Therapeutic Effect of Local Injection of VX765 Sodium Alginate Nanogel on Myocardial Infarction

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Research Article

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Abstract

Objectives: To determine the effect of polyethyleneimine/sodium alginate composite nano-gel (AG/PEI-VX765NGs) coated with VX765 on cardiac function in rats with myocardial infarction (MI).

Methods: VX765-polyethyleneimine nano-microspheres (PEI-VX765 NP) were encapsulated by sodium alginate (AG) nanogel (NGs) to construct AG/PEI-VX765 NGs. The morphological observation was performed under scanning electron microscope (SEM). The viability was evaluated by using CCK-8 assay in vitro. Then, 24 male SPF Sprague-Dawley rats were randomly divided into 4 groups: Sham, MI, PEI-VX765NP, and AG/PEI-VX765NGs. After 28 days, rats in each group were subjected to assessment of cardiac function by echocardiography. The myocardial infarct size was evaluated by TTC test, and the differences in cardiac fibrosis and cardiomyocyte apoptosis between groups were analyzed by histological methods.

Results: The prepared NGs shows a porous structure, while PEI-VX765 NP is uniformly distributed in the AG NGs samples. AG/PEI-VX765 NGs with a concentration of VX765 (range: 0-1000 μ M) displayed no significant toxicity to cells. Meanwhile, we observed a relatively more persistent release of VX765 from AG/PEI-VX765 NGs compared with PEI-VX765. LVIDs and LVIDd in both PEI-VX765 and AG/PEI-VX765NGs groups were significantly smaller than those in MI group, while ejection fraction (EF) and short-axis shortening rate (FS) were markedly increased in the above-mentioned two groups. Compared with MI group, PEI-VX765 and AG/PEI-VX765NGs groups exhibited a significant reduction in the infarct size, degree of fibrosis, and the rate of TUNEL positive cells.

Conclusion: AG/PEI-VX765NGs can significantly improve the cardiac function of rats with MI.

1. Introduction

Heart disease has become one of the major diseases that threaten human health. Particularly, myocardial infarction (MI) has a high incidence and a mortality rate reaching 50% within 5 years, while the age of onset is getting younger[1–3]. Although numerous treatment options including drug therapy, interventional therapy, heart transplantation, and other treatment strategies are available in clinic, it is impossible to completely repair damaged heart tissue due to extremely limited ability of cardiomyocyte regeneration [2, 4]. Therefore, development of new treatment strategies is needed for preventing cardiomyocyte death, reducing MI size, improving left ventricular function, and impeding heart failure.

Studies have shown that while pyroptosis is related to a variety of cardiovascular diseases, one of the main signaling pathways involved in pyroptosis is mediated by caspase-1[5, 6]. Caspase-1 inhibitor VX765 has been shown to have a protective effect on rat model of acute myocardial ischemia reperfusion (IR) [7], suggestive of a potential of VX765 for cardiac protective therapy [8]. However, drug degradation and loss following traditional drug administration remain to be a problem. In order to improve the bioavailability and concentration of drugs in the lesion area, many different combinations of drugs and carriers are being studied. Nanogel (NGs) is a three-dimensional network of hydrogel particles

composed of hydrophilic or amphiphilic polymer chains by physical or chemical cross-linking, which has the characteristics of hydrogel materials and nanomaterials. NGs has been widely used in biomedical fields such as drug delivery, genetic engineering and tissue engineering[9, 10]. In the field of experimental drug delivery in heart repair, efforts have been made to maximize drug delivery to the heart through a variety of targeted strategies and methods. Intramyocardial injection has the advantage of local treatment while minimizing the potential systemic effects. Several NGs acting as injectable delivery carriers designed for targeting the injured site have been tested in the treatment of MI[11, 12]. Paul et al[12] developed an injectable and biocompatible hydrogel that can effectively deliver graphene oxide (GO) and vascular endothelial growth factor 165 (VEGF) angiogenic gene nanocomposites for myocardial therapy.

