Electrical Stimulation Enhances Functional Assembly of Engineered Myocardium

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Functional tissue engineering of myocardium depends on our ability to reproduce *in vitro* developmental cues normally found *in vivo*. We hypothesized that electrical stimulation can enhance the formation of functional myocardium *in vitro*. Collagen sponges (6 x 8 x1.5mm) were seeded with neonatal rat ventricular cells ($5(10^6)$) using Matrigel (n = 91, 9 experiments). Between days 3 and 8 of cultivation, constructs were stimulated with two parallel electrodes to contract synchronously using a cardiac stimulator (supra-threshold biphasic pulses, 2 ms, 60 bpm). Statistics was done using Tukey's test with ANOVA. Contraction amplitude, measured by video imaging of stimulated constructs, increased with time of stimulation and at the end of cultivation (8 days) was 7-fold higher in stimulated than non-stimulated constructs. Maximum capture rate was higher in stimulated (579 (34 bpm) than non-stimulated (416 (23 bpm, p=0.001) and excitation threshold was lower in stimulated (1.98 (0.09 V) than non-stimulated (2.18 (0.12 V) constructs. Stimulated constructs and native neonatal rat heart contained aligned, elongated cells staining for ⍺-actin, cardiac troponin I, α-myosin heavy chain (MHC), β-MHC and connexin-43 in contrast to non-stimulated constructs that had disorganized cells (by immunostain) (1). Amounts of α-MHC, connexin 43, creatin kinase-MM and cardiac troponin I were higher in stimulated than non-stimulated constructs (by Western blot). Expression of all markers was confirmed by RT-PCR. In stimulated samples, sarcomeres were remarkably well developed and comparable to native neonatal rat hearts whereas non-stimulated constructs had scattered sarcomeres (TEM). Volume fractions of sarcomeres and mitochondria and frequencies of intercalated discs

and gap junctions in stimulated constructs (e.g. sarcomeres, 32 (2%) were comparable to native hearts (41 (3%) and higher than non-stimulated constructs (10 (1%) (TEM, morphometry). These studies strongly suggest that electrical stimulation during *in vitro* culture improved the properties of engineered myocardium at the ultrastructural, cellular, and tissue levels and enhanced contractile function presumably due to enhanced excitation-contraction coupling.

1. Carrier R. L., Papadaki M., Rupnick M., Schoen F. J., Bursac N., Langer R., Freed L. E., Vunjak-Novakovic G. (1999), Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization, *Biotechnol Bioeng.* **64**(5):580-589.