In Situ Crosslinkable Gelatin Hydrogel for *Ex Vivo* Organ Culture of Cardiac Tissue

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Introduction: Synthetic hydrogels have been demonstrated to be an adequate three-dimensional (3D) microenvironment that is structurally and biomechanically similar to native extracellular matrix (ECM) topology and provides a rich ligand landscape to influence cellular behaviors. Especially, *in situ* cross-linkable hydrogels have attracted a great deal of attention as 3D cell/tissue culture templates or scaffolds for tissue engineering due to easy encapsulation of cells or tissues under mild condition as well as easy application based on minimally invasive technique. In our previous study, we developed *in situ* crosslinkable gelatin hydrogels with excellent bioactivity and tunable physicochemical properties.

In this study, *ex vivo* myocardium culture system using the gelatin-based hydrogels has been established and relationship between mechanical property of the matrix and cellular behaviors has been investigated.

Methods: Gelatin-poly(ethylene glycol)-tyramine (GPT) conjugate was synthesized by coupling amine reactive PEG−TA with gelatin.[1] The chemical structure of the GPT graft copolymer was characterized using ^{1}H NMR and UV spectroscopy. Hydrogels were formed *via* enzyme-mediated crosslinking reaction using horseradish peroxidase (HRP) in the present of hydrogen peroxide (H_2O_2). For the organ culture of cardiac tissue, myocardium of rats was encapsulated within hydrogel matrices with different mechanical strength (figure 1). Cells were isolated by treatment of collagenase solution and the isolated and subcultured outgrown cells (OCs) were characterized the immunophenotype using immunofluorescence staining. In addition, *in vitro* differentiation potential of the OCs into adipogenic and cardiomyogenic lineages was investigated.

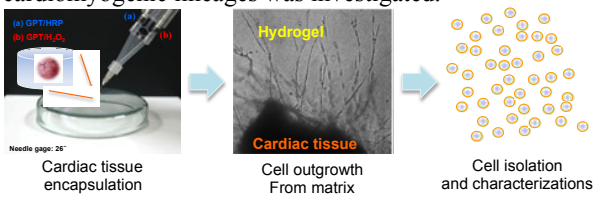

Figure 1. Schematic representation of *ex vivo* cardiac tissue culture system using *in situ* crosslinkable gelatin-based hydrogels.

Results: Gelatin-based hydrogels were successfully prepared and matrix stiffness of hydrogels was controlled by varying the H_2O_2 concentration (1800~8100 Pa). To confirm the influence on cell outgrowth from cardiac tissue depending on mechanical strength, rat heart tissue was fragmented and combined with various gelatin hydrogels. As shown in figure 2, despite the OCs were spindle-like appearance in all type of hydrogels, most of cells were not fully elongated except 2900 Pa, demonstrating mechanical properties influenced OCs migration and cell outgrowth was enhanced when encapsulated within softer hydrogels.

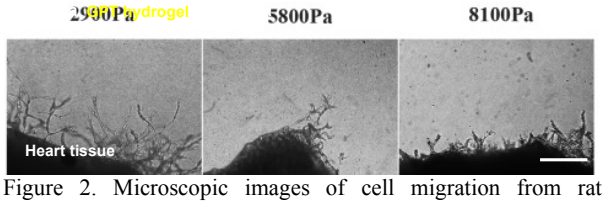

Figure 2. Microscopic images of cell migration from rat myocardium: the softer hydrogels provides substratum as a biocompatible extracellular microenvironment for cell migration (scale bar = 200 μm).

To investigate the phenotypical characteristics of OCs, we adapted cardiac specific genes and stem cell markers (figure 3). OCs consistently expressed GATA-1 and Nkx2.5 as well as nestin. On the order hand, late cardiac genes including troponin I (TNI), and α-sarcomeric actinin (α-SA) and endothelial marker (CD34) were not expressed in subcultured OCs. These results demonstrated that the isolated cells maintained their phenotype as cardiac stem cells (CSCs).

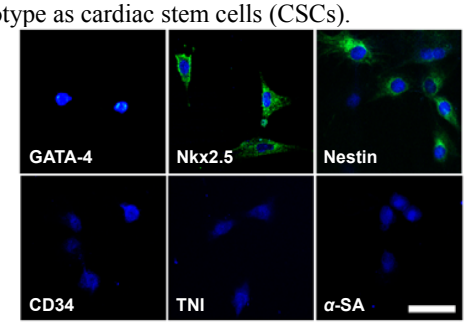

Figure 3. Immunophenotypical characterization of subcultured OCs. Representative images of immunofluorescence staining of OCs isolated from GPT hydrogel (Scale bar = 50 μm).

Conclusions: Novel biocompatible *in situ* crosslinkable gelatin hydrogel has been designed and the biological efficacy using *ex vivo* myocardium culture system has been validated. Optimized hydrogel provided the 3D extracellular microenvironment for cell adhesion, migration, and proliferation. In addition, it could be useful to isolate putative CSCs from heart tissue. Our innovative system is able to enrich the stem cell population, which are promising application in tissue regeneration.

Reference:

1. Park KM, Ko KS, Joung YK, Shin H, Park KD. *J. Mater. Chem.* 2011; 21:13180-13187.

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