Does function follow form? Differences in excitation contraction coupling of hiPSC-cardiomyocytes cultured in 3D vs 2D

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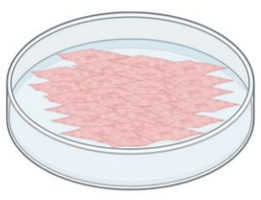
Background

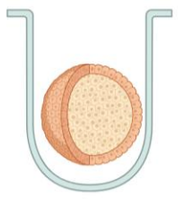
- Human induced pluripotent stem cell derived-cardiomyocytes (hiPSC-CMs) are widely used as an in vitro cardiac model, typically in monolayers, but these are attached to a rigid surface and have poor cell-cell interactions.
- There is increasing interest in 3D culture models of hiPSC-CMs such as cardiac spheroids, but it is unclear whether the different structure and arrangement of cells in 3D results in altered excitation-contraction (EC) coupling.

Cells growing on a 2D dish



- High stiffness
- Spreading of cells
- Restricted cell-cell interactions
- Very small diffusion gradient
- 2D contraction

Cells growing in a 3D spheroid

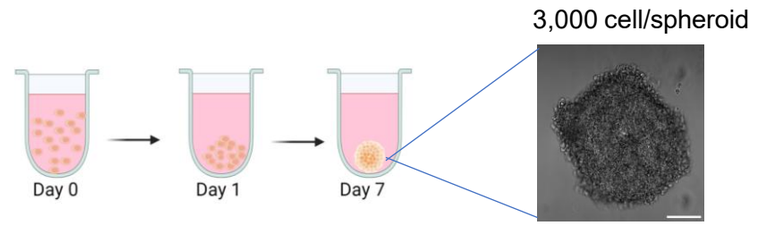


- Lower stiffness
- Restricted spreading of cells
- Higher cell-cell interactions
- Larger diffusion gradient
- 3D contraction

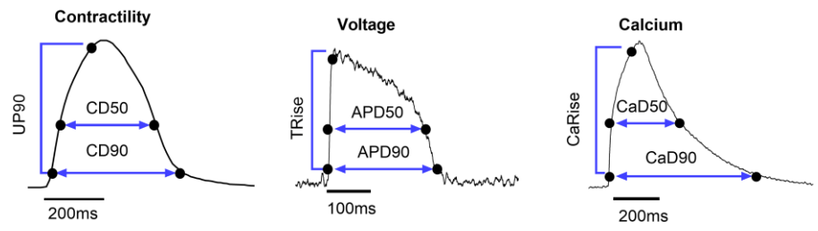
Aim: Characterise EC coupling of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) cultured in 3D (spheroids) vs 2D (monolayers)

Methodology

hiPSC-CM spheroid generation



Electrophysiological parameters



- Contractility measured using MUSCLEMOTION[1].
- Voltage and calcium measured using optical fluorescent dyes (FluoVolt and Cal520) on CellOPTIQ system.

