Cardiomyocyte Response to Varying Composition of ECM Proteins and Stiffness on a Semi-Synthetic Hyaluronan Hydrogel

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Introduction

Each year 8 million Americans have suffered from an acute myocardial infarction (MI) and treatment costs for cardiovascular disease are estimated at over 250 billion USD[1]. Promising cardiac tissue engineering strategies involve the replacement of the injured and/or infarcted myocardium with new cells and /or bioactive materials[2]. Cells for tissue engineering require a particular substrate. This study was designed to optimize the response of cardiomyocytes to engineered ECM proteins of varying composition and stiffness[3].

Materials and Methods

Neonatal ventricular myocytes were cultured on a semi-synthetic hyaluronan (HA) hydrogel (HyStem). HyStem hydrogels were prepared with either fibronectin (Fn), Collagen type I, fibrinogen or varying combinations of mentioned proteins in concentrations ranging from 100 ug/mL to 1 mg/mL. The stiffness of the hydrogel was also varied by using different concentrations of PEGDA (Extralink)[4]. After 48 hours of culture cells were fixed and stained for DAPI and f-actin. Additionally, the culture was analyzed for beating percentage and spreading morphology. The gel stiffness was measured by an AR 500 controlled stress rheometer.

Results

After 48 hours cells cultured on HyStem with Fn and 2% Extralink (Figure 1A) had a higher spread area and beating percentage (Table 1, condition A). Cardiomyoctye cultures on HyStem with collagen and 2% Extralink (Figure 1E) resulted in no quantifiably beating cells (Table 1, condition E). The Young's modulus (stiffness) of the gels was found to range between 161 to 331 Pa.

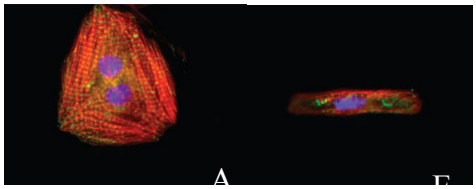

Fig. 1 (A) Cardio myocyte cultured on HyStem with Fn and 2% Extralink. (E) Cardio myocyte cultured on HyStem with collagen and 2% Extralink.

Discussion and Conclusions

Hydrogels containing fibronectin resulted in favorable myocyte attachment and spreading, however, hydrogel with covalently linked Fn had a significantly increased percentage of beating cells. The cell shape, myofibril assembly and organization on hydrogels containing collagen was highly attenuated implicating the importance of ligand type and activity on myocyte remodeling. Surprisingly, myocyte structure and function (contractility) on relatively soft (300 Pa) Fn containing HyStem (HA) gels was highly preserved. These findings provide the rationale for engineering soft (injectable) semi-synthetic bioactive materials for reconstituting the myocardial microenvironment. Future studies include further exploration of cardiomyocyte response to other ECM proteins and stiffnesses as well as eventual *in vivo* studies with HyStem encapsulated cardiomyocytes in infarct myocardium models.

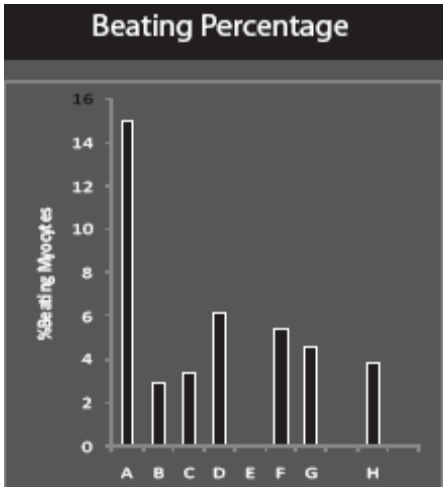

Table. 1. The percentage of beating cardiac myocytes on HyStem hydrogels with stiffness and incorporated ECM proteins.

References List

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Acknowledgments

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