Electronic Supplementary Material (ESI) for Analyst.
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Harvesting primary cardiac myocytes

Primary cultures of neonatal rat cardiac myocytes were established as described previously. [8] In brief, hearts from newborn rat pups were digested using the Worthington cardiomyocyte isolation system (Worthington Biochemical Corporation, Lakewood, New Jersey, USA) and after 2 hours preplating on tissue culture plates in plating medium (68% DMEM, 16% M199, 10% horse serum, 5% fetal calf serum, 4mM glutamine, 1% penicillin-streptomycin; all sera from PAA (Linz, Austria), all other cell culture components from Sigma Aldrich) to get rid of contaminating non muscle cells, the supernatant containing the isolated cardiomyocytes was transferred to the cell electrospinning unit. Prior to cell electrospinning the cells were mixed with a modified matrigel rich collagen biopolymer and subsequently subjected to the cell electrospinning process.

Experimental equipment set-up and operation

The cell electrospinning equipment arrangement explored in these investigative studies was very similar to our previous studies. [2-5] This essentially consisted of a coaxial needle system having an outer needle with inner and outer diameters of ~1.35 and ~1.65 mm, respectively, and an inner needle with inner and outer diameters of ~0.6 and ~0.9 mm held in an insulating needle holder. The needles were connected to a high voltage power supply capable of supplying a constant applied voltage of 0-30kV with a maximum drawing current of ~4mA. The needles were fed with their respective solutions with individual syringes and pumps capable of supplying a consistent flow rate ranging from ~10^{-15}-$10^{-5}$$m^3s^{-1}$. A large operational space was investigated for setting up the equipment for generating living cardiac fibers with these primary rat cardiac myocytes. Hence we traversed an applied voltage range

from 1-15kV and 10^{-12}-$10^{-9}m^3s^{-1}$ for the flow rate. Note during these studies we also optimized the distance between the cell electrospinning needle system and the grounded electrode. An addition to the experimental set-up in these studies were where we employed a highly sensitive Picoammeter system (9130 Picoammeter, RBD Instruments, Inc Oregon, United States) capable of measuring currents from as low as 2nA. This instrument was connected between the coaxial needle system and the grounded element to measure the real time current throughout the duration of the experiment.

Bright field and fluorescent Microscopy

In these studies cells were phenotyped, and the cell electrospun scaffolds were imaged using a Leica DM inverted microscope system. This microscope allowed the combination of brightfield and florescent microscopy to be simultaneously used for imaging of GFP expressing cardiac cells within the electrospun living cardiac fibers and scaffolds.

Flow cytometry

Flow cytometry is a fluorescence-activated cell sorting (FACS) method used for quantifying cellular features by optical analysis. In summary, the FACS technique and system employed can quantify both general cell death (necrosis) and programmed cell death (apoptosis). In those cells undergoing apoptosiss, the cellular membrane phospholipid phospatidylserine (PS) is translocated from the inner to the outer layers of the plasma membrane. During the initial stages of a cell undergoing apoptosis the cell membrane remains intact, whilst at the time of necrosis the cellular membrane loses its integrity and becomes permeable to the vital dye propidium iodide (PI).

Staining with Annexin V which has a high affinity for PS in conjunction with PI allows the identification of living cells (Annexin − PI−), cells undergoing early apoptosis (Annexin + PI−), cell undergoing necrosis (Annexin + PI+) and cellular debris (Annexin − PI +) to give an accurate measurement of the cellular viability.

Several single cell suspensions were prepared from each of the cell samples in both groups. Cells were mixed with Annexin V FITC (final concentration 1 μ g ml−1) (Pharmingen, UK) and PI (final concentration 1 μ g ml−1) (Sigma, UK) in the presence of 1.8mM calcium. Subsequently the cells were incubated at room temperature for ~15 min prior to quenching in calcium containing binding buffer (Pharmingen, UK) and were analyzed. A Dako Cytomation CyAn ADP flow cytometer was used to interigate data for 10000 events. Flow was carried out with the excitation set at 488 nm using an argon laser; the FITC emission was collected with a 525 ± 20 nm band pass filter and PI with a 675 ±20 nm filter. The data was analyzed using the software Summit 4.3 (Dako Cytomation, UK). Thus, in our studies the controls and cell electrospun primary cells were passed through the flow apparatus to analyze the number of living and dead cells through to cells, which were undergoing programmed cell death. Using the above flow cytometry approach, sevaral samples in both groups where analyzed.

GFP adenoviral transduction

The green fluorescent protein adenovirus was propagated in HEK 293 cells and was purified with a couple of cesium chloride gradients and its viral titer was established by a plaque assay. Cardiac myocytes were removed in ~0.5% trypsin solution centrifuged at 300g for ~5mins and resuspended in DMEM with 10% Fetal Bovine

Serum. The cardiac cells were then infected while in solution with the GFP adenovirus at a MOI of 50 and were subsequently cell electrospun to form the scaffolds that were digitally micrographed for demonstrating the GFP expressing cardiac cells within the fibers and scaffolds.

Immunofluorescence and confocal microscopy

Post-electrospinning the processed and control cells were plated in plating medium (see above) on tissue culture plates and the cardiomyocytes were allowed to adhere and spread. Following 24 hours in culture, the medium was removed, the cells were washed once with PBS and fixed for 10 min at room temperature in 4% paraformaldehyde/PBS (Agar Scientific, Stansted, UK). After permeabilisation in 0.2% Triton X100/PBS for 5 minutes, sequential immunostaining was performed as described previously. [8] As primary antibodies polyclonal rabbit anti MyBP-C (kind gift from Prof Mathias Gautel, King's College London), polyclonal rabbit anti connexin-43 (Invitrogen) and monoclonal mouse anti sarcomeric alpha-actinin (clone EA-53, Sigma) and monoclonal mouse anti myomesin (clone B4) were used. [9] Secondary antibodies were Cy3-conjugated goat anti mouse immunoglobulins (multilabelling quality) and Cy2-conjugated goat anti rabbit immunoglobulins (multilabelling quality; both from Jackson Immunochemicals via Stratech Scientific, UK). DAPI to visualize the nuclei was purchased from Sigma, Alexa633-conjugated phalloidin was bought from Invitrogen. Visualisation was carried out on a Leica SP5 confocal microscope that was equipped with a blue diode and argon as well as helium neon lasers, using a 63x/NA 1.4 oil immersion lens. Images were assembled using Adobe Photoshop.