreasing Bcl-2 expression in H9c2 cells. Compared with the Ang-II group, treatment with free AS or PEG/ZIF-8@AS NPs significantly reduced Bax and cleaved caspase-3 expression and increased Bcl-2 expression, indicating effective suppression of apoptosis. As shown in Fig. 5D and Supplementary Fig. 5, the p-Akt/Akt ratio was significantly decreased in the Ang-II group compared with the Control group. In contrast, treatment with free AS or PEG/ZIF-8@AS NPs markedly increased the p-Akt/Akt ratio relative to the Ang-II group, suggesting that activation of the PI3K/Akt signaling pathway may contribute to the cardioprotective effects of AS against HF. Furthermore, as shown in Fig. 5D and Supplementary Fig. 5, the expression levels of VEGFR2, VEGF, and HIF-1 α were significantly elevated in the Ang-II group compared with the Control group. Notably, treatment with free AS or PEG/ZIF-8@AS NPs further enhanced the expression of VEGFR2, VEGF, and HIF-1 α . These findings indicate that AS promotes angiogenesis and that PEG/ZIF-8@AS NPs further potentiate this pro-angiogenic effect.

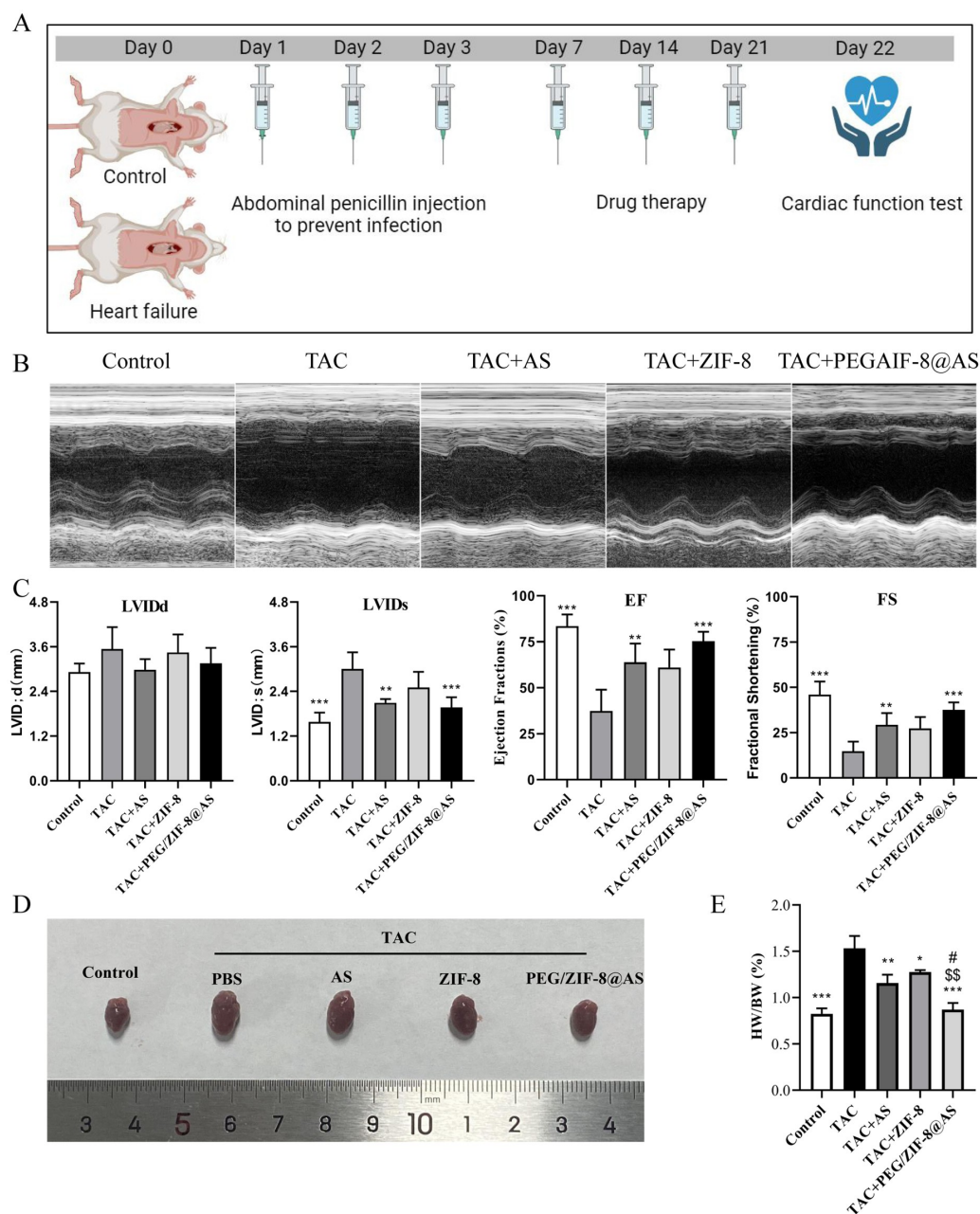


Fig. 3. The impact of PEG/ZIF-8@AS NPs on the heart function of TAC mice. (A) Experimental timeline for *in vivo* experiments. (B) Evaluation of heart function in mice using echocardiography, and calculation of (C) LVIDd, LVIDs, EF, and FS values. (D and E) Measurement of