Introduction
Novel cell therapies hold great promise for preventing heart failure during post-infarct healing. However, inconsistent results and failed clinical trials suggest an incomplete understanding of underlying mechanisms, partly due to inadequate model systems bridging traditional 2D cell cultures and live animal experiments. To address the need for realistic in vitro 3D model systems for studying cardiac regeneration and repair, the objective of this study was to establish a novel cryo-injured engineered cardiac tissue (ECT) model of the post-infarct myocardial microenvironment.

Materials and Methods
Spontaneously beating, trabecular muscle shaped ECT (9-mm long, 0.5-mm dia.) were created from primary neonatal rat ventricular cardiocytes (15x10^6 cells/ml) in matrix solution (2 mg/ml type-I collagen; 0.9 mg/ml Matrigel), and cultured in custom PDMS molds with integrated flexible posts (0.5-mm dia.) at each end to anchor the tissue and induce cell alignment during gel compaction and culture. Elastic beam theory was used to calculate contractile force of beating ECT from optically measured end-post deflections. On culture day 9, ECT (n = 3 per group) were locally injured using a frozen (CRYO) or room temperature (SHAM) 1.6-mm diameter steel pin laid across the tissue for 5 sec. ECT were monitored up to 12 days post-injury during 2 Hz field stimulation at 37 °C. Local finite strain was analyzed using triads of titanium dioxide surface markers in the injured middle region (M) and the left (L) and right (R) uninjured regions. Statistical comparisons were made using ANOVA with α = 0.05.

Results
Pre-injury, SHAM and CRYO ECT contracted uniformly (p > 0.6 vs. region) and indistinguishably (p > 0.4 vs. group). Within 10 min of injury, calcein AM stain revealed a well-defined necrotic region in CRYO, but not in SHAM. At day 1 post-injury, cTn-I in culture media was increased by 33% over baseline in CRYO versus 18% increase in SHAM. Regional strain patterns were clearly altered by injury (Fig. 1). The M region of SHAM exhibited systolic shortening of -1.3 ± 0.51 % versus -3.0 ± 0.76 % for L and R (p = 0.05), indicating minor damage due to sham injury. By contrast, the M region in CRYO stretched paradoxically by 1.4 ± 0.66 % (p = 0.007 vs. SHAM M), with substantial fibroblast migration into the injury zone by 12 days post-injury. Such patterns are qualitatively similar to strain measurements in animal models of myocardial infarction (MI).

Discussion and Conclusions
Cryo-injured ECT provides a simplified 3D model system with controlled biocomplexity yielding functional characteristics that mimic key aspects of classical animal models of MI. Such a platform may enhance in vitro testing of strategies for cardiac repair and regeneration.

References

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Disclosures
The authors have nothing to disclose.