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Cell –Cell Interaction in Engineered Cardiac Tissue

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Despite a dramatic decline over the last 20 years, cardiovascular disease remains the leading cause of death and disability in the western world. A common outcome is congestive heart failure due to insufficient pumping capacity of the heart, which is the result of scaring. The only long-term treatment is transplantation and this treatment option is only available on a limited basis. In an attempt to develop a treatment alternative, we are attempting to tissue engineer a segment of cardiac muscle *in vitro* that has the three-dimensional structure of normal cardiac muscle. Ultimately, the goal is to graft the piece of artificial muscle into the heart and replace the scar tissue, thus restoring the pumping capacity. A key feature of this artificial muscle necessary to achieve this goal of transplantation and integration is the formation of appropriate cell-cell contacts.

Based on the structure, function and composition of the endogenous matrix of the heart, the logical choice for a scaffold material to construct an artificial myocardium would be collagen. However, collagen has not always proven to have the correct physical properties in other systems. What are the necessary properties for a scaffold for artificial muscle? From a biological standpoint, the material must be noncytotoxic, be biodegradable, the byproducts biodegradation must be nontoxic, and the scaffold must promote or at least support the maintenance and synthesis of new tissue. Collagen meets these requirements. From a mechanical standpoint, the scaffold must have an appropriate modulus, possess flexural rigidity, be flexible, have memory, have a surface energy that is not too hydrophobic or to hydrophilic, and thus provide a mean pore size and pore volume fraction to support the cells and fluid necessary to form a tissue. In nature, collagen possesses all of these features. The challenge is to recreate them in vitro.

Extruded collagen substrates in the form of a sheet were prepared as a scaffold (figure 1). Myocytes were isolated from 2-4 day old neonatal rats by collagenase digestion, plated on the collagen substrates, then placed either in a Rotating Wall Bioreactor (RCCS, Synthecon) or in a culture dish and cultured in DMEM containing 10% newborn calf serum, 1% penicillin, streptomycin and 4ug/ml cytosine arabinocide. After seeding in the RCCS bioreactor, cardiac myocytes align along the pattern of the extruded collagen and begin beating individually after 1-2days. Cell-cell connections formed in 7-10 days (Figures 2 and 3) resulting in synchronized beating of the cells. At this point the substrates contract as a whole and some patches have been maintained for up to 10 weeks in vitro. Fresh cells were added to both sides of the sheets once a week, which increased the vigor of contraction over time *in vitro*. Confocal microscopy indicated that the cardiac myocytes had extensive myofibrillar arrays (Figure 4) and connexin 43 position gap junctions.

Importantly, we have recorded spontaneous local current fluctuations from cardiac patches. Local electrical responses were recorded from a cardiac patch (voltage-clamp via Axopatch 200B amp) by a closely positioned 2 MΩ patch pipette. In this configuration, local electrical responses in the preparation were recorded as "current" fluctuations. A platinum electrode (insulated except for the very tip), connected to a Grass stimulator, was positioned at the far side of the preparation (~20 mm). Stimulation was initiated above threshold amplitude, which led to propagated electrical responses. Successful stimulation was possible between 1-6 Hz (not shown). The patch was superfused with Tyrode's solution, and gently restrained to avoid motion artifacts.

Intracellular Ca^{2+} levels were measured as Fluo-3/AM fluorescence (Ex λ 488 nm, Leica TCS SP2 microscope). Calcium transients responded to changes in stimulation frequency (both peak and "diastolic" Ca^{2+}), and increased with isoproteronol (10^{-6} M), as expected. Nifedipine (10μM) largely abolished Ca^{2+} signals, suggesting a role for L-type Ca^{2+} channels in Ca^{2+} homeostasis of these cardiac patches.

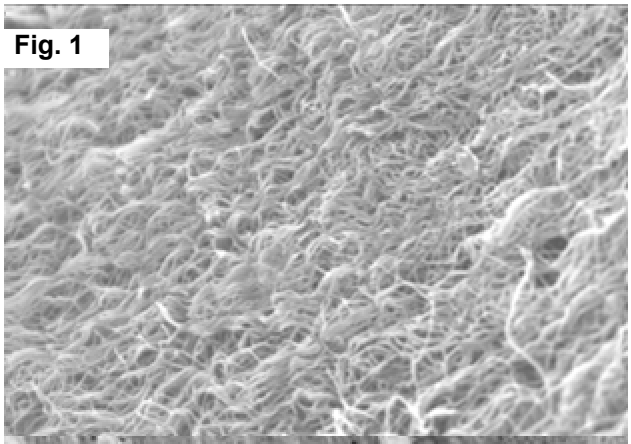
Fig. 1

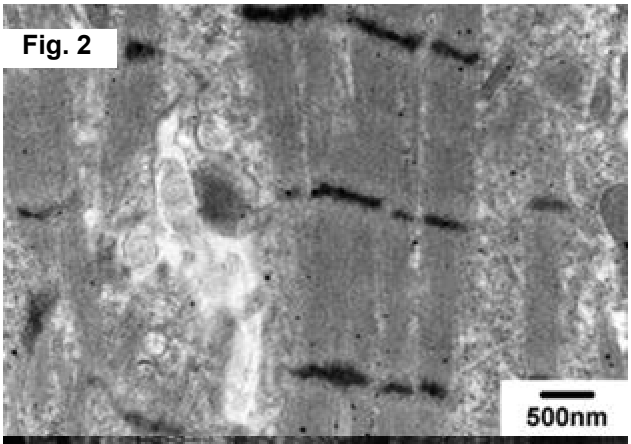

Fig. 2

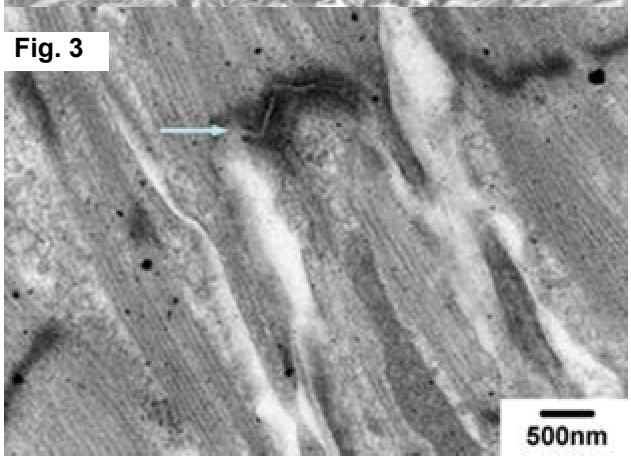

Fig. 3

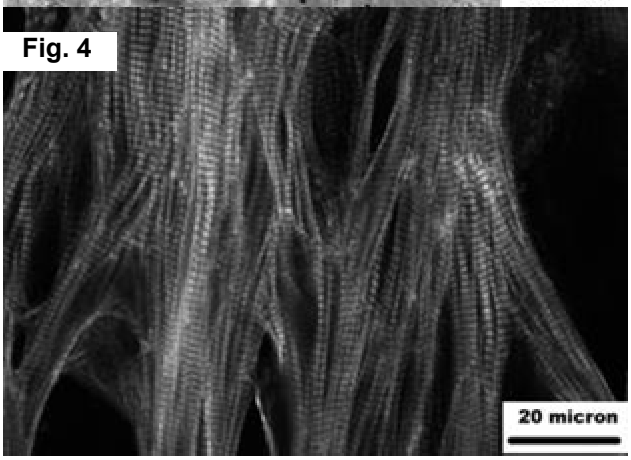

Fig. 4

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