Biodegradable, Imagable, Injectable, and Thermosensitive Hydrogels for Cardiac Cell Therapy

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Statement of Purpose: Stem cell therapy is a promising approach for cardiac tissue regeneration. Injection based minimally invasive approach has been used to deliver stem cells into infarcted hearts. To improve therapeutic efficacy, stem cells are encapsulated into injectable hydrogels. The hydrogels can increase cell retention in the tissue due to its higher viscosity than saline. They also improve stem cell survival by promoting cell attachment and growth, and blocking proinflmmatory factors such as TNFα and IL-1β, and reactive oxygen species. During hydrogel degradation, stem cells are gradually released from the hydrogel and integrated with the host tissue. To non-invasively monitor stem cell release process in order to determine the time period of complete stem cell release, it is attractive to use degradable and imagable hydrogels as cell carriers. Hypericin (HYP), which was found from plants of genus, was regarded as a natural photosensitizing and generally reported as a non-porphyrin photodynamic drug [1]. In this study, we have developed a family of imagable and non-toxic hydrogels by conjugating HYP molecules with injectable and thermosensitive hydrogels via poly(1-Vinyl-2-pyrrolidinone). The biocompatibility and efficacy of the developed imagable hydrogels were examined in vitro and in vivo. The results demonstrated that these hydrogels are attractive cell carriers.

Methods: Materials used in the study include N-isopropylacrylamide (NIPAAm), acrylate polylactide (APLA), 2-Hydroxyethyl methacrylate (HEMA) and 1-vinyl-2-pyrrolidinone (VP) and benzoyl peroxide (BPO). The hydrogel was synthesized by copolymerization of NIPAAm, HEMA, APLA and VP (molar ratio 77/10/8/5 and 77/10/8/10) using free radical polymerization. ^{1}H-NMR spectrum was used to confirm the structure of synthesized hydrogel polymer. Hypericin (HYP) and the hydrogels were mixed together to form the complexes with the ratio of 1:8 in an ice bath. Hydrogel complexes properties, such as lower critical solution temperature (LCST), gelation time, mechanical properties, water content and degradation rate, were characterized following our previously established methods [2]. Upon the formation of the hydrogel complexes, the unconjugated HYP molecules were removed by dialysis in DI water for 3 days and the water was changed every 10 hours. The fluorescent spectrum of the HYP/hydrogel complexes was determined by UV-Vis instrument scanned from wavelength 500 – 700 nm. To examine biocompatibility, rat mesenchymal stem cells (RMSCs) were encapsulated in the hydrogel and cultured with growth medium for 14 days. dsDNA content (for live cells) was measured by a Quant-iT™ PicoGreen® dsDNA Assay Kit. Gene expression was characterized by real-time RT-PCR. For in vivo study, the hydrogels were injected into nude mice to test fluorescent intensity at days 0, 3, 7 and 14. To test in vivo biocompatibility, the hydrogels were injected into regular mice. After four weeks, the samples were collected, fixed and sectioned for Hematoxylin and Eosin (H&E) staining and immunofluorescence (IF) staining using rat anti-mouse F4/80 antibody.

Results: The ^{1}H-NMR spectrum confirmed the structure of the synthesized hydrogel. The monomer feed ratio of NIPAAm/HEMA/APLA/VP was consistent with molar ratio. UV-Vis spectrum confirmed the conjugation of HYP molecules with hydrogels. The hydrogel solutions were flowable at 4°C and can be readily injected through 26G needles. The hydrogel solutions exhibited sol-gel temperature around room temperature. At 37°C, the hydrogels were highly flexible with modulus from 16 to 39 kPa. dsDNA content results showed that cell number in the hydrogels increased during the 14-day culture period. The HYP conjugated hydrogels did not induce substantial inflammation response. IVIS Spectra demonstrated that the fluorescent signals remained detectable with the high epi-fluorescence after 14 days.

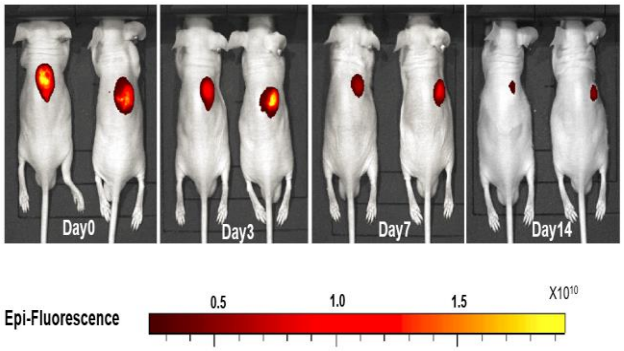

Figure.1 Nude mice imaging with injection of HYP/hydrogel complexes (left mouse: HYP/VP5%, right mouse: HYP/VP10%).

Conclusions: HYP-based imagable, injectable and thermosensitive hydrogels were developed for cardiac stem cell therapy. The hydrogels supported RMSC growth. In vivo studies demonstrated that fluorescent signal within the hydrogels can be detected for at least 14 days. The hydrogels showed good biocompatibility.

References:

[1] Kubin A. Current pharma design. 2005; 11:233-53.
[2] Zhenqing Li. Biomaterials. 2012; 33: 5914-5923.