The influence of fibroblast co-culture and 3D structures on cardiac action potentials of human stem cell derived cardiomyocytes.

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Introduction

Adult myocardium operates as a 3D syncytium of cardiomyocytes (CMs) and fibroblasts yet 2D mono-cultures of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) are routinely used for cardio-tox to predict pro-arrhythmic potential. Fibroblasts (FB) are known to affect cardiac phenotype via direct and paracrine actions. 3D electronic influences via gap-junctions between CMs are known to alter electrical activity of CMs within the syncytium. Therefore, both co-culture and 3D structures may be important in determining electrical behaviour of individual CMs.

Methodology

The relative influence of co-culture and 3D structure on the electrophysiology and contractility of CMs was examined using a voltage sensitive dye (Di-4-ANEPPS) and high resolution camera on the CellOPTIQ platform (Clyde Biosciences Ltd) 8 days post culture. hiPSC-CMs (iCell2® CDI Inc) and human cardiac fibroblasts (hCFBs) were cultured into 3D micro-tissues (approx. 0.3mm diameter) using hanging drop technology (InSphero AG); monolayers were cultured on fibrinogen coated glass bottom wells both as monocultures (hiPSC-CMs only) and co-cultures using a range of FB:CM ratios (1:20-1:4).

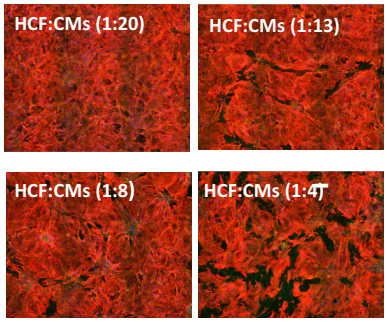

Fig. 1: The representative Immunofluorescence images demonstrate the presence of fibroblasts using the specific cardiac marker Anti-α-Actinin (sarcomeric) (red). Those cells negative for this marker and positive for actin (green) are fibroblasts. The number of fibroblasts being proportional to the ratio.

Results

Recordings from micro-tissues 8 days in co-culture displayed regular spontaneous activity with an action potential duration at 90% repolarisation (APD90) of 307±6ms (n=6 microtissues) at a spontaneous cycle length (1330±20ms). 2D monoculture of CMs using the same batch of hiPSC-CMs 8 days after plating showed APD90 values of 504±7ms (n=6 wells) at an average cycle length of 2876±118s. Co-culture with hCFBs caused a prolongation of APD90 across the range of FB:CM ratios used (1:20 – 1:4), at 1:8 the APD90 was 756±56ms (n=6 wells) at a cycle length of 4341±636s (n=6 wells). Over the range of FB:CMs used there was no clear dose dependence of the effects.

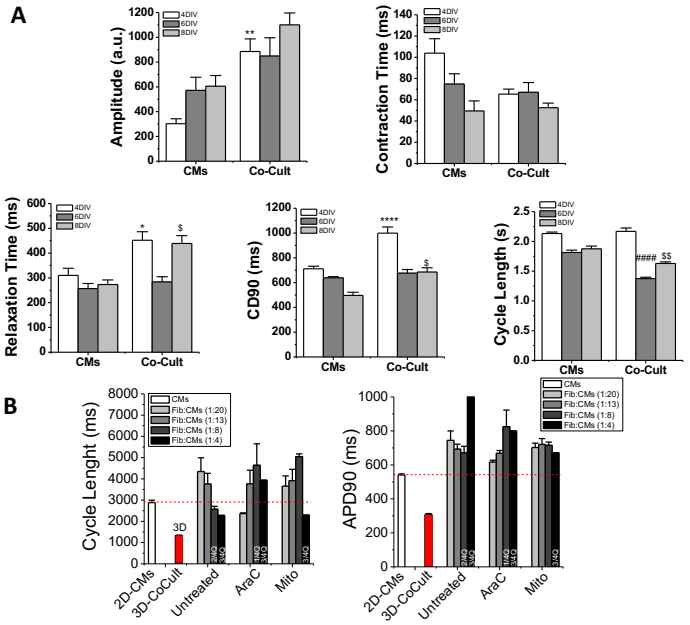

Fig. 2: Contractile and electrophysiological behaviour of co-culture of HCFav:hiPSC-CMs at day 8. A) Contractile behaviour of 1:10 hiPSC-CMs:HCFav showing the amplitude of the contraction, time to reach contraction, duration of 90% of contraction, cycle length. B) The electrical activity 8DIV showing APD90 and cycle length at different ratios. *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

Conclusions

- The effects were associated with changes in cycle length, but these were insufficient to explain APD changes observed.
- Co-culture of fibroblasts in 2D culture result in a significantly prolonged APD when compared to monoculture.
- Direct or paracrine influences may be responsible for the effect of hCFBs, the latter being less likely due to the exchange of culture media every 48hrs.
- The dramatically shorter APD90 seen in 3D co-culture must arise from the unique culture conditions of limited extracellular volume and 3D geometry of the tissue and is not simply the presence of human FBs.

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