Compared with other biomaterials, sodium alginate (AG) has attracted more and more attention in the fields of drug carrier, tissue engineering and regenerative medicine because of its excellent biocompatibility, biodegradability and good gel properties[13, 14]. Studies have shown that injection of calcium alginate solution cross-linked with calcium into MI site leads to the phase transition to hydrogel at the infarct site, which provides temporary physical support for the damaged heart tissue while preventing poor cardiac remodeling and dysfunction after MI in rats[15]. In addition, polyethyleneimine (PEI), also known as polyazacyclopropane, is a water-soluble polymer, and its nanoparticles have a potential of prolonging the retention time, reducing the clearance rate, and improving the bioavailability of drugs.

Here, we prepared injectable VX765-polyethyleneimine/sodium alginate nanogel (AG/PEI-VX765 NGs) by using sodium alginate nanogel (AG NGs) and PEI carrier with good biocompatibility, and investigated the effects of AG/PEI-VX765 NGs on cardiac function, tissue fibrosis, cardiomyocyte apoptosis and infarct area in rats with MI. This study laid a foundation for potential clinical application of VX765 nanogel.

2. Materials & Methods

2.1. Ethics Approval

Protocols involving the use of the animals were approved by the Wenzhou Medical University Animal Policy and Welfare Committee (Approval documents: wyd2019-0206). We have taken all steps to minimize the animals' pain and suffering.

2.2. Preparation of AG/PEI-VX765 NGs

Aqueous solution of VX765-polyethyleneimine nanospheres (PEI-VX765 NP) was prepared by self-assembly method[16]. The prescribed quantity of PEI (branched, relative molecular weight of 600, batch number F1518083, Shanghai Aladdin Reagent Company, Shanghai, China) was dissolved in 0.5% acetic acid solution. VX765 drug solution (catalogue number: HY-13205; MedChemExpress, New Jersey, USA) at a concentration of 0-1000 μ M was slowly dripped into the above PEI solution under stirring, and aqueous solution of PEI-VX765 NP was obtained by stirring at a constant temperature of 30°C for 10 min.

AG/PEI-VX765 NGs was prepared by double emulsification method. Firstly, AG (batch number S22278, American FMC Company) was dissolved in ultra-pure water to obtain AG aqueous solution. 3 mL of AG aqueous solution was placed in a reaction bottle, and 8.25 mg EDC was then added to the solution. The mixture was stirred for 3 h to activate the carboxyl group on AG skeleton chain. Thereafter, the activated solution was dripped into DCM solution with dissolved AOT (2.5 wt%, 6 mL), and the mixed solution was stirred at 1000 rpm for 5 min to form water in oil emulsion. The Wamp O emulsion was then dripped drop by drop into aqueous solution of PVA (45 mL 2 wt%), and the solution was stirred at 1000 rpm and mixed for 10 min to form a water-in-oil-in-water (W/O/W) emulsion. Then, the prepared aqueous solution of PEI-VX765 (10 mg / mL) was dropped into the W/O/W emulsion drop by drop, and the mixture was first stirred at 1000 rpm overnight for evaporation and removal of organic solvent DCM, then centrifuged at 8000 rpm, and washed three times to remove the surfactant. Finally, the product was re-dispersed in ultra-pure water or PBS buffer to obtain AG/PEI-VX765 NGs. The preparation of AG/PEI NGs was performed using the same method as described above, except for the drug administration step. The morphology of alginate brine gels was observed under the scanning electron microscope (SEM, S-4800; Hitachi, Tokyo, Japan).

2.3. Cell viability

In this study, CCK-8 assay was carried out to evaluate cytocompatibility of AG/PEI-VX765 NGs using H9C2 cells. H9C2 rat cardiomyocytes were purchased from the American Type Culture Collection (Rockville, Maryland, USA) and cultured in DMEM/ high glucose (HyClone, USA) with 10% fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) at 37 °C under 5% CO₂. AG/PEI-VX765 NGs with various concentrations of VX765 (0, 25, 50, 100, 200, 400, 800 and 1000 µM) were added respectively to the cultured cells, rinsed twice with sterilized PBS, and then soaked in the medium overnight. The cells were inoculated on the hydrogel in a 24-well plate at a density of 2 × 10⁵ cells per well. In order to analyze the effect of hydrogel composition on cell proliferation, 10% of (v/v) cell counting kit solution (CCK-8, Kumamoto, Japan) was added to the culture supernatant, and the mixture was reacted for 2 hours at 37 °C under 5% CO₂. The absorbance of each hole at λ = 450 nm was detected, and the cell viability was then determined.

