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Nitric oxide releasing hydrogel enhances the therapeutic efficacy of mesenchymal stem cells for myocardial infarction

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ABSTRACT

Stem cell therapy has been proved to be an effective approach to ameliorate the heart remodeling post myocardial infarction (MI). However, poor cell engraftment and survival in ischemic myocardium limits the successful use of cellular therapy for treating MI. Here, we sought to transplant adipose derived-mesenchymal stem cells (AD-MSCs) with a hydrogel (NapFF-NO), naphthalene covalently conjugated a short peptide, FFGGG, and β-galactose caged nitric oxide (NO) donor, which can release NO molecule in response to β-galactosidase. AD-MSCs, either from transgenic mice that constitutively express GFP and firefly luciferase (Fluc), or express Fluc under the control of VEGFR2 promoter, were co-transplanted with NapFF-NO hydrogel into murine MI models. Improved cell survival and enhanced cardiac function were confirmed by bioluminescence imaging (BLI) and echocardiogram respectively. Moreover, increasing VEGFR2-luc expression was also tracked in real-time *in vivo*, indicating NapFF-NO hydrogel stimulated VEGF secretion of AD-MSCs. To investigate the therapeutic mechanism of NapFF-NO hydrogel, cell migration assay, paracrine action of AD-MSCs, and histology analysis were carried out. Our results revealed that condition medium from AD-MSCs cultured with NapFF-NO hydrogel could promote endothelial cell migration. Additionally, AD-MSCs showed significant improvement secretion of angiogenic factors VEGF and SDF-1α in the presence of NapFF-NO hydrogel. Finally, postmortem analysis confirmed that transplanted AD-MSCs with NapFF-NO hydrogel could ameliorate heart function by promoting angiogenesis and attenuating ventricular remodeling. In conclusion, NapFF-NO hydrogel can obviously improve therapeutic efficacy of AD-MSCs for MI by increasing cell engraftment and angiogenic paracrine action.

1. Introduction

Stem cells therapy has been proved as a promising strategy for myocardial infraction (MI) treatment [1]. However, the low retention and survival rate of administrated stem cells in ischemic zone discounts the therapeutic effect dramatically [2]. The strategy to co-transplant stem cells with synthetic biomaterials that are designed to mimic the *in vivo* microenvironments provides not only a scaffold for cell anchorage, but also a supportive niche for cell engraftment [3–5]. Hydrogels, which are polymeric materials distinguished by high water content and diverse physical properties, can be engineered to resemble the extracellular environment [6]. Hydrogel products have been widely used as vehicles for stem cell therapy and drug-delivery devices in preclinical practice [6–8].

Recent studies have revealed that delicate designed synthetic polymers and self-assembling peptides, which can form hydrogel under certain circumstance, were developed for stem cells delivery into MI heart and the cell retention rate got effective improvement

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[9–11]. Given the hostile environment in ischemic region, many bioactive factors, such as vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1α (SDF-1α), were combined with the materials in order to further improve the therapeutic effect of hydrogel in animal MI models [12–14].

Nitric oxide (NO) is a vital messenger that mediates many important signaling pathways, including circulation system development and cardiac protection [15]. Furthermore, intramyocardial administration of exogenous NO donors can activate guanylyl cyclase-protein kinase G signaling pathway, leading to the mitochondrial ATP-sensitive K channel open, which finally resulted in cardiac protection [16]. Meanwhile, NO molecule also could promote the expression of pro-angiogenic cytokines in stem cells, which is favorable for angiogenesis [17]. However, the short half-life much limits NO utilization in MI treatment [18]. Therefore, delivering NO in a controllable manner is essential for its application *in vivo*.

With the capabilities for visualization, characterization, and measurement of biological processes at the molecular and cellular levels in the intact animal or human, molecular imaging offers the potential for non-invasive assessment of therapeutic mechanisms and real-time monitoring of therapeutic responses simultaneously [19,20]. Among many imaging modalities, bioluminescence imaging (BLI) system holds relatively high sensitivity, which makes it possible that even 100 cells can be detected *in vivo* [2]. Upon gene modification, both cellular behaviors (e.g. proliferation, migration, differentiation, and paracrine action) and molecular behaviors (e.g. gene expression, protein–protein interaction, and post translational modification) of stem cells can be real-time monitored longitudinally using BLI system [21], leading to comprehensive application of BLI technology in stem cell research.

In this study, we synthesized a hydrogel by combination of naphthalene conjugated short peptide, FFGGG, and β-galactose caged nitric oxide (NO) donor (NapFF-NO), which can release NO molecule in response to β-galactosidase [22,23]. Here, we hypothesize that this controlled nitric oxide releasing hydrogel could enhance the therapeutic efficacy of mesenchymal stem cells (MSCs) for MI. To test this hypothesis, we transplanted adipose derived-mesenchymal stem cells (AD-MSCs) along with NapFF-NO hydrogel into the infracted myocardium in mice, and evaluated the therapeutic effect of AD-MSCs. Moreover, cell retention and paracrine activity were monitored by molecular imaging respectively.