Results

1. HiPSC-CMs from the same cell line have different EC coupling when cultured as spheroids or monolayers

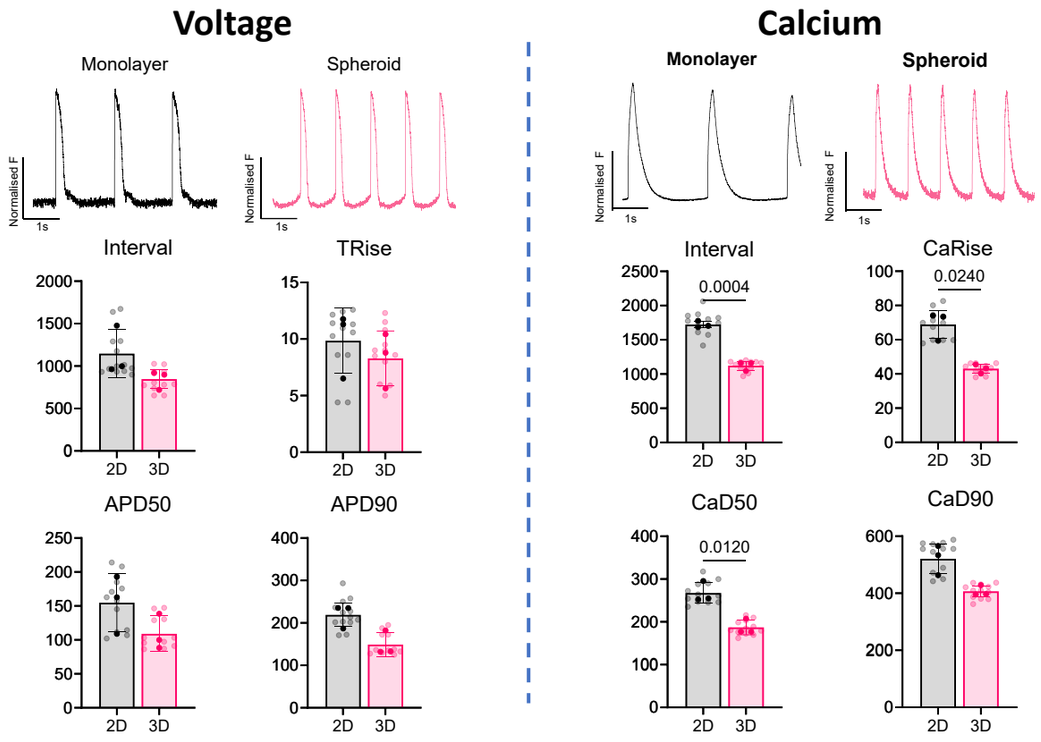


Figure 1: Characterising spheroid voltage and calcium. Representative raw traces and mean values of voltage (Left, A-D) and calcium parameters (Right, E-H). N_{exp}=3, , $n_{monolaye/apheroidr}$=12.

Cardiac spheroids have a **faster spontaneous beat rate and faster calcium dynamics** compared to monolayers

