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A π-π conjugation-containing soft and conductive injectable polymer hydrogel highly efficiently rebuilds cardiac function after myocardial infarction

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ABSTRACT

Previous studies suggested that a stiffer hydrogel system exhibited a better performance to promote heart function after myocardial infarction (MI). However, the nature of myocardium, a tissue that alternately contracts and relaxes with electrical impulses, leads us to hypothesize that a soft and conductive hydrogel may be in favor of mechanical and electrical signals transmission to enhance heart function after MI. In this work, π-π conjugation interaction was first employed to produce a soft injectable hydrogel with conductive property. Melamine with π-π conjugation ring was used as a core to synthesize a multi-armed crosslinker PEGDA700-Melamine (PEG-MEL), which could crosslink with thiol-modified hyaluronic acid (HA-SH) to form an injectable hydrogel rapidly. By incorporating graphene oxide (GO), the injectable PEG-MEL/HA-SH/GO hydrogel exhibited a soft (G' = 25 Pa) and anti-fatigue mechanical property and conductive property ($G = 2.84 \times 10^{-4}$ S/cm). The hydrogel encapsulating adipose tissue-derived stromal cells (ADSCs) was injected into MI area of rats. The significant increase in α-Smooth Muscle Actin (α-SMA) and Connexin 43 (Cx43) expression confirmed that the gel efficiently promoted the transmission of mechanical and electrical signals. Meanwhile, a significant improvement of heart functions, such as distinct increase of ejection fraction (EF), smaller infarction size, less fibrosis area, and higher vessel density, was achieved.

1. Introduction

Currently, myocardial infarction (MI) still remains one of the leading causes of morbidity and mortality worldwide, posing a treat therapeutic challenge [1]. The native myocardium is an electroactive tissue capable of transferring electrical signal [2] and stimulating a heart to beat [3], while MI leads to scar formation of myocardium [4]. The loss of mechanical and electrical signals transmission results in impaired cardiac function and eventually heart failure [5]. Numerous hydrogels designed with a broad range of mechanical properties have been reportedly employed to rebuild myocardial function so far [6–9]. The work by Burdick et al. on the influence of injectable hydrogel on infarct expansion and post-infarction remodeling suggested that the high-modulus hydrogel (43 kPa) showed better functional outcomes (infarction size) than the low-modulus hydrogel (8 kPa) [6]. A decellularized myocardial matrix hydrogel with relatively higher modulus was shown to achieve the best functional outcomes in treating MI [7]. Most of these studies seemed to suggest that a stiffer hydrogel was more appropriated for rebuilding cardiac function. However, if we look deep into the nature of myocardium, a tissue that alternately contracts and relaxes with electrical impulses [2,10], an injectable soft, fatigue-resistant and conductive hydrogel system may be in favor of cyclic mechanical and electrical signals transmission to facilitate cardiac repair after MI due to its unique ability to coordinate the contraction of the myocardium tissue and transmit mechano-electric signals.

Over the past decade, conductive polymers have been developed as cardiac tissue engineering scaffolds, such as polypyrrole (PPy), polyaniline (PANI), and poly(thiophene-3-acetic acid) (PTAA) [11–13]. However, these rigid conductive polymers are non-water soluble, and thus extremely difficult to fabricate a soft injectable hydrogel. It has been identified that π-π conjugation could achieve long-range electron transport [14–16], and thus was used to increase the conductivity of polymers [13,17–19]. It is well accepted

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that π-π conjugation in conventional PPy and PANI conductive polymers tends to result in rigid products. To fabricate a soft but mechanically stable conductive hydrogel, we linked the flexible multi-armed polyethylene glycol to the rigid core of π-π conjugation via Michael addition reaction. The emanating flexible PEG chains from the core were anticipated to offset the rigidity of π-π conjugation. To the best of our knowledge, there has been no report utilizing π-π conjugation to fabricate an injectable and conductive polymer hydrogel directly for rebuilding cardiac function thus far.

In this proof of concept study, melamine with π-π conjugation ring served as a core to synthesize a multi-armed crosslinker PEGDA700-Melamine (PEG-MEL), which was then used to crosslink thiol-modified hyaluronic acid (HA-SH) by "thiol-ene" click reaction. Graphene oxide (GO) was employed to improve the stability, anti-fatigue, and conductive properties of the hydrogels. After encapsulating adipose derived stem cells (ADSCs), the hydrogel was injected to the MI area to rebuild the impaired myocardium (Fig. 1).

2. Materials and methods

2.1. Materials

Thiol-modified hyaluronic acid (HA-SH) was purchased from ESI BIO, USA. Poly(ethylene glycol) diacrylate (Mw = 700) (PEGDA700), melamine (MEL), tri(aminoethyl)amine (TAA), diethyl ether, graphene oxide (GO, synthesized by using a modified Hummers method, <20 μm), triethylamine, penicillin-streptomycin, anti-α-SMA antibody, and anti-Cx43 antibody were supplied by Sigma. 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl tetrazolium bromide (MTT, 98%) was supplied by Fluka (Buchs, Switzerland). Dulbecco's Modified Eagle Medium (DMEM) and fetal calf serum were supplied by Giboco. Calcein-AM and Propidium Iodide (PI) were provided by Invitrogen. Rat adipose tissue-derived stromal cells (ADSCs) were obtained from the Department of Biomedical Engineering of Jinan University (Guangzhou, China). Ultrapure water (Millipore, 18.25 Ω cm) was used to prepare solutions. All the other reagents were of analytical grade and used without further purification.

