Effect of Substrate Stiffness on Differentiation and Electrophysiology of Cardiomyocytes

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Statement of Purpose: The stiffness of a substrate on which adhesive cells are grown has been shown to affect the differentiation of stem cells[1], the force generation of maturing cardiomyocytes[2] and the beating rate of embryonic cardiomyocytes.[3] The purpose of this study was to investigate the use of polyacrylamide gels of different stiffness to specifically influence the differentiation of stem cells into cardiomyocytes and the electrophysiological properties of maturing cardiomyocytes.

Methods: Polyacrylamide gels coated with covalently bound type I collage were made using previously published techniques.[2] The concentrations of acrylamide monomer and bisacrylamide crosslinker were varied to produce gels with elastic modulus that spanned the physiologic range from 1 kPa to 50 kPa.

For differentiation studies, amniotic fluid-derived stem cells (AFSC), which previous studies have shown to express cardiac markers in cardiogenic conditions,[4] were cultured on gels of varying stiffness for 10 days in cardiogenic conditions (60% DMEM, 28% MCDB-201, 10% FBS, 100 units/ml penicillin, 100 mg/ml streptomycin, 10^{-9}M dexamethasone, 10^{-4}M ascorbate phosphate, 10 mg/L insulin, 5.5 mg/L transferrin, 5 µg/L selenium, 0.5mg/L BSA, 4.7 µg/L linoleic acid)[5] and the expression levels of the mesodermal and myocyte transcription factors Nkx2.5 and GATA-4, and the cardiomyocyte marker sarcomeric α-actinin were measured using Western Blot.

For electrophysiologic studies, neonatal rat ventricular myocytes (NRVM) were isolated using previously published techniques[2] and cultured on polyacrylamide gels of 5 and 25 kPa, which are either softer or stiffer than native myocardium, estimated at 10-15 kPa.[6] The spontaneous beating rate was recorded for two weeks after plating and the action potential voltage was measured by current clamp at one week after plating.

Results: At 10 days of differentiation, AFSC showed lower expression of Nkx2.5 and greatly increased expression of GATA-4 and sarcomeric α-actinin. However, expression of these markers did not correlate to substrate stiffness on which they were grown (Fig. 1).

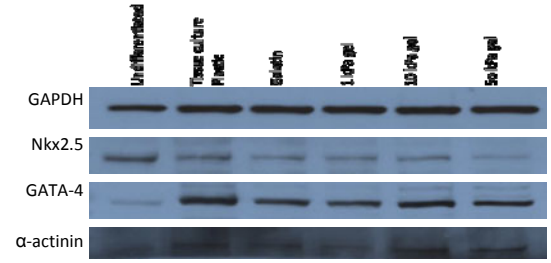

Figure 1: Western blot shows decrease in Nkx2.5 and increase in GATA-4 and α-actinin. Gel stiffness does not strongly affect expression.

For the two weeks after plating, the beating rate between cells grown on different stiffness gels was not significantly different at any time point (Fig. 2).

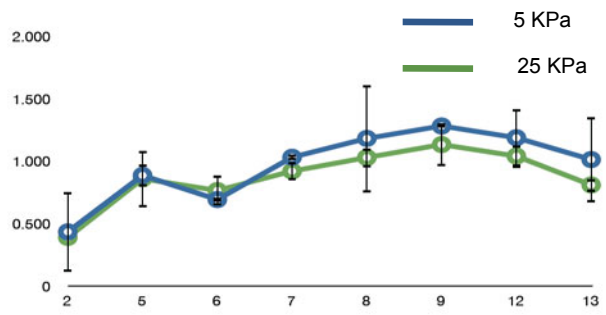

Figure 2: Spontaneous beating rate of NRVM on 5 and 25 kPa gel is not significantly different at any time point.

At one week after plating, NRVM show significant differences in action potential shape (Fig. 3). Specifically, in cells with equivalent resting potentials, matched for ease of comparison, the cells on the softer gels had longer repolarization times and pronounced plateaus.

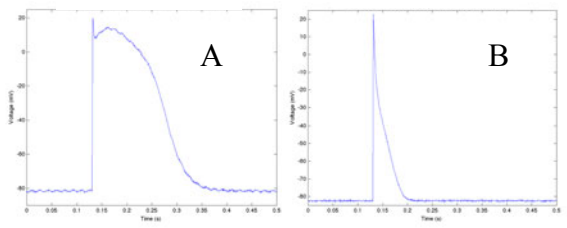

Figure 3: Representative single action potential of NRVM on gels of 5 kPa (A) and 25 kPa (B).

Conclusions: Substrate stiffness in the range of 1 to 50 kPa does not significantly affect the differentiation of AFSC into cardiomyocytes over the first 10 days of differentiation. However, a smaller range of stiffness, from 5 to 25 kPa, does significantly affect the electrophysiology of maturing cardiomyocytes, with cells that have longer action potentials with longer decay times on gels softer than the native myocardium.

References:

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