# Bioengineering of Cardiac Tissue Constructs with Adult Cardiomyocytes

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**Statement of Purpose:** Cardiac tissue constructs have been bioengineered with fetal cardiomyocytes (CMs), neonatal CMs, and embryonic and induced pluripotent stem cells-derived CMs. However, there are no published reports of 3D heart tissue constructs bioengineered using adult CMs, which would be useful not only for heart developmental studies but for guiding stem cell differentiation research and assessing CM development and maturation. Therefore, in this study we fabricated cardiac tissue constructs using adult rat CMs on a fibrin gel matrix.

**Methods:** Adult rat CMs were isolated with a collagenase type II buffer using a modified Langendorff perfusion system. Cardiac constructs were fabricated with dedifferentiated CMs by culturing freshly isolated CMs for one week (indirect plating) or with freshly isolated CMs (direct plating) on fibrin gel. The gel matrix was made by plating 1 ml media containing 5 U thrombin, adding 0.5 ml saline containing 5 mg fibrinogen in 35-mm culture plates.

**Results:** The current protocol generated (3.1 ± 0.5) × $10^6$ (n=5) fresh CMs from a single heart isolation. Tissue constructs obtained by both types of plating could contract up to 30 days, and ECG signals and contractile twitch forces were detected. The constructs bioengineered by indirect plating produced an ECG R wave amplitude of 15.1 ± 5.2 μV (n=7), a twitch force of 70-110 μN, and a spontaneous contraction rate of about 400 bpm, which is equivalent to an adult rat heart. The constructs bioengineered by direct plating generated an ECG R wave amplitude of 6.6 ± 2.3 μV (n=5), a twitch force of 40-60 μN, and a spontaneous contraction rate of about 220 bpm. The pacing response frequency from indirect plating (4.1 ± 0.8 Hz, n=8) was greater than that from direct plating (2.9 ± 0.7 Hz, n=7). The immunofluorescence staining was positive for α-actinin, a CM maker. Positive staining of vimentin and collagen type I revealed that the cardiac fibroblasts maintained the potential for extracellular matrix production and support. Positive staining for connexin 43 illustrated that the cardiac tissues maintained electromechanical coupling, which sustains electrical propagation between cardiac cells.

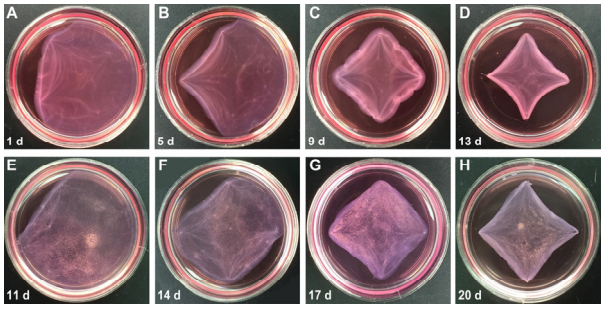


**Figure 1**. Schematic representatives show cardiac tissue formation after indirect plating (**A-D**) and direct plating (**E-H**) of CMs.

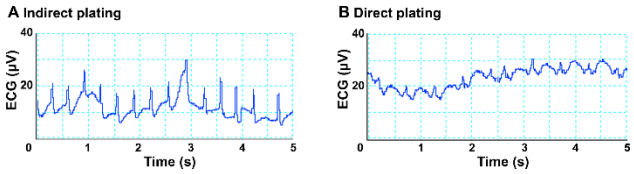


**Figure 2**. Representative ECG signals from indirect plating (**A**) and direct plating (**B**) of CMs.

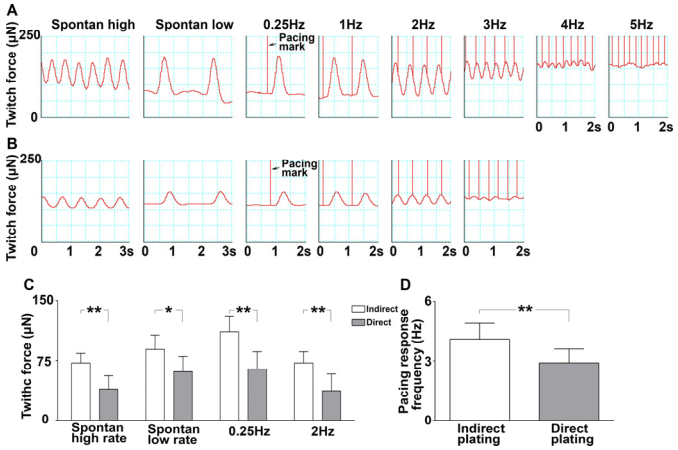


**Figure 3**. Representative plots of spontaneous and electrical paced contractile twitch force from indirect plating (**A**) and direct plating (**B**), and pacing response frequency (**D**). Values are mean ± SD. * $P$<0.05, ** $P$<0.01.

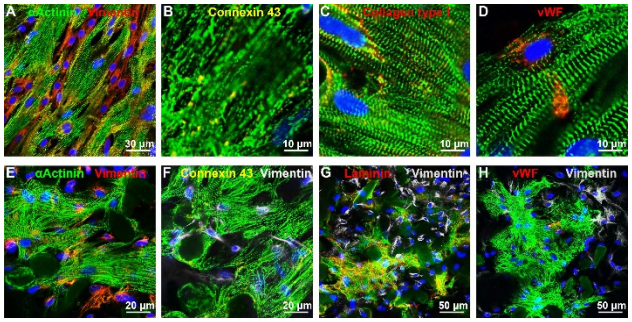


**Figure 4**. Immunofluorescence staining show CMs, fibroblasts, collagen type I, electromechanical coupling and endothelial cells of cardiac constructs from indirect plating (**A-D**) and from direct plating (**E-H**) of CMs.

**Conclusions:** This study is the first report to bioengineer a 3D functional heart tissue construct using adult CMs. The constructs fabricated using dedifferentiated CMs can beat as fast as a normal rat heart. The current adult CM constructs add a new potential cell source for treating heart defects, and furthermore, are useful for heart developmental studies, and guiding stem cell differentiation research and assessing CM development and maturation.