heart mass and body mass to calculate the heart mass/body mass ratio, indicating cardiac hypertrophy. # $p < 0.05$, vs. TAC + AS; ## $p < 0.01$, vs. TAC + ZIF-8; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, vs. TAC. $n = 5$. LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole; EF, ejection fraction; FS, fractional shortening. Graphs were generated using GraphPad Prism 8.0, and figure assembly was performed using Microsoft PowerPoint.

Biocompatibility

We explored the feasibility of *in vivo* application of PEG/ZIF-8@AS NPs. We first assessed the blood compatibility of PEG/ZIF-8@AS NPs through a hemolysis assay. In the animal experiment, the administration dose of PEG/ZIF-8@AS was approximately 8 mg/kg based on the DLC of 24.6%, with the body weight of the mice around 20 g. This corresponds to a total drug amount of approx-

imately 160 μg in the bloodstream. Given that the total blood volume of the mice is about 1.5 mL, the concentration of 300 $\mu\text{g}/\text{mL}$ is higher than the actual blood concentration. The results showed that even at 300 $\mu\text{g}/\text{mL}$, the hemolysis rate did not exceed 5%, indicating excellent blood compatibility of PEG/ZIF-8@AS NPs (Fig. 6A). Furthermore, histological examination of liver, kidney, spleen, and lung tissues revealed no obvious pathological abnormalities following PEG/ZIF-8@AS administration (Fig. 6B). This fur-