2.2. Synthesis of multi-armed crosslinker

PEG-MEL was synthesized as a multi-armed crosslinker. Briefly, PEGDA700 (30 wt% in DMSO) and MEL (10 wt% in DMSO) solutions (2:1 M ratio of double bond to active hydrogen) were mixed into a glass bottle which was placed in an oil bath under stirring (600 rmp) for 12 h at 100 °C. Triethylamine was added to the reaction as a catalyst. Then, the reaction mixture was extracted with iced diethyl ether to remove the unreacted monomer. Further purification was performed by repeated precipitation and the final product was dried in vacuum. PEG-TAA was prepared with the same method.

2.3. Characterization of PEG-MEL

Gel Permeation Chromatography (GPC, TDA305, UK), Nuclear Magnetic Resonance Spectrometer (NMR, 500 MHz, Varian INOVA) and attenuated total reflection Fourier transform infrared (FTIR) spectrometry (PerkinElmer spectrum 100, USA.) were used to confirm the chemical structure of PEG-MEL.

The particle size distribution of PEG-MEL (5 wt%) was analyzed by dynamic light scattering (DLS) using a Nicomp 380 particle size

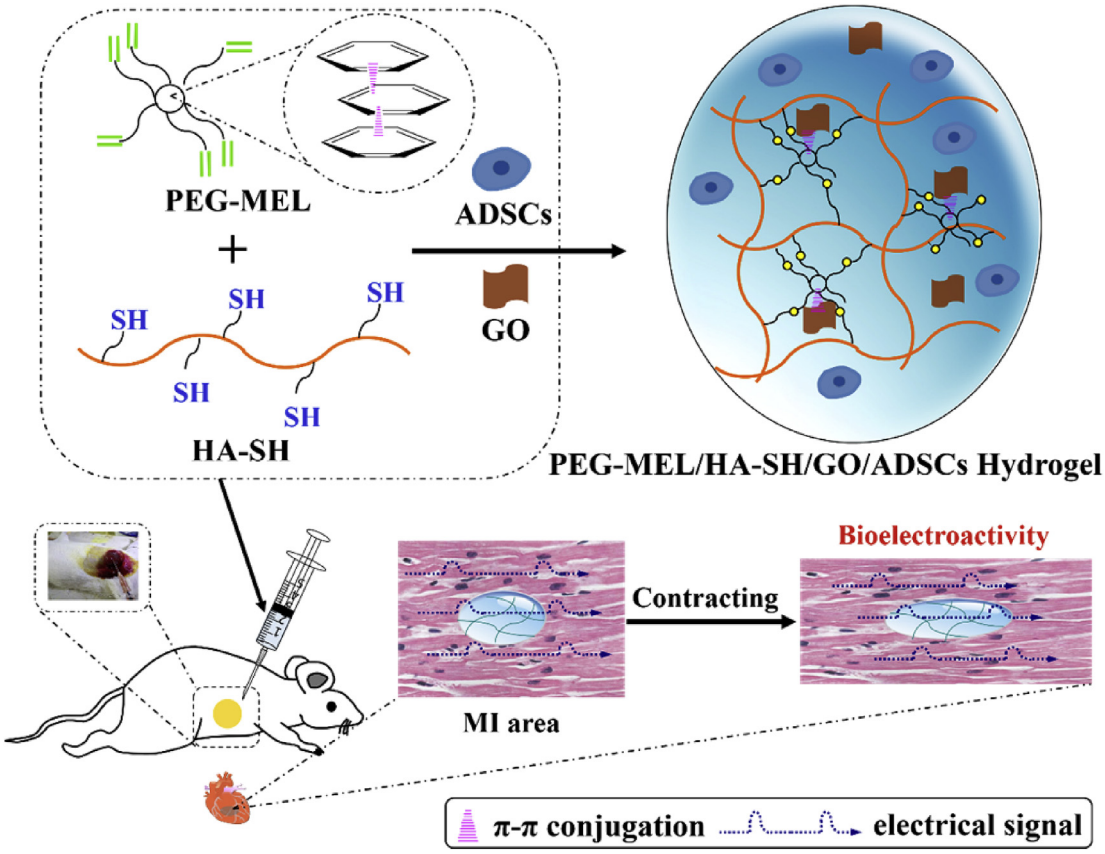

Fig. 1. Schematic sketch illustrating the application of this soft and conductive PEG-MEL/HA-SH/GO hydrogel system encapsulating ADSCs for cardiac repair by injection into the MI area of SD rats, with the objective of enhancing the transmission of mechanical and electrical signals to rebuild cardiac function.