Thermosensitive hydrogels induce cardiosphere derived cells differentiation into cardiac lineage

Zhenqing Li[1], Xiaolei Guo[1], Satoshi Matsushita[2], James S. Forrester[2] and Jianjun Guan[1,*]
[1] Department of Materials Science and Engineering, Ohio State University, Columbus, Ohio 43210, [2] Division of Cardiology, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, California, USA.

Statement of Purpose: Cell therapy for myocardial infarction (MI) has attracted extensive attention in recent years. Cardiosphere derived cell (CDC) is an emerging cell type that has great potential to be used for cardiac regeneration (1). CDCs are isolated from endocardium biopsies by a fast and convenient protocol. They have high proliferation rate, making it possible to use autologous CDCs for cell therapy. More importantly, CDCs are capable of differentiating into cardiomyocytes and endothelial cells in vivo (1,2). To deliver CDCs into heart, a suitable carrier is needed to retain the cells in the myocardium and support cell survival. Ideally, microenvironment of the carrier should actively regulate the fate of delivered CDCs (3). For example, the microenvironment should direct CDCs differentiation into cardiomyocytes. To address these, we have developed a family of hydrogels with the following properties, 1) injectable and thermosensitive, allowing the hydrogels to be readily delivered into the heart; 2) form highly stretchable hydrogels with stiffness matching that of the myocardium; 3) biodegradable and the biodegradation products are nontoxic; and 4) hydrogels differentiate CDCs into cardiomyocyte lineage in vitro.

Methods: Hydrogel polymers were synthesized by ATRP using bromided polycaprolactone as an initiator, and CuCl and Me_6TREN as catalysts. Monomers include N-isopropylacrylamide (NIPAM), 2-hydroxyethyl methacrylate (HEMA) and dimethyl-γ-butyrolactone acrylate (DBA). A family of hydrogel polymers were synthesized by varying monomer ratio and molecular weight. Polymer structure and composition were verified by ^{1}H-NMR. Polymer molecular weight and polydispersity were quantified by GPC. Lower critical solution temperatures (LCST) were determined by DSC for 20% (w/v%) polymer solutions. After solidification at 37°C for 24 h, the hydrogels were subjected to tensile tests. The tests were conducted in a 37°C water bath using an Instron load frame (cross-head speed is 50 mm/min). Hydrogel degradation was conducted in PBS at 37°C for an 8-week period. CDCs were isolated from mouse endocardium, proliferated to passage thirteen in vitro and then encapsulated into the hydrogels. The formed hydrogel/CDC constructs were cultured for two weeks. Cell growth was quantified by dsDNA PicoGreen assay after 1, 7 and 14 days of culture. Total RNA was isolated and cardiac specific genes cardiac troponin T (cTnT), myosin heavy chain 6, cardiac (MYH6) were assessed by real-time RT-PCR. For immunohistochemistry, gel pellets were cryo-fixed and sectioned into 10μm thick sections, followed by staining using mouse monoclonal anti-cardiac troponin I (cTnI) (Abcam) and Alexa488 conjugated goat anti-mouse IgG. Cells cultured on culture plate were used as control.

Results: All hydrogels had compositions close to their feed ratios. Hydrogel possessed LCSTs from 18-22°C, depending on the DBA content. An increase in DBA content decreased LCST. In contrast, molecular weight did not affect LCSTs significantly. Hydrogels were highly flexible with breaking strains >300% and moduli in the range of 17-63 KPa. These moduli match those of the myocardium. Hydrogels showed a gradual degradation profile with weight losses ranging from 10 to 34% over 8 weeks. The degradation products were nontoxic as examined by culturing fibroblasts supplemented with degradation products containing medium. CDCs were found to proliferate within the hydrogels during a 2-week culture period. Real time RT-PCR results demonstrated that cardiac transcription factors cTnT and MYH6 were greatly up-regulated in the 3-D hydrogel compared to 2-D plate culture (Figure 1). The gene expression was related to the hydrogel modulus. A higher modulus (31 KPa) led to higher cTnT and MYH6 expressions. Immunohistochemistry showed a continuous expression of cTnI in all hydrogels.

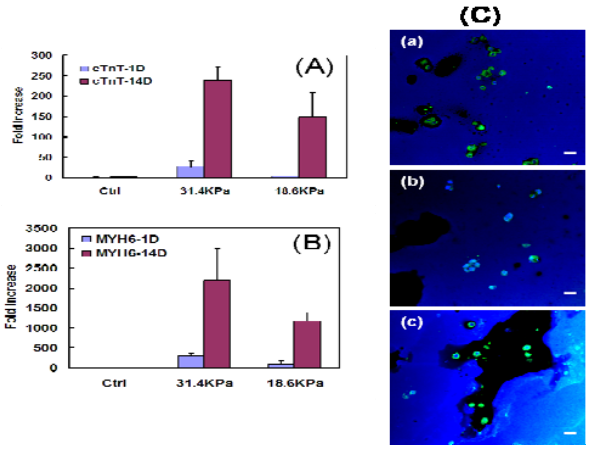

Figure 1. CDC cultured in 3-D hydrogel up-regulated cTnI (A) and MYH6 (B) gene expression at days 1 and 14 compared to those cultured on 2-D plate ("Ctrl" group); (C) IHC staining of cTnI (green) and nucleus (blue) of CDCs at day 1 (a), day 7 (b) and day 14 (c);

Conclusions: A family of thermosensitive, injectable and biocompatible hydrogels were synthesized. The hydrogels supported CDCs growth and induced them differentiation into cardiomyocyte lineage. These results demonstrated the potential of developed hydrogels to be used for cardiac cell therapy.

References:

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