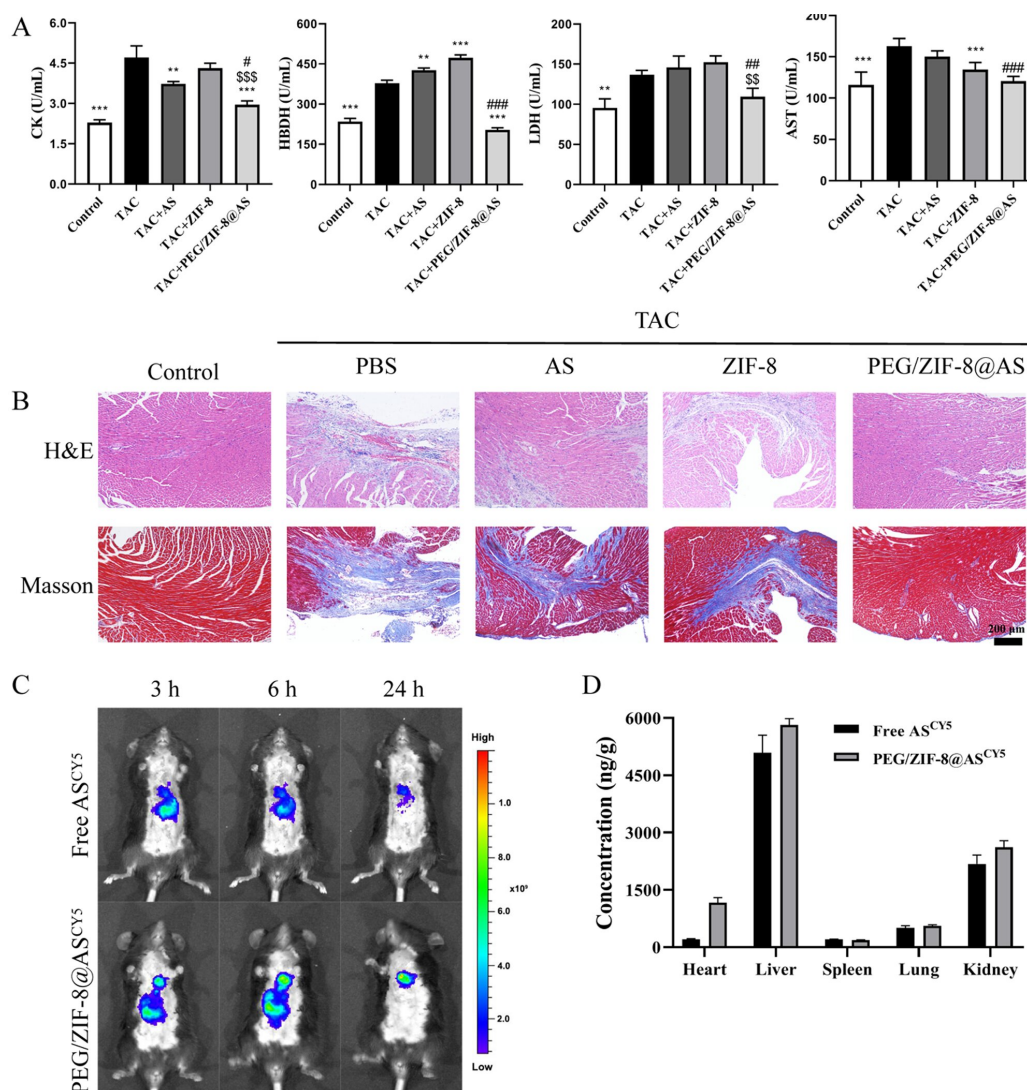


Fig. 4. The therapeutic effects of PEG/ZIF-8@AS NPs on mice cardiac injury. (A) Detection of classic biomarkers (CK, HBDH, LDH, AST) in serum reflecting cardiac damage using an automated biochemical analyzer. (B) Evaluation of the restorative effect of PEG/ZIF-8@AS NPs on heart tissue in mice through H&E and Masson staining of mice's heart tissue sections. (C) *In vivo* fluorescence imaging of Cy5-AS biodistribution in mice. (D) Quantification of Cy5-AS accumulation in major organs. Scale bar = 200 μ m. # p < 0.05, ## p < 0.01, ### p < 0.001, vs. TAC + AS; \$\$ p < 0.01, \$\$\$ p < 0.001, vs. TAC + ZIF-8; ** p < 0.01, *** p < 0.001, vs. TAC. n = 5. CK, creatine kinase; HBDH, hydroxybutyrate dehydrogenase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase. Graphs were generated using GraphPad Prism 8.0, and figure assembly was performed using Microsoft PowerPoint.

ther demonstrates the high biocompatibility of PEG/ZIF-8@AS NPs.

