An injectable hydrogel with oxygen release to augment cardiosphere-derived cell survival under ischemic conditions

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Statement of Purpose: Myocardial infarction (MI) is a major cardiovascular disease affecting more than 8 million people in the US alone. MI causes massive cell death and partial loss of heart function. Stem cell therapy has the potential to improve heart function. However, cells delivered to the heart experience limited engraftment, due to ischemic condition (low oxygen and low nutrient) in the infarct heart tissue [1]. This prevents widespread clinical application of stem cell therapy. Exploring approaches that augment cell survival under ischemic condition is critically necessary to improve the efficacy of current cardiac stem cell therapy. Our previous study demonstrated that a microsphere-based oxygen release system can promote cell survival under ischemic condition. In this study, a new biodegradable and thermal sensitive hydrogel was developed to serve as a carrier for microspheres. The efficacy of this new oxygen releasing system in improving cell survival under ischemic conditions was examined in vitro and in vivo.

Methods: Materials used in the study include 2-hydroxyethyl methacrylate (HEMA), N-isopropylacrylamide (NIPAAm). Poly(lactide-coglycolic acid) (PLGA, LA/GA ratio 50/50, inherent viscosity 0.55~0.75), hydrogen peroxide, catalase (2000~5000 units/mg), and acrylate polylactide (APLA). The hydrogel was synthesized by copolymerization of NIPAAm, APLA and HEMA (molar ratio 86/4/10) using free radical polymerization. ^{1}H-NMR spectrum was used to confirm the structure of synthesized hydrogel polymer. Hydrogel properties, such as gelation time, thermal transition temperature, water content, tensile properties, and degradation rate, were characterized following our previously established methods [2]. The core-shell oxygen releasing microspheres were fabricated by co-axial electrospraying with PLGA as shell and PVP/H_2O_2 as core. The microspheres were characterized by SEM and confocal microscope. Oxygen release kinetics was conducted for 2 weeks by detecting intensity of oxygen sensitive fluorescent [2]. To fabricate oxygen releasing system, cardiosphere-derived cells and oxygen releasing microspheres were encapsulated into a 20 wt% hydrogel solution containing 1 mg/ml catalase. The system was then solidified in a 37^{o}C water bath, and cultured in a culture medium without FBS at 1% oxygen (mimicking ischemic condition) for 7 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. Oxygen concentration in cells was determined by electron paramagnetic resonance (EPR). For in vivo study, the system was injected into infarct rat hearts after acute MI. CDCs were labeled with live cell tracker CMFDA before injection. The rat hearts were harvested after 2 weeks of implantation, and fixed and sectioned. Live cell images were taken with confocal microscope and density of surviving cells was calculated based on the images.

Results: The ^{1}H-NMR spectrum confirmed structure of the synthesized hydrogel. Molar ratio of NIPAAm/APLA/HEMA in the polymer was consistent with the monomer feed ratio. The hydrogel solution was injectable through a 26-gauge needle. The core-shell oxygen release microspheres with different H_2O_2/VP ratios were fabricated. Sustained oxygen release was observed during a 2-week release period (Figure 1). After 4 days of release, the microspheres with H_2O_2/VP ratio of 4.5/1 reached around 20% oxygen level, and remained at this level for over 10 days. dsDNA content results showed that cell number in the hydrogel without oxygen release dramatically decreased after 7 days of culture, while it significantly increased in the hydrogel with oxygen release. The enhanced cell survival is a result of oxygen content increase in the cells, as the oxygen level in CDCs with oxygen release (23.8 mmHg) was much higher than that without oxygen release (2.6 mmHg). In vivo study demonstrated that cell engraftment was significantly improved using oxygen release system.

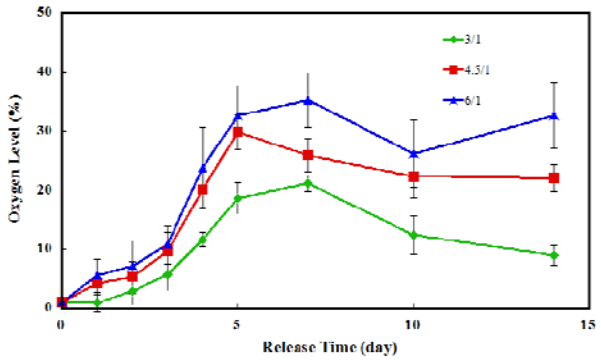

Figure.1 Oxygen release kinetics with three H_2O_2/VP ratios for 14 days.

Conclusions: An injectable hydrogel based oxygen release system was developed to improve cell survival under ischemic conditions. The system was readily injectable and capable of sustainedly releasing oxygen for 2 weeks. Cell survival in the developed system was significantly enhanced in vitro and in vivo under ischemic conditions.

References:

[1] Friehs I. Ann Thorac Surg. 2003; 75: S678-84.
[2] Zhenqing Li. Biomaterials. 2012; 33: 5914-5923.