Protocol for CardioCluster Creation

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Method Article

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Abstract

Cellular therapy to treat heart failure is an ongoing focus of intense research, but with limited progress due to limitations in engraftment and persistence of injected cells. Engineered augmentation of established cellular effectors overcomes impediments, enhancing reparative activity. Such ‘next generation’ implementation includes delivery of combinatorial cell populations working together synergistically to improve heart function following injury. Concurrent isolation and expansion of 3 distinct cardiac-derived interstitial cell types from human heart tissue prompted design of a 3D structure that maximizes cellular interaction, allows for defined cell ratios, controls size, enables injectability, and minimizes cell loss. Here we describe a method wherein, mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs) and c-Kit^{+} cardiac interstitial cells (cCICs) when cultured together spontaneously form scaffold-free 3D microenvironments we have termed 'CardioClusters'.

Introduction

Reagents

Cardiac Interstitial Cell Medium F12 HAM’s (1x) (SH30026.01, HyClone) 10% ES FBS (16141079, Gibco) 1% Penicillin-Streptomycin-Glutamine (100X) (10378016, Gibco) 5 mU/mL human erythropoietin (E5627, Sigma-Aldrich) 10 ng/mL human recombinant basic FGF (HRP-0011, Biopioneer) 0.2 mM L-Glutathione (66013-256, Sigma-Aldrich) EBM-2 Basal Medium (CC-3156, Lonza) **Endothelial Progenitor Cell Medium** EGM-2 Kit Supplements and Growth Factors: (CC-4176, Lonza): · 0.5 mL Human Epidermal Growth Factor · 0.5 mL Insulin-Like Growth Factor-1 · 0.5 mL Vascular Endothelial Growth Factor · 0.5 mL HEPARIN · 0.5 mL Gentamicin Sulfate Amphotericin-B · 0.5 mL Ascorbic Acid · 2.0 mL Human Fibroblast Growth Factor-B · 2.0 Hydrocortisone · 10 mL FBS **Mesenchymal Stem Cell Medium/CardioCluster Medium** 10.1 g/L Minimum Essential Medium Eagle, Alpha Modification (M0644, Sigma-Aldrich) 20% FBS (FB-01, Omega Scientific, inc.) 1% Penicillin-Streptomycin-Glutamine (100X) (10378-016, Gibco) Cell Culture Grade Water **Cell Dissociation** Cellstripper (Corning, catalog #25-056-CI) TrypLE Express (1X) (Thermo Fisher Scientific, catalog #12604-013)

Equipment

Cell Culture Dishes TC Treated Dishes, 100 x 20mm (Cat# 25-202, GenClone, Genesee Scientific) 96 well, ultra-low attachment multiwell round-bottom plates (CLS7007, Corning)

Procedure

CardioCluster Formation 1. Dissociate cCICs and MSCs from TC culture dishes using a 1:1 mixture of Cellstripper and TrypLE Express (1X). Centrifuge resulting suspensions at 350 *g* and resuspend in CardioCluster Media. 2. cCICs and MSCs compose the inner core of the CardioCluster in a 1:2 ratio (approximately 100 MSCs and 150 cCICs - per 500 cell CardioCluster). Count each cell population and

dilute to the appropriate concentration. 3. Seed the cCICs and MSCs into 96 well, ultra-low attachment multiwell round-bottom plates in 100 µL/well CardioCluster media. Store plates for 24 hours at 37°C in a 5% CO_2 incubator. 4. The following day, dissociate EPCs from TC culture dishes using a 1:1 mixture of Cellstripper and TrypLE Express (1X). Centrifuge resulting suspensions at 350 *g* and resuspend in CardioCluster Media. 5. The outer EPC layer is created using a cell number equal to the number of cells used to create the central core (approximately 250 EPCs - per 500 cell CardioCluster). Count the EPC population and dilute to the appropriate concentration. 6. Add EPCs to the 96 well plates that contain cCIC/MSC central cores using an additional 50 µL/well CardioCluster media. Incubate the 96 well plates at 37°C in 5% CO_2 for 24 hours until CardioCluster 3D structure forms.

Troubleshooting

Time Taken

48 hours

Anticipated Results

The formation of a CardioCluster approximately 150 µm in diameter, composed of a total of 400±100 cells.

References

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