2.4. Release of VX765 from nanogel

5 mL of PBS buffer solution was applied to AG/PEI-VX765 NGs and well mixed by shaking. Thereafter, the samples were taken at various time points (0, 24, 48, 72, 96 and 120 h) for centrifugation at 12000 rpm for 10 min. and the supernatant was tested for ultraviolet absorption at the wavelength of 480 nm. Subsequently, 5 mL of PBS buffer solution was re-added to the centrifuge tube, and the mixture was kept in a constant temperature water bath oscillator. To ensure data accuracy, each sample was measured at least three times.

2.5. Preparation of rat MI model and injection of grouping materials

24 male SPF Sprague-Dawley rats aged 6–8 weeks (weighting 250 g ± 20 g) were obtained from the Wenzhou Medical University Animal Center. Animals were fed in a standard laboratory with controlled

room temperature ($22 \pm 1^\circ\text{C}$), humidity (65–70%), and 12 hours of light-dark cycle, while having free access to food and water. Anesthesia was performed by intraperitoneal injection of 2% pentobarbital sodium (30 mg/kg), and the anesthetized rats received ventilator-assisted respiration for small animals (inspiratory/expiratory ratio of 1/2, tidal volume of 3 mL/100g, and respiratory rate of 50–70 beats/min). The fourth and fifth intercostal skin of the left edge of the sternum was cut laterally, and the length of incision was about 1.5 cm. The subcutaneous tissue, pectoralis major muscle and pectoralis minor muscle were bluntly separated, and the intercostal muscle was cut. Meanwhile, the intercostal incision was opened using the eyelid device, and the pericardium was bluntly torn open to fully expose the heart. The left anterior descending coronary artery was ligated with a 6 – 0 silk suture, while the ligation site was located at the midpoint of the line connecting the pulmonary artery cone with 1–2 mm at the lower edge of left atrial appendage, and the ligation depth was about 2.0 mm. During the ligation, the color change of the ventricle wall was monitored. A swift change from red to purplish white via a cyanosis in the color of the anterior wall of left ventricle indicates a successful surgery for the MI model. Rats in the sham operation group underwent the same surgical operation without ligating the coronary artery. In this study, rats were divided into four groups with six for each group according to the random number table method: Sham group, MI group, PEI-VX765 NP group, and AG/PEI-VX765 NGs group. Injection of respective materials for each group was performed on 5 different sites at the edge of the MI region of rats, with 2 μL of liquid injection volume for each site. The chest cavity was closed after the injection. The muscles and skin were sutured layer by layer, and the tracheal intubation was removed. And the rats were raised in a single cage after their autonomous activities were resumed.

2.6. Echocardiographic detection

Echocardiography was conducted on the SD rats at the 28th day after operation to evaluate the improvement of cardiac function. Rats were anesthetized by intraperitoneal injection of 2% pentobarbital sodium (30mg/kg) and examined by Vevo2100 ultrasound (VisualSonics, Toronto, Canada). The selected measurement parameters were as follows: left ventricular end-systolic diameter (LVSDs), left ventricular end-diastolic diameter (LVSDd), ejection fraction (EF), and short-axis shortening rate (FS).

2.7. Triphenyl tetrazolium chloride (TTC) staining

After echocardiographic examination, euthanasia was administered by an overdose of pentobarbital sodium via intraperitoneal injection according to the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals, 2013 Edition. The heart was harvested and cut into 2–3 mm thick slices along the sagittal section of the heart. And the slices were kept in TTC solution (37°C, 1%, pH7.4) for 25 min, and then fixed in 10% formaldehyde solution overnight. Finally, images were taken with a digital camera, and the infarct area was analyzed by using ImageJ software.

2.8. Histological analysis

The heart tissue was immersed in 4% paraformaldehyde at 4°C for 48 hours, subjected to alcohol gradient dehydration, embedded in paraffin, and sectioned in 5 μm thick slice. Hematoxylin eosin (HE) and Masson trichromatic staining were performed on the slices, and apoptosis was detected based on

TUNEL staining using fluorescein in situ cell death detection kit (Roche). The images were taken by microscope and quantified by ImageJ software. The percentage of infarct area was calculated as follows: infarct area (%) = fibrotic area / Total area X100%.

