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Dissociated Cardioids Assembly Using 30% Matrigel Droplets

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Matured cardiomyocytes are needed for disease modelling and drug screening. In this protocol we describe a microfluidic way of reassembling dissociated cardiac organoids back into cardiac organoids.

30% Matrigel Droplet Generation and Dissociated Cardioid Assembly

1 Thaw frozen stock of dissociated cardioids in a waterbath set to 37C for two minutes. Don't allow it to thaw completely.

2 Transfer into 15ml Falcon tube and resuspend in 5ml RMPI 1640+B27 supplement.

3 Centrifuge at 200xg for 5 min and decant the supernatant.

4 Add 1ml RPMI 1640+B27 to the cell pellet and resuspend.

5 Measure cardiomyocytes viability by taking 10ul of cell suspension and adding it to 10ul of Trypan blue (1:1). Count using an automated countess 3 haemocytometer.

6 Prepare 30% Matrigel by diluting down stock Matrigel with RPMI 1640+B27.

7 Resuspend 2-5 million dissociated cardioids in 30% Matrigel and use for droplet generation.

8 Generate about 300um droplets off-chip into 1.5ml eppendorf tube prefilled with 100-500ul of RPMI 1640+B27.

9 Aliquot 2ml of RMPI 1640+B27 into each well of 6-well plate.

10 Pipette 100ul of 30% Matrigel-cardiomyocytes droplets into each well of the 6-well plate.

11 Swell the plate gently making sure not to merge the droplets.

12 Incubate in a 37C/5% CO2 incubator and change media everyday.

13 Take images and videos everyday beginning Day 0 until Day 10. On each day check for viability by staining with Calcein-AM (Add 1.5ul of Calcein-AM to 1.5ml of RPMI 1640+B27 and use that to stain the droplets for 2 hours)

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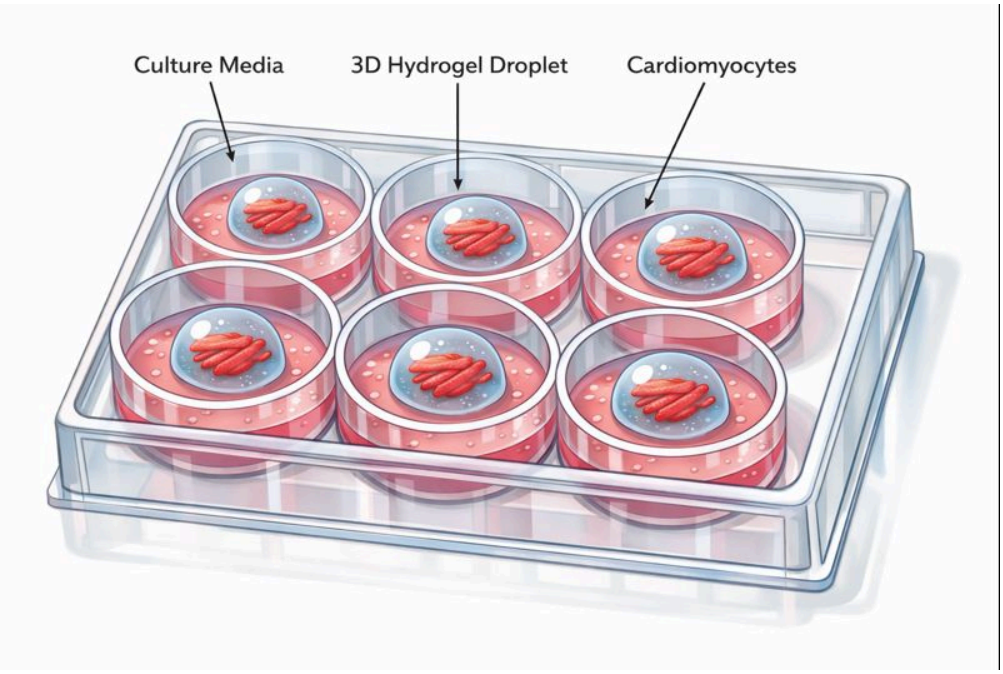

Schema of encapsulated dissociated cardioids in 30% Matrigel