**Substrate Stiffness Affects Cardiomyocyte Action Potential**

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**Statement of Purpose:** Previous studies have shown that the maturation of neonatal rat ventricular myocytes (NRVM) is affected by the stiffness of the substrate on which they are cultured, with the overall effect that cardiomyocytes develop a functional sarcoplasmic reticulum when cultured on gels with an elastic modulus near that of the native myocardium (around 10 kPa), resulting in higher intracellular calcium levels during contraction and therefore higher contractile force. However, effects of substrate stiffness on the electrophysiology and ion handling of cardiomyocytes are unknown. The purpose of this study was to evaluate the effect of 7 day culture on substrates of varying stiffness on the action potential of NRVM, and to specifically measure calcium currents and expression of calcium ion channels in these cells.

**Methods:** NRVM were isolated from 1 to 2-day-old Sprague-Dawley rats using enzymatic digestion with a purchased kit (Cellutron, Highland Park, NJ). Polyacrylamide gels were manufactured using varying concentrations of acrylamide monomer (3%, 5% and 7%) and bisacrylamide crosslinker (0.15%, 0.25% and 0.35%) to create gels with elastic moduli of approximately 1, 10 and 50 kPa. These gels were coated with covalently-bound type I collagen through a heterobifunctional crosslinker, sulfo-SANPAH. Techniques are similar to those used in Jacot et al. (2008). Cells were cultured on these gels in DMEM media with 5% horse serum and 1% fetal bovine serum for 7 days. For patch clamping experiments, cells were bathed in Tyrode's buffer at 32°C and patch clamped using current clamping techniques, with voltage traces collected using an Axopatch amplifier and pClamp software (Axon Instruments, Union City, CA).

**Results:** Action potentials from cardiomyocytes on 1 kPa and 50 kPa gels tended to have longer plateau phases, like NRVM, while cardiomyocytes cultured on 10 kPa gels had action potentials without plateaus, similar to adult rat cardiomyocytes (**Fig 1A**). The presence of a plateau in action potential tracings is generally seen in cardiomyocytes from neonatal rats but not in cardiomyocytes from adult rats (Kilborn, 1990), suggesting that cells may be maturing toward an adult rat phenotype when on gels of physiologic stiffness.

No significant difference was observed in the beating rates of neonatal rat cardiomyocytes on gels with stiffnesses of 1 to 50 kPa between 2 and 13 days in culture (**Fig 1B**). The mean action potential decay time was significantly lower in cardiomyocytes cultured on 10 kPa gels, approximately the elastic modulus of native cardiac tissue[3], compared to 1 kPa and 50 kPa gels (**Fig 1C**). Average action potential decay time was inversely correlated with contractile force, calcium transient magnitude and internal calcium stores reported in previous studies[1]. Decay slope and rise slope were correlated with contractile force, while activation threshold was highest for the stiffest gels (**Fig 1E-G**).

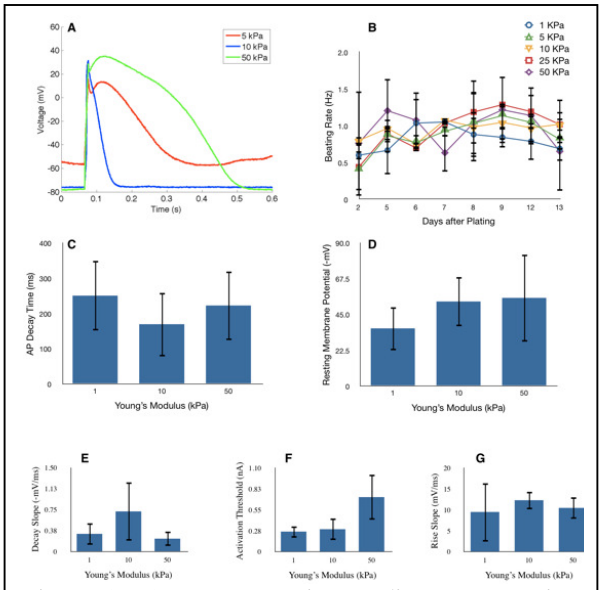


Figure 1. (A) Representative cardiomyocyte action potential recordings from whole cell patch clamping using an Axon instruments amplifier and pCLAMP software. (B) Average cardiomyocyte beating rates at various time points. Beating rates were calculated by counting the number of contraction in a 20-second period. N=20. (C) Action potential decay time, (D) resting membrane potential, (E) mean decay slope, (F) rise slope and (G) activation threshold for cardiomyocytes on various substrate stiffnesses. N=5, 4 and 4, respectively. Error bars represent standard deviation.

**Conclusions:** Action potential decay time is significantly faster when cardiomyocytes are cultured on gels with approximately the stiffness of the native myocardium, compared to both softer and stiffer gels. Action potential shortening on physiological stiffness gels resembles the in vivo shortening of action potential duration during postnatal development in rats.

In future studies, Western blotting will be used to examine the relative expression of ion channels known to change expression levels during postnatal development in rats. These ion channels include L-type calcium, inwardly rectifying potassium, sodium-calcium exchanger, and transient outward potassium channels. Individual ion currents will be recorded using voltage clamp techniques. Optical techniques will be used to measure action potential duration. Data presented here will be compared to action potentials obtained by patch clamping freshly isolated neonatal and adult rat cardiomyocytes.

**References:**

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Kilborn MJ. J Physiol. 1990;430:37-60.