PORCINE CARDIOMYOCYTE ISOLATION PROTOCOL

Day Prior to Isolation:

1. Ensure stock solutions made: 2L of 0-Ca^{2+} Physiologic Saline Solution (PSS) and 1L of MEM
2. Ensure large animal enzyme aliquot made (see solution supplement)

Day-of, Prior to Isolation:

3. Make day-of solutions (see solution supplement)
   a. Wait to add BSA to solution 3 until heart arrives – pre-measure what you need and store in a falcon tube in the fridge for ease
4. Check oxygen tank levels, switch tanks as needed, and make sure they are secured.
5. Run 1L of DI water through isolation rig
   b. Cover top reservoir until use
6. Ensure vacuum flasks
7. Check water level in recirculation pump
   c. Check bath temp
8. Collect and inspect: Cannula, tissue pins, Sylgard disk, Cheese cloth mesh (for solution filtering), bulldog clamps
9. Tie double knot sutures and leave in weigh boat (~6)
10. Set up drainage port and solution recycler (funnel, mesh, beaker).
11. Set up 1L Millipore water to boil for rig cleaning rig after isolation – wait until heart arrives
12. One hour before heart arrives, turn on water bath and recirculation pump.
13. Add solution 1 to top reservoir and turn on oxygen to bubble (about 30 minutes before heart arrives).
    d. Once heart arrives, perfuse solution 1 through the system, and remove as many bubbles as possible.

Isolation Protocol:

14. Cannulate left anterior descending coronary artery in left-ventricular wedge and secure with pre-tied suture; begin Solution 1 perfusion
15. Solution 1 typically perfuses ~10-15 minutes: use this time to check for and tie off all leaks.
16. When Solution 2 hits the heart, ligate subsequent leaks
17. Once Solution 2 is in the heart, drain the bath completely from solution 1 to ensure recycled solution is only enzyme solution.
18. During digestion, continue basting heart with bath solution.
19. Solution 2 perfusion is ~28-30 minutes. Do a test cut into the LV tissue at ~ 25 minutes to check digestion. Tissue should be orange in color and soft.
20. Post-digestion, acquire tissue (and subsequent cells) from only the mid-myocardium. Endocardium and the epicardium are ablated and discarded.
    a. Move the entire wedge into a weigh boat with BSA Solution. Remove a large chunk of tissue from the LV and move to a new dish of BSA.
    b. From the LV chunk, cut off the epi and endocardium and discard. Move the LV tissue to a new dish with fresh BSA.

c. From the midmyocardium LV tissue, cut into a few pieces and gently slough off cells.
21. Cells are filtered into 50ml falcon tubes through 100 µm mesh filter
22. Excess tissue can be discarded into a glove, tied up, and put in the fridge for incineration.

Following Isolation (this can be done while plating/dye loading cells):

23. Once heart is removed from rig: oxygen, water bath, and recirculation pump can be turned off.
24. Wash rig (make sure vacuum dish is open):
    a. Flush with ~500ml of boiling Millipore water through rig
    b. Flush with ~500ml of 70% ethanol
    c. Flush again with ~500ml of boiling Millipore water
    d. Flush a final time with Millipore water to ensure all ethanol is removed
25. After all solution has been flushed, turn off hotplate and vacuum.

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