2.9. Statistical analysis

Experimental data for each group were expressed by mean $\pm$ standard deviation. In all experiments, one-way ANOVA (ANOVA) and Tukey's multiple comparisons were used to compare the differences between samples. Data processing was conducted by using SPSS20.0 software. $p < 0.05$ is considered statistically significant.

3. Results

3.1. Characterization and performance evaluation of NGs

SEM images show that NGs possess a porous structure, while PEI-VX765 NP is uniformly distributed in the sample of AG NGs (Fig. 1A). As shown in Fig. 1B, H9C2 cells treated with AG/PEI-VX765NGs containing various concentrations of VX765 had a viability of more than 85%, indicating that the tested AG/PEI-VX765 NGs display no significant cytotoxicity rather a good cytocompatibility. Moreover, we observed a relatively more persistent release of VX765 from AG/PEI-VX765 NGs compared with PEI-VX765 NPs (Fig. 1C).

3.2. Evaluation of cardiac function and infarct size

Postoperative echocardiography was conducted to measure cardiac function of rats in each group (Fig. 2A). As shown in Fig. 2B, the left ventricular end-systolic diameter (LVSDs) and left ventricular end-diastolic diameter (LVDD) in both PEI-VX765 and AG/PEI-VX765NGs groups were significantly smaller than those in MI group, whereas ejection fraction (EF) and short-axis shortening rate (FS) were markedly increased in the above-mentioned two groups. Compared with MI group, PEI-VX765 and AG/PEI-VX765 NGs groups exhibited a significant reduction in myocardial infarct size (Fig. 3).

3.3. Histological observation

Fibrosis was measured by trichromatic staining of H&E and Masson 28 days after injection of AG/PEI-VX765 NGs. The histological studies revealed that the degree of fibrosis in both PEI-VX765 and AG/PEI-VX765 NGs groups was significantly lower than that in MI group (Fig. 4).

3.4. Detection of cardiomyocyte apoptosis

TUNEL immunofluorescence assay was performed to assess apoptosis in myocardial tissue treated with various intervention methods. In the experiments, while the nucleus was stained with DAPI (blue), apoptotic cells were visualized by TUNEL staining (green). As depicted in Fig. 5, the proportion of TUNEL

positive cells was significantly declined in both PEI-VX765 and AG/PEI-VX765 NGs groups as compared to MI group, while AG/PEI-VX765 NGs group had the lowest rate of TUNEL positive cells. These observations indicated that AG/PEI-VX765 NGs could mitigate MI-caused apoptosis and protect cardiomyocytes.

4. Discussion

MI is a kind of heart injury disease mainly caused by ischemia and hypoxia. Local ischemia can cause irreversible loss of cardiomyocytes, leading to inflammation, fibrosis and cardiac dysfunction[17]. Given that mature myocardium cannot regenerate itself, one major challenge in the treatment of cardiovascular diseases is the restoration of damaged myocardium. At present, there are no treatments available for restoring the cardiac function after MI[4]. Therefore, myocardial tissue engineering has become a hot spot in the treatment of damaged myocardium[9]. Injectable NGs provides an effective minimally invasive method to restore the development of functional myocardium, showing great significance for the treatment of cardiovascular diseases[11]. It can effectively deliver drugs to infarcted myocardial tissue, solving the major problem for the nanotherapeutic strategy, while enhancing the effect of conventional therapy[9, 11]. In this study, we showed that AG/PEI-VX765NGs displays a protective effect on MI, as reflected by the improvement of cardiac function as well as reduction in cardiac fibrosis, apoptosis, and MI size.