2. Materials and methods

2.1. Animals

Adult wild type FVB and C57BL/6 mouse (8–10 weeks old) were purchased from the Laboratory Animal Center of the Academy of Military Medical Science (Beijing, China). The transgenic FVB mouse, which constitutively expressed firefly luciferase (Fluc) and green fluorescent protein (GFP) through the whole body [24], and the C57BL/6 background transgenic mice, which expressed Fluc under the promoter of vascular endothelial growth factor receptor 2 (VEGFR2-luc) [25], were obtained from Xenogen Corporation (Hopkinto, MA, USA). Protocols were approved by the Nankai University Animal Care and Use Committee guidelines, which conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8th edition, 2011).

2.2. Hydrogel preparation

The hydrogelator, β-galactose caged nitric oxide (NO) donor covalently combined with a naphthalene molecule (Nap) modified 5 amino acids peptide, F (phenylalanine), F, G (glycine), G, G (Nap-FFGGG-"NO"; Fig. 1A), was synthesized and purified as previously reported [22]. The Nap-FFGGG-"NO" (NapFF-NO) molecular hydrogel can consistently release NO molecule in the presence of β-galactosidase. And the Nap-FFGGG (NapFF) hydrogel, without the NO donor, was used as control in the whole experiment.

For *in vitro* test, all the powder was exposed to the UV light for 30 min for sterilization. Then, 1.0 mg of NapFF-NO or NapFF hydrogelator and 0.15 mg of sodium carbonate (Na_2CO_3) were suspended in 200 μl phosphate buffer saline (PBS, pH = 7.4), thereafter the suspensions were heated to form clear solutions. The gels (0.5% concentration) would form after cooling back to room temperature within 5 min. The release of NO from NapFF-NO hydrogel was measured as previously described [22].

2.3. MTT assay

For the cytotoxicity test of NapFF-NO hydrogel, NIH 3T3 mouse fibroblast cells were seeded into 96-well plates at a density of 10,000 cells per well and incubated for 24 h. The hydrogelator powder was dissolved in DMEM (Gibco, Grand Island, NY, USA) medium, and added into the cells (final concentration were 100, 200, 400, and 800 μM). After 24 h' incubation, MTT test was performed as previously described [23].

2.4. Cell proliferation assay

For proliferation test, each well of a 12-well cell culture plate was coated with 20 μl 0.5% NapFF-NO or NapFF hydrogel. AD-MSCs were seeded onto the hydrogel coated or uncoated tissue culture plates at a density of 100,000 cells/well with 300 U/L β-galactosidase (Sigma–Aldrich, Milwaukee, WI, USA) in the medium. 1 μM LH-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ, Sigma) was used to block the NO signal pathway in this experiment. At different time point (24 h, 48 h and 72 h), AD-MSCs were harvested and cell numbers were determined by cell counting. The methods for AD-MSCs isolation, cultivation, and characterization can be found in Supplementary Materials section.

2.5. Optical imaging of VEGFR2-luc AD-MSCs in vitro

It has been reported that the therapeutic effect of MSCs is mainly attributed to their paracrine action and the expression of pro-angiogenic cytokines [26]. To investigate whether NapFF-NO hydrogel could enhance the pro-angiogenic activity, AD-MSCs isolated from VEGFR2-luc transgenic mice were used. VEGFR2-luc AD-MSCs were seeded onto the hydrogel coated or non-coated 12-well cell culture plates at a density of 70,000 cells/well. β-galactosidase solution was added into the medium after the cells attachment at final concentration of 300 U/L. 11 h later, culture medium was changed to FBS free α-MEM contained 100 μM hydrogen peroxide (H_2O_2) and 300 U/L β-galactosidase, to mimic the oxidative damage caused by MI *in vivo*. One hour later, the cells were washed with PBS and 100 μl D-Luciferin (15 g/L; Biosynth International) was added. The signal of cells was collected by *in vivo* Imaging System IVIS Luminar (Xenogen Corporation, Hopkinto, MA). For normal condition, AD-MSCs were cultured for 12 h and then the signal of cells was measured by the imaging system. The experiments were performed in triplicate.

2.6. Real-time PCR

AD-MSCs were seeded into 6-well plates which were coated with hydrogel as described above. 11 h after β-galactosidase addition, oxidative damage was induced by H_2O_2 solution for one hour. For normal condition, cells were cultured for 12 h. RNA of the AD-