Dual Delivery of TGFβ Receptor 2 Binding Peptide and Oxygen to Control Cardiac Fibrosis

Zhaobo Fan, Minghuan Fu, and Jianjun Guan.
Material Science and Engineering, Ohio State University.

Statement of Purpose: In the US, there are more than 8 million people suffering from myocardial infarction (MI). Following MI, cardiac fibroblast, a major cell type in the myocardium, differentiates into myofibroblast. The myofibroblasts initially secrete extracellular matrix (ECM) in order to strengthen the injured heart tissue. However, the excessive ECM deposition leads to cardiac fibrosis, which progressively decreases cardiac function and evolves the infarcted hearts into heart failure [2]. TGFβ signaling pathway has been demonstrated to be a major driving force for cardiac fibroblast differentiation. Therefore, blocking TGFβ signaling pathway is a potential approach to control cardiac fibrosis. In this report, we investigated whether blocking the initial step of TGFβ signaling pathway - TGFβ binding to TGFβ receptor II, can efficiently inhibit cardiac fibroblast differentiation. Decrease of TGFβ expression after MI has been considered as an effective approach to attenuate cardiac fibrosis. After MI, hypoxic condition in injured heart tissue activates hypoxia-inducible factor 1(HIF-1) and induces TGF-β expression [1]. In this study, we hypothesized that relief of hypoxia by controlled oxygen release will decrease TGFβ expression, which in concert with blocking TGFβ receptor II will efficiently inhibit cardiac fibrosis.

Methods: A thermosensitive and injectable hydrogel was used as a drug delivery vehicle. The hydrogel was synthesized from N-isopropylacrylamide, acrylate-polylactide and 2-hydroxyethyl methacrylate (the hydrogel was abbreviated as APLA). Hydrogel thermal transition temperature was measured using DSC. The injectability of the hydrogel solution was tested by injecting a 4% hydrogel solution through a 26-gauge needle at 4ºC. Peptide ECGLLPVGRPDRNWRWLC (abbreviated as TBP) was used as a TGFβ receptor II inhibitor and its release kinetics from the hydrogel was determined at 37ºC using PBS as release medium. Oxygen release microspheres (ORMs) were fabricated by electrospraying with PVP/H_2O_2 as core and PLGA as shell. The oxygen release kinetics was measured in hypoxic condition at 37ºC as previously reported [3]. To investigate the effect of TBP and oxygen release on cardiac fibroblast differentiation, a 3D in vitro model was established by seeding the rat cardiac fibroblasts (RCF) in collagen gel, and injecting hydrogel encapsulated with TBP and ORMs. To test the efficacy in vivo, a MI model was created by LAD ligation of Sprague-Dawley rats, and the hydrogel encapsulated with TBP or TBP+ORM was then injected into the infarcted region. The hearts were harvested 4 weeks after injection.

Results: APLA hydrogel demonstrated a lower critical solution temperature of 26.5±0.2ºC. At 4ºC, the hydrogel solution (4%, w/v) can be readily injected through a 26-gauge needle. The encapsulated TBP can be gradually released from the hydrogel during a 28-day study period. The release kinetics is dependent on TBP loading. The fabricated ORM was able to release molecular oxygen under hypoxia for at least 28 days, with a peak oxygen level of 24.7%. In vitro, the release of TBP or TBP/oxygen significantly decreased cardiac fibroblast αSMA expression. In vivo, the myofibroblast density was significantly decreased after injection of hydrogel with TBP. It was further decreased after injection of hydrogel with both TBP and ORMs. Interestingly, the injection of TBP and TBP/ORMs significantly increased vessel density in infarcted area. Four weeks after injection, the ejection fraction and fractional shortening were significantly improved.

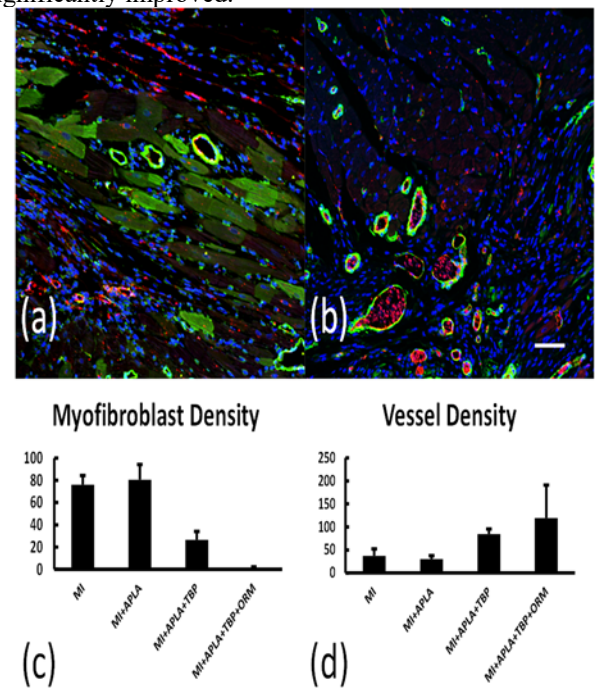

Figure 1. *In vivo* study after injection of TBP and ORM. (a) injection of TBP, (b) injection of TBP/ORM. (c) and (d), density of myofibroblast and blood vessel, respectively. (Red: VWF, Green: αSMA, Blue: Hoescht) (Scale bar = 60μm).

Conclusions: Hydrogel based TBP and TBP/ORM release systems were created to control cardiac fibrosis. TBP and oxygen were able to release for 28 days. In vitro and in vivo results demonstrated that the release systems significantly decreased cardiac fibrosis with TBP/ORM system exhibiting greater efficacy.

References:

[1] Ammirante M. Proc Natl Acad Sci. 2014;111:14776-81.
[2] Pfeffer JM. Am J Physiol. 1991;260:H1406-H14.
[3] Li Z. Biomaterials. 2012;33:5914-23.