The strategy for improving heart failure after MI by injection of biomaterials into the infarcted myocardium has proved successful. The composition and physicochemical properties of injected biomaterials mainly determine the repair effect in the infarcted myocardium. In this study, AG and PEI were used as the main materials. Because there are abundant carboxylic acid groups in the AG polymer chain, AG/PEI-VX765NGs is chemically cross-linked with the carboxylic acid group on AG through the surface amino group of PEIs, thereby being wrapped in AG. We observed that AG/PEI-VX765 NGs with a concentration of VX765 in the range of 0-1000 μ M has good cytocompatibility, laying a foundation for subsequent evaluation in vivo. The above observation is consistent with the previous studies. The ultraminiature iron oxide (Fe_3O_4) NP prepared by Hao et al[10] was coated with PEI, and the alginate (AG) NGs colloid formed by double emulsion was stable and displayed cell compatibility. In addition, sustained release of the nano-gel provides sufficient time for the hydrogel to interact with cells and produce a promoting effect on regeneration. The sustained-released effect of nanogel has also been demonstrated in previous studies[12, 18, 19].

Myocardial fibrosis after MI can induce malignant cardiovascular diseases[20]. It is of great clinical significance to determine the mechanism of myocardial fibrosis and undertake individualized targeted therapy. Fibrin-specific poly (N-isopropylacrylamide) nanogels comprising core-shell colloidal hydrogels can inhibit cardiac fibrosis after IR injury[21]. Consistent with previous studies, histological analysis (Hype and Masson) of cardiac tissue after MI in this study suggests that while interstitial and perivascular fibrosis is inhibited, administration with AG/PEI-VX765NGs reduces myocardial fibrosis. Moreover, echocardiography and TTC detection revealed that AG/PEI-VX765NGs treatment remarkably

improves the cardiac function of rats with MI, indicating that nanogels play a protective role in the heart, as shown in previous studies [12, 21].

Apoptosis is an important form of cell death in the early stage of MI, which can elicit death of many cardiomyocytes and other types of cells, affecting ventricular remodeling to reduce cardiac function. In the present study, the NGs was found to reduce apoptosis in vivo. As an inhibitor of caspase-1, VX765 may play a very important role in pyrolysis, an inflammatory form of caspase-1-dependent programmed cell death[5]. It has been shown that inhibition of Caspase-1-dependent pyrolysis may attenuate copper-induced apoptosis[22]. Moreover, while VX-765 inhibits the scorching death of vascular smooth muscle cells (VSMCs) in the process of atherosclerosis, targeting caspase-1 activity may serve as a potential therapeutic strategy for atherosclerotic cardiovascular diseases[23].

5. Conclusions

In this study, we demonstrated for the first time that injection of AG/PEI-VX765NGs promotes myocardial recovery and reduces apoptosis, cardiac fibrosis, and MI area, resulting in significant cardiac functional recovery. These data therefore indicated that AG/PEI-VX765NGs has a therapeutic potential for myocardial repair, while in vivo application of injectable nanogels may serve as an effective strategy for MI treatment.

Abbreviations

AG, sodium alginate; NG, nanogel; PEI, polyethyleneimine; MI, myocardial infarction; SEM, scanning electron microscope; CCK-8, Cell Counting Kit-8; LVIDs, left ventricular end-systolic diameter; LVIDd, left ventricular end-diastolic diameter; EF, ejection fraction; FS, short-axis shortening rate; TTC, Triphenyl tetrazolium chloride; HE, Hematoxylin eosin

Declarations

Acknowledgements

Not applicable.

Authors' Contributions

TQX, LJL drafted the article or revised it critically for important intellectual content. TQX, LJL, CQ, ZMX had substantial contributions to conception and design, acquisition of data or analysis and interpretation of data. All authors reviewed and approved the final version of article and agree 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 are appropriately investigated and resolved All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Data Availability Statement

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

Ethics approval and consent to participate

All the animal procedures used in the present study were approved by Wenzhou Medical University Animal Policy and Welfare Committee (Approval documents: wyd2019-0206) and were carried out in compliance with Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines. We have taken all steps to minimize the animals' pain and suffering. Euthanasia was administered by an overdose of pentobarbital sodium via intraperitoneal injection according to the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals, 2013 Edition.

Consent for publication

Not applicable.

Declaration of 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.

