Supplemental Figures

Supplementary Figure 1: (Left) Protein coverage was assessed on various hydrogels coated with CY5 conjugated fibronectin. The average fluorescence intensity was measured using an Olympus confocal microscope and subtracted from the intensity of an uncoated gel. 5-6 images of each gel were taken and normalized (± S.D.); (Right) average spread area of myocytes on 300Pa polyacrylamide gels coated with different fibronectin concentration.

Supplementary Methods

Atomic Force Microscopy (AFM) was performed as described previously (Byfield et al., 2009). Briefly, AFM was performed with a DAFM-2X Bioscope (Vecco, Woodbury, NY) mounted on an Axiovert 100 microscope (Zeiss, Thomwood, NY) using silicon nitride cantilevers (196 μm long, 23 μm wide, 0.6 μm thick) with a 1 μm bead tip for indentation and a spring constant of 0.01 N/m (Novascan, Ames, IA). The deflection measurements taken were fit to the Hertz model for a beaded tip in order to obtain the elastic modulus of the gel. For the first 500 nm of tip deflection, the Hertz model for the deflection of a sphere is:

$$f_{bead} = k * d_{cantilever} = \frac{4}{3}\frac{E}{1-v^2}\sqrt{R}\delta^{\frac{3}{2}}$$

where f_{bead} is the force on the bead, k is the spring constant of the cantilever, $d_{cantilever}$ measured by the AFM, E is the Young's modulus, v is the Poisson's ration, R is the radius of the bead, and δ is the vertical indentation of the material, determined by subtracting $d_{cantilever}$ from the distance traveled by the cantilever during the indentation process. Ten to fifteen measurements were taken at different points on the gels.

Supplementary Figure 2: Stiffness measurements of 7 day old hyaluronan gels coated with collagen I or fibronectin using atomic force microscopy. Stiffness measurements were made at different locations throughout the gel.

Supplementary figure 3: NVRM cultured for 48 hours on 10μm (A) and >200μm thick 1800Pa hyaluronan gels and stained for F-actin (red), alpha-actinin (green) and nucleus (blue). Myocytes on thin HA gels where the stiffness of the underlying plastic substrate dominates, were less efficient in re-assembling myofibrils.