Discussion

AS, one of the principal bioactive constituents of *As-tragalus membranaceus*, possesses well-documented cardioprotective properties. However, its clinical translation has been limited by poor aqueous solubility, rapid systemic clearance, and insufficient accumulation within diseased cardiac tissue. In this study, we encapsulated AS in nanoparticles for transport and found that PEG/ZIF-8@AS NPs had a significantly better therapeutic effect than direct AS injection. It showed superior outcomes in terms of my-

ocardial injury, myocardial fibrosis, myocardial hypertrophy, and cardiac dysfunction, which can be attributed to the selective release of PEG/ZIF-8@AS NPs in the acidic microenvironment, where they exert inhibitory effects on cell apoptosis and promote angiogenesis (Fig. 6C).

Physicochemical characterization confirmed the successful fabrication of PEG/ZIF-8@AS NPs with a uniform particle size distribution, high drug-loading efficiency, excellent colloidal stability, and pH-responsive drug release behavior. The acid-responsive release profile is particularly relevant because pathological cardiac remodeling and HF are often associated with metabolic disturbances and mild extracellular acidification [22]. The initial rapid re-

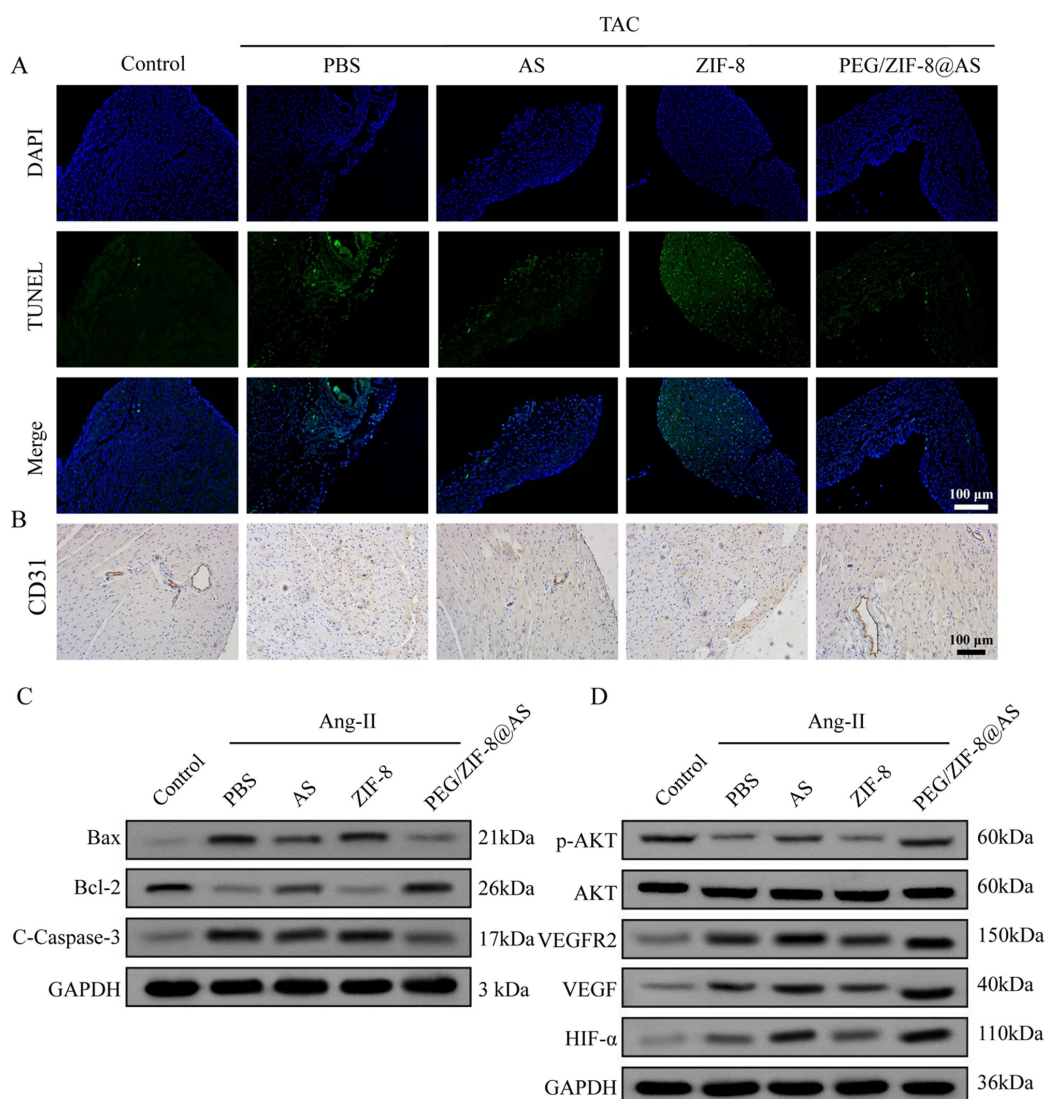


Fig. 5. Effects of PEG/ZIF-8@AS NPs on apoptosis and angiogenesis in mouse heart tissue. (A) Detection of cell apoptosis in mouse heart tissue by TUNEL assay. Scale bar = 100 μ m. (B) Expression of the angiogenesis marker CD31 in mouse heart tissue assessed by immunohistochemistry. $n = 5$, Scale bar = 100 μ m. (C) Expression of apoptosis-related proteins in H9c2 cells determined by Western blotting. (D) Expression of PI3K/Akt signaling pathway- and angiogenesis-related proteins in H9c2 cells determined by Western blotting. $n = 3$. Graphs were generated using GraphPad Prism 8.0, and figure assembly was performed using Microsoft PowerPoint.