References

1. B PVA, B DKA, B MMA, B KCA, B HVAA, B SAA, B PBA, B RWS, A, Abhijeet Dhoble AB: Outcomes of Acute Myocardial Infarction in Patients with Influenza and Other Viral Respiratory Infections - ScienceDirect. *The American Journal of Medicine* 2019, 132(10):1173-1181.
2. Saleh M, Ambrose JA: Understanding myocardial infarction. *F1000 Research* 2018, 7(9):1378.
3. I R, J P, J P, J M: [Myocardial infarction in the young - our results and experience]. *Vnitřní Lékarství* 2012, 58(7-8):536-543.
4. Cahill TJ, Choudhury RP, Riley PR: Heart regeneration and repair after myocardial infarction: translational opportunities for novel therapeutics. *Nature Reviews Drug Discovery* 2017, 16(10):699-717.
5. Jia C, Chen H, Zhang J, Zhou K, Zhuge Y, Niu C, Qiu J, Rong X, Shi Z, Xiao J: Role of pyroptosis in cardiovascular diseases. *International Immunopharmacology* 2019, 67(2):311-318.

6. Stefano T, Mauro AG, S CZ, Antonio A: Inflammasome, pyroptosis, and cytokines in myocardial ischemia-reperfusion injury. *AJP Heart and Circulatory Physiology* 2018, 315(6):H1553-H1568.
7. Carmo D, Helison, Arjun, Sapna, Petrucci, Orlando, Yellon, Derek M, Davidson, Sean M: The Caspase 1 Inhibitor VX-765 Protects the Isolated Rat Heart via the RISK Pathway. *Cardiovascular drugs and therapy* 2018, 32(2):165-168.
8. Audia JP, Yang XM, Crockett ES, Housley N, Haq EU, O'Donnell K, Cohen MV, Downey JM, Alvarez DF: Caspase-1 inhibition by VX-765 administered at reperfusion in P2Y₁₂ receptor antagonist-treated rats provides long-term reduction in myocardial infarct size and preservation of ventricular function. *Basic Research in Cardiology: Official Journal of the German Association of Cardiovascular Research* 2018, 113(7):32-46.
9. Li Y, Maciel D, Rodrigues J, Shi X, Tomás H: Biodegradable Polymer Nanogels for Drug/Nucleic Acid Delivery. *Chemical Reviews* 2015, 115(16):8564-8608.
10. Hao X, Xu B, Chen H, Wang X, Zhang J: Stem cell-mediated delivery of nanogels loaded with ultrasmall iron oxide nanoparticles for enhanced tumor MR imaging. *Nanoscale* 2019, 11(11):4904-4910.
11. Nguyen MM, Gianneschi NC, Christman KL: Developing injectable nanomaterials to repair the heart. *Curr Opin Biotechnol* 2015, 34(8):225-231.
12. Paul A, Hasan A, Kindi HA, Gaharwar AK, Rao VTS, Nikkhah M, Shin SR, Krafft D, Dokmeci MR, Shum-Tim D: Injectable graphene oxide/hydrogel-based angiogenic gene delivery system for vasculogenesis and cardiac repair. *Acs Nano* 2014, 8(8):8050-8062.
13. Xue Y, Xia X, Yu B, Luo X, Cai N, Long S, Yu F: A green and facile method for the preparation of a pH-responsive alginate nanogel for subcellular delivery of doxorubicin. *RSC Advances* 2015, 5(90).
14. Ching SH, Bansal N, Bhandari B: Alginate gel particles-A review of production techniques and physical properties. *Crit Rev Food Nutr* 2017, 57(6):1133-1152.
15. Leor J, Tuvia S, Guetta V, Manczur F, Castel D, Willenz U, Petneházy r, Landa N, Feinberg MS, Konen E: Intracoronary Injection of In Situ Forming Alginate Hydrogel Reverses Left Ventricular Remodeling After Myocardial Infarction in Swine. *Journal of the American College of Cardiology* 2009, 54(11):1014-1023.
16. Yang L, Zheng Z, Qian C, Wu J, Liu Y, Guo S, Li G, Liu M, Wang X, Kaplan DL: Curcumin-functionalized silk biomaterials for anti-aging utility. *Journal of Colloid & Interface ence* 2017, 496(6):66-77.
17. Sumanth, Prabhu, Nikolaos, Frangogiannis: The Biological Basis for Cardiac Repair After Myocardial Infarction: From Inflammation to Fibrosis. *Circulation Research* 2016, 119(1):91-112.
18. Sahu P, Kashaw SK, Kushwaha V, Jain S, Iyer AK: pH responsive biodegradable nanogels for sustained release of bleomycin. *Bioorg Med Chem* 2017, 25(17):4595-4613.