2. Cells within a spheroid have distinct differences in cell shape, size and arrangement than cells within a monolayer

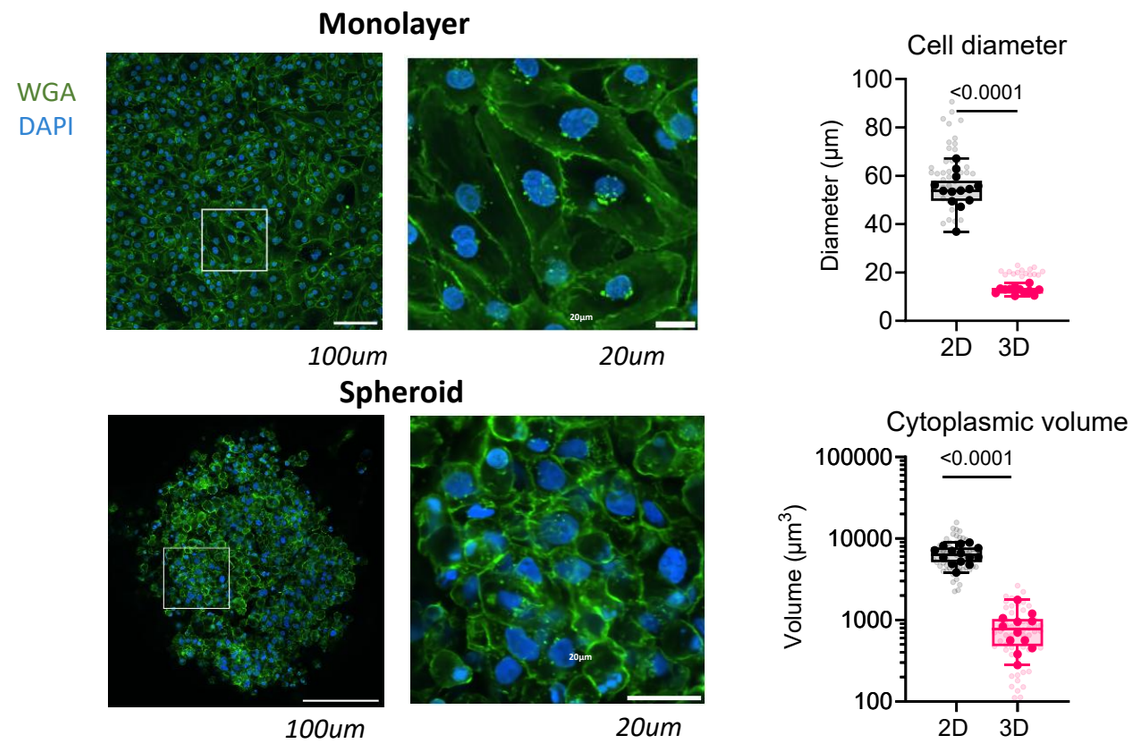


Figure 2: Differences in cell geometry could explain faster EC-coupling in spheroids. WGA and DAPI stained hiPSC-CMs monolayer and spheroid imaged on a Nikon AX-R. Nwell=12-14, ncell=60-70

Cells within a spheroid are smaller, more circular and have a **cytoplasmic volume 8X smaller** than cells in a monolayer

3. In silico modelling suggests cell shape alone does not explain EC coupling differences

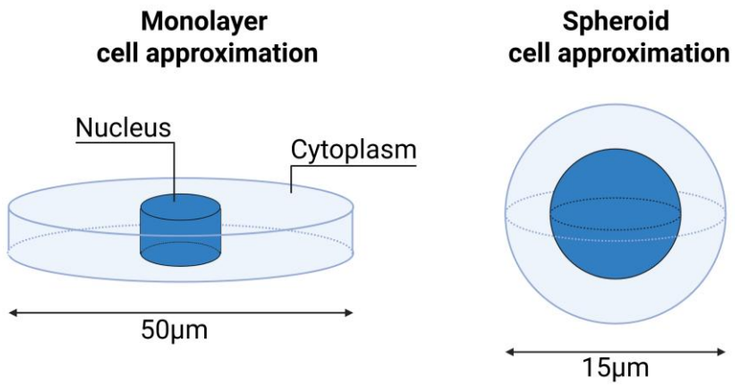
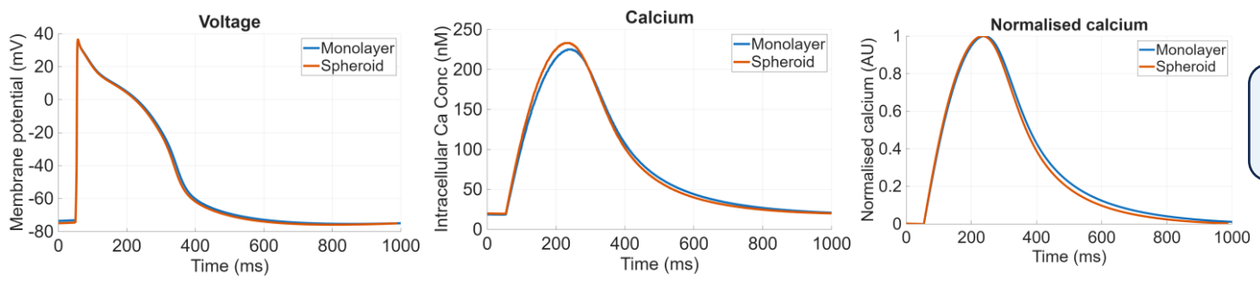