lease may stem from the AS adsorbed on the NPs surface or within the PEG layer, as the zinc-imidazole coordination bonds of ZIF-8 are stable, making the encapsulated drug less likely to release further. Additionally, PEG modification hinders the diffusion of the drug from the ZIF-8 channels. This suggests that PEG/ZIF-8@AS NPs can remain relatively stable at physiological pH while undergoing enhanced degradation and drug release within the pathological cardiac microenvironment associated with HF. Compared with free AS, PEG/ZIF-8@AS NPs exerted substantially stronger cardioprotective effects both *in vitro* and *in vivo*. These benefits were manifested by enhanced cardiomyocyte viability, reduced expression of hypertrophic markers, attenuation of ventricular remodeling, and improved cardiac function. Notably, *in vivo* fluorescence imaging

demonstrated significantly enhanced cardiac accumulation and prolonged retention of AS following nanoparticle encapsulation. This extended myocardial exposure likely contributes to the superior therapeutic efficacy observed in the PEG/ZIF-8@AS group. In addition, PEGylation may have contributed to the improved *in vivo* performance of the nanopatform. PEG chains on the nanoparticle surface are known to form a hydrophilic steric barrier that reduces protein adsorption and recognition by the mononuclear phagocyte system, thereby prolonging systemic circulation and enhancing nanoparticle stability in biological fluids [23]. Although a PEG/ZIF-8 control group was not included in the present study, the enhanced cardiac retention observed for PEG/ZIF-8@AS NPs may be partially attributable to the favorable delivery characteristics conferred by PEGylation.

