Gelatin-based Hydrogels as Potential Cellular Delivery Systems for Cardiac Tissue Engineering

Gulden Camci-Unal[1,2], Neslihan Alemdar[1,2] and Ali Khademhosseini[1,2]

[1]Division of Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, 02139, USA; [2]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA.

Statement of Purpose: Cardiac failure is one of the leading cause of death throughout the world. A major reason for failure of current approaches in regenerative cardiac tissue engineering is the inability to repair the myocardial tissue upon injuries. Therefore, there is a high demand to develop effective therapies to treat myocardial infarction (MI). It has been reported that transplantation of cell suspensions to the MI area may potentially be used to regenerate the cardiac tissue.[1] However, due to the physiological stress conditions and lack of a 3D biomimetic environment, more than 95% of the transplanted single cells die few hours after the injection. In this context, it may be of benefit to use flexible hydrogels to entrap cells inside and maintain them in place to increase retention rates, protect them from direct exposure to harsh physiological conditions, and provide them flexible 3D surroundings. Herein, we encapsulated cardiac side population cells (CSPs) within photocrosslinkable gelatin-based hydrogels at different photoinitiator and polymer concentration to optimize their cellular behavior *in vitro*. We studied the viability, metabolic activity, and proliferation of CSPs under physiological stress conditions, such as oxidative stress and hypoxia as a function of H_2O_2 concentration. GelMA hydrogels with cell adhesive functional groups provided 3D ECM-mimetic microenvironments for CSPs with potential *in vivo* applications.

Methods: We synthesized photocrosslinkable methacrylated gelatin (GelMA) using a previously reported protocol.[2] CSPs were cultured in alpha-modified Eagle Medium (alpha-MEM) media containing 20% FBS, 1% PenStrep and 20 mg/L glutamine in a 37 °C incubator supplemented with 5% CO_2. To prepare the polymer precursor solution, 5 or 10 % (w/v) GelMA was dissolved in phosphate buffered saline (PBS), which contained 0.1 or 0.5% (w/v) photoinitiator. Viability was assessed by CalceinAM/Ethidium homodimer staining to visualize live and dead cells, respectively. Metabolic activity was determined by an MTS assay. Proliferation of CSPs was determined by a dsDNA content kit. Oxidative stress was induced by the addition of different doses of H_2O_2 (0-800 uM) in the CSP culture media. To induce hypoxia, samples were cultured in a hypoxia chamber at 1% oxygen level upto 3 days. CSPs were also stained for visualization of their cytoskeleton and nuclei. Statistical analyses were carried using GraphPad Prism by means of ANOVA analyses with Bonferroni post-hoc tests.

Results: In this study, we generated biomimetic constructs by encapsulating CSPs within GelMA in 3D as a hydrogel-based cell delivery system for MI tissue. Although previously single cell suspensions have been delivered to damaged cardiac tissues, an ECM-mimic flexible environment is essential for cells to maintain their phenotype and ability to function properly. Furthermore, degradation products from hydrogels may improve regeneration potential of the myocardium. Due to these reasons, we proposed that GelMA-based hydrogels may provide a bimimetic microenviroenment and potentially be used as a cellular-delivery system. We report that decreasing photoinitiator concentration from 0.5 to 0.1% (w/v) made a significant difference in CSP viability and spreading behavior within hydrogel constructs. Addition of H_2O_2 resulted in significantly lower metabolic activity. Exposure to hypoxia up to three days did not significantly damage the CSPs within GelMA hydrogels in 3D. To demonstrate *in vivo* applicability of CSP encapsulated hyrogels, different sizes and shapes were microfabricated by photolithography (Figure 1). Delivery of CSPs in biodegradable hydrogels to the MI tissue may increase the survival rate of CSPs *in vivo*, and therefore help regenerating the cardiac tissue and restoring the muscle function.

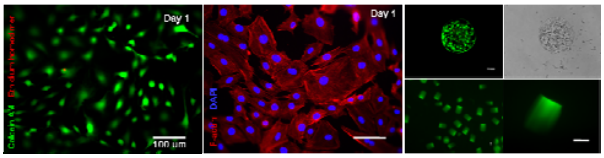

Figure 1. CSPs seeded in 2D and encapsulated in 3D within GelMA hydrogels. In addition, CSP-laden hydrogels were photopatterned to generate cellular delivery modules.

Conclusions: We reported the generation of microfabricated CSP-laden hydrogel constructs using a simple photocrosslinking approach. We encapsulated CSPs in photocrosslinkable gelatin-based hydrogels at different photoinitiator and polymer concentrations to optimize their viability, metabolic activity and spreading behavior *in vitro*. We characterized the cellular behavior of CSPs under physiological stress conditions, such as oxidative stress at different doses of H_2O_2 or hypoxia. GelMA hydrogels with cell adhesive functional groups resulted in 3D flexible ECM mimetic microenvironments for CSPs. The results of this study may be of benefit for clinical cases where transplantation of CSPs to the MI area is required for myocardial regeneration. The future work will include electrical stimulation of CSPs within GelMA hydrogels to promote their differentiation and *in vivo* trials in mouse models.

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