19. Chao, Qin, Yaqi, Chaoran, Jingjing, Lifang, Yin, Wei: Lipid-bilayer-coated nanogels allow for sustained release and enhanced internalization. *International journal of pharmaceutics* 2018, 551(1-2):8-13.
20. Talman V, Ruskoaho H: Cardiac fibrosis in myocardial infarction—from repair and remodeling to regeneration. *Cell and Tissue Research* 2016, 365(3):563-581.
21. Emily M, Huang K, Erin S, Cheng K, Brown AC: Targeted Treatment of Ischemic and Fibrotic Complications of Myocardial Infarction Using a Dual-Delivery Microgel Therapeutic. *Acs Nano* 2018, 12(8):7826-7837.
22. Jianzhao, Liao, Fan, Yang, Zhaoxin, Tang, Wenlan, Yu, Qingyue, Han: Inhibition of Caspase-1-dependent pyroptosis attenuates copper-induced apoptosis in chicken hepatocytes. *Ecotoxicology and environmental safety* 2019, 174(2):110-119.
23. Li Y, Niu X, Xu H, Li Q, Zhang Z: VX-765 attenuates atherosclerosis in ApoE deficient mice by modulating VSMCs pyroptosis. *Experimental Cell Research* 2020, 389(1):111847.

Figures

Figure 1

Material characterization of AG/PEI-VX765 NGs. (A) Scanning electron microscope (SEM) test (Left, X18.0K, right, X30.0K). (B) CCK-8 assay-based detection of drug toxicity. (C) Drug release curves. Grouping was as follows: AG/PEI blank NP samples, VX765 drugs, and AG/PEI-VX765 NGs samples.

Figure 2

Echocardiographic detection of AG/PEI-VX765 NGs (n=3/group). (A) Data on typical electrocardiogram (ECG). (B) Ultrasonic indices were shown as follows: left ventricular end-systolic diameter (LVIDs), left ventricular end-diastolic diameter (LVIDd), ejection fraction (EF), and short-axis shortening rate (FS). *p < 0.05, **p < 0.01, and ***p < 0.001 compared with sham group, ##p < 0.01 and ###p < 0.001 compared with MI group. Rats were randomly divided into 4 groups: Sham, MI, PEI-VX765 NP, and AG/PEI-VX765 NGs.

Figure 3

Injection of AG/PEI-VX765 NGs reduced myocardial infarct size (n = 5/group). (A) Myocardial infarct size, the infarction zone (white) and risky zone (red) were shown. (B) Myocardial infarct size was expressed as the percentage of the infarct area relative to the total at-risk area after MI. ***p < 0.001 compared with sham group, and ###p < 0.001 compared with MI group. Rats were randomly divided into 4 groups: Sham, MI, PEI-VX765 NP, and AG/PEI-VX765 NGs.

Figure 4

Histological observation of AG/PEI-VX765 NGs after injection (n = 3/group). (A) Hematoxylin and eosin (H&E) staining of myocardial tissue. (B) Myocardial section stained with Masson's trichrome. (C) Statistical analysis of Masson's trichrome staining. ***p < 0.001 compared with sham group, and ##p < 0.01 compared with MI group. Rats were randomly divided into 4 groups: Sham, MI, PEI-VX765 NP, and AG/PEI-VX765 NGs.

Figure 5

The effect of AG/PEI-VX765 NGs on myocardial apoptosis was assessed by TUNEL immunofluorescence ($n = 3/\text{group}$). (A) Microscopic observation of TUNEL-stained samples. The nucleus was stained with DAPI (blue), while apoptotic cells were visualized by TUNEL staining (green). (B) Statistical analysis of TUNEL staining. $*p < 0.05$ and $***p < 0.001$ compared with sham group, $##p < 0.01$ and $###p < 0.001$ compared with MI group. Rats were randomly divided into 4 groups: Sham, MI, PEI-VX765 NP, and AG/PEI-VX765 NGs.