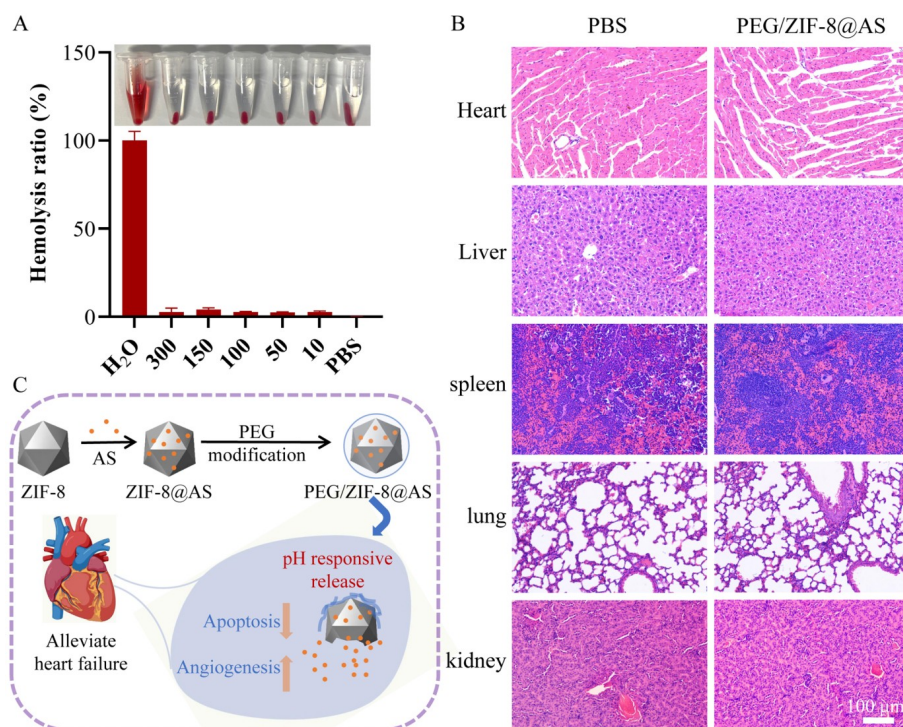


Fig. 6. Investigation of the biocompatibility of PEG/ZIF-8@AS NPs. (A) Assessment of the hemolysis rate of blood cells in mice treated with different concentrations of PEG/ZIF-8@AS NPs. (B) Analysis of the impact of PEG/ZIF-8@AS NPs on the liver, spleen, lungs, and kidneys of mice using H&E staining. $n = 5$, Scale bar = 100 μm . (C) Schematic diagram of the study. Graphs were generated using GraphPad Prism 8.0, and figure assembly was performed using Microsoft PowerPoint.

Several delivery strategies have recently been explored to improve the therapeutic performance of AS in cardiovascular diseases. For example, cardiac extracellular matrix-derived hydrogels have been developed for localized and sustained AS release after myocardial infarction, thereby enhancing angiogenesis and cardiac repair [24]. In addition, AS-loaded exosomes have demonstrated promising cardioprotective effects by promoting angiogenesis and suppressing apoptosis [25]. Nevertheless, these approaches generally require local administration or rely on biologically derived carriers, which may present challenges in large-scale manufacturing, quality control, and storage stability. Compared with these systems, PEG/ZIF-8@AS integrates high drug-loading capacity, systemic administration, pH-responsive release, and prolonged myocardial retention within a single nanoplatform, potentially offering distinct advantages for HF therapy.

Cardiomyocyte apoptosis is a critical contributor to the progressive deterioration of cardiac function during HF. Excessive apoptosis accelerates cardiomyocyte loss and promotes adverse ventricular remodeling [26]. Our findings demonstrated that PEG/ZIF-8@AS significantly inhibited apoptosis, consistent with previous reports describing the anti-apoptotic properties of AS [7,27]. Importantly, nanoparticle-mediated delivery further amplified these protective effects. In addition to apoptosis inhibition, enhanced angiogenesis also appeared to contribute to cardiac recovery.

Adequate vascularization is essential for maintaining oxygen and nutrient supply to the failing myocardium. We observed increased expression of CD31, VEGF, VEGFR2, and HIF-1 α following PEG/ZIF-8@AS treatment, indicating activation of angiogenic signaling pathways. These findings are in agreement with previous studies showing that AS promotes endothelial function and neovascularization [28]. The enhanced angiogenic response may improve myocardial perfusion and thereby facilitate functional recovery. At the mechanistic level, activation of the PI3K/Akt signaling pathway may represent an important mediator of these beneficial effects [29,30]. Ang-II stimulation markedly suppressed Akt phosphorylation, whereas PEG/ZIF-8@AS treatment restored the p-Akt/Akt ratio. Given the central role of PI3K/Akt signaling in regulating both cell survival and angiogenesis, activation of this pathway may simultaneously inhibit apoptosis and promote vascular regeneration. Nevertheless, the causal relationship between PI3K/Akt activation and the observed biological effects remains to be established through studies employing pathway-specific inhibitors or genetic approaches.