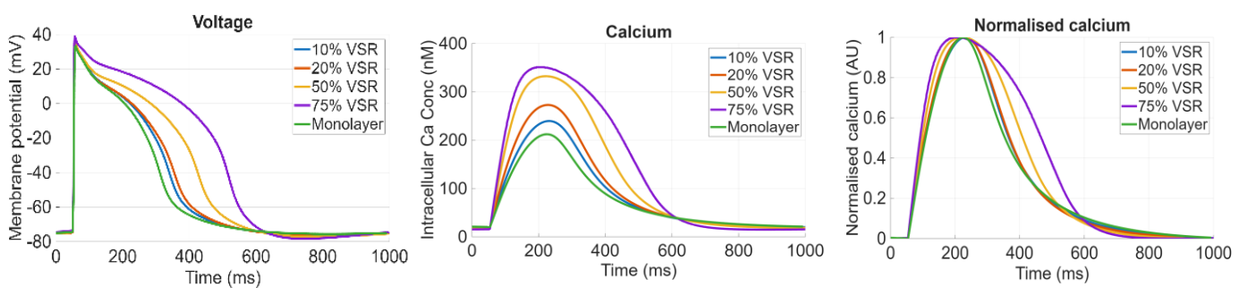
Figure 3: Differences in cell geometry could explain faster EC-coupling in spheroids. Diagram of cell approximation in a monolayer and a spheroid. Dimensions measured from confocal images were input into the Paci 2013 hiPSC-CM cell model to assess the mechanism of altered EC coupling[2].

Model 1: Altered cell size and rate



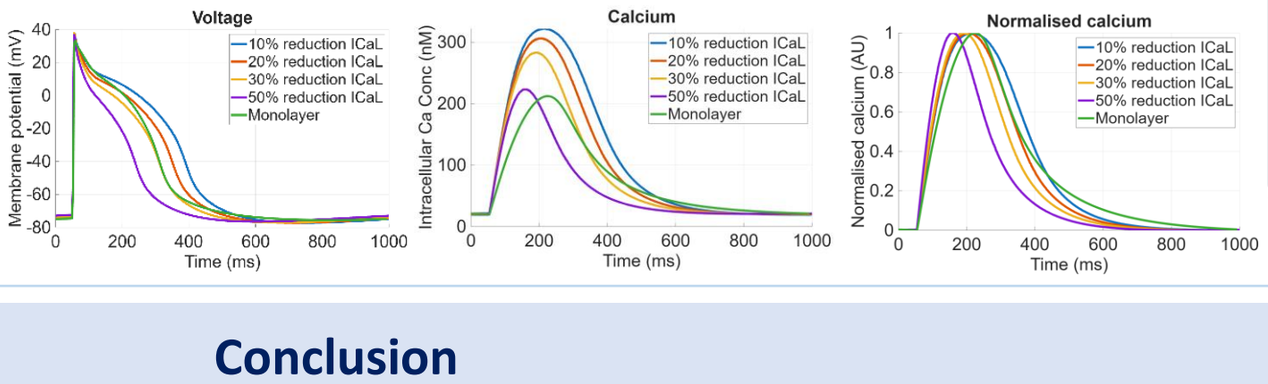
No difference

Model 2: Effect of reduced cytoplasmic volume/SR volume ratio



APD ↑
CaD ↑

Model 3: Effect of reduced I_{CaL}/I_{kr} ratio



APD ↓
CaD ↓
CaRise ↓

Reduced cytosolic volume/SR volume ratio + reduced I_{CaL}/I_{kr} expression in the spheroid could explain EC coupling differences

Conclusion

- Experimental data shows hiPSC-CMs have different EC coupling and cell geometry when cultured as 2D monolayers or 3D spheroids
- In silico modelling suggests reduced cytosolic volume and reduced I_{CaL} expression of cells within the spheroid could explain these EC coupling differences
  Understanding how EC coupling of hiPSC-CM spheroids may differ from monolayers is an important consideration before 3D culture models are routinely used in fields such as drug testing, disease modelling and cardiac regeneration.

References: 1. Sala et al, 2018, *Circ Res. 2.* Paci et al, 2013, *Ann Biomed Eng.*

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