Biocompatibility represents a critical consideration for the clinical translation of nanomedicine. Although ZIF-8 nanoparticles have been reported to exhibit Zn²⁺ associated cytotoxicity at high concentrations [14], PEGylation has been shown to improve colloidal stability and reduce nonspecific interactions with biological components

[31]. Consistent with these observations, PEG/ZIF-8@AS nanoparticles exhibited negligible hemolytic activity and caused no detectable histopathological toxicity in major organs, supporting their favorable biosafety profile. Several limitations of the present study should be acknowledged. First, although *in vitro* studies using H9c2 cells demonstrated protective effects on cardiomyocytes, immortalized cell lines may not fully recapitulate the complex microenvironment and multicellular interactions present in the *in vivo* myocardium. Consequently, the cellular mechanisms observed may differ in primary cardiomyocytes or human tissues. Second, the TAC model primarily reflects pressure overload-induced HF and may not fully recapitulate other etiologies, such as ischemic or diabetic cardiomyopathy. Future studies should address these limitations while further evaluating the long-term safety and therapeutic efficacy of PEG/ZIF-8@AS nanoparticles.

Conclusions

In summary, we encapsulated AS within PEG/ZIF-8 nanoparticles using a one-step synthesis method. PEG/ZIF-8@AS NPs exhibited high drug loading, stability, and selective drug release in acidic microenvironments. *In vitro* studies demonstrated that PEG/ZIF-8@AS NPs increased cell viability and improved cardiomyocyte hypertrophy, showing stronger therapeutic effects compared to AS alone. *In vivo* studies suggest that PEG/ZIF-8@AS NPs may alleviate HF by inhibiting cardiomyocyte apoptosis and promoting angiogenesis. Compared to free AS, they are more effective in restoring cardiac function, reducing myocardial hypertrophy, treating myocardial injury, and mitigating myocardial fibrosis in HF mice. In conclusion, encapsulating AS within PEG/ZIF-8 nanoparticles resulted in improved therapeutic efficacy, providing a safe and effective treatment strategy for HF.

List of Abbreviations

HF, heart failure; AS, Astragaloside IV; MOF, metal-organic framework; TAC, transverse aortic constriction; NPs, nanoparticles; PEG, polyethylene glycol; TEM, transmission electron microscopy; XRD, X-ray diffraction; FTIR, Fourier-transform infrared spectroscopy; DLC, drug loading content; HPLC, high-performance liquid chromatography; FBS, fetal bovine serum; PBS, phosphate-buffered saline; WB, Western blotting; RT-qPCR, real-time quantitative polymerase chain reaction; LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole; EF, ejection fraction; FS, fractional shortening; CK, creatine kinase; HBDH, hydroxybutyrate dehydrogenase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; H&E, hematoxylin and eosin; ZIF-8, zeolitic imidazolate framework-8; Ang-II, Angiotensin II; IHC, immunohistochemistry; ANOVA, analysis of variance; DMEM, Dulbecco's Modified Eagle's Medium; DAPI, 4',6-diamidino-2-phenylindole; CCK-8,

Cell Counting Kit-8; HPLC-MS, High-Performance Liquid Chromatography–Mass Spectrometry; TUNEL, Terminal deoxynucleotidyl transferase dUTP Nick End Labeling.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

LFL and ZPS contributed to the design of this work. LFL, XJJ, SJC, LLH, YWH, and ZZG contributed to the investigation, interpretation of data, and analyzed the data. LFL and XJJ drafted the work. ZPS revised critically for important intellectual content and contributed to the funding acquisition. All authors read and approved the final manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Ethics Approval and Consent to Participate

The animal experiments was approved by Experimental Animal Ethics Committee of Wenzhou Medical University (wydw2025-0262).

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.22203/eCM.v059a05>.

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