Rabbit Myocyte Isolation

Preparation:

- Autoclave the following items:
  1. 2-250 ml beaker  
  2. 2-150 ml beaker  
  3. 2-50 ml beaker  
  4. 3-1 L bottles  
  5. 2-100 ml bottles  
  6. cellctor  
  7. tritutators  
  8. cannula  
  9. 8x8 baking dish w/surgical instruments: large and small scissors  
  large and small forceps  
  large retraction hemostat

- Prepare solutions as shown on protocol sheet. Pre-warm all solutions except Solution 1 (ice-cold for mounting).

- Wash out perfusion apparatus:
  1. Turn on water bath.  
  2. 70% ethanol  
  3. Sterile dH20 x 2  
  4. Sterile dH20 + P/S  
  5. Fill left syringe with Solution 2 and right syringe with Solution 3.

- Set up dissecting area:
  1. Place blue pads and black bag over work surface.  
  2. Set up bucket of ice with petri dish and 150 ml beaker (filled with Solution 1 to rinse and mount heart).  
  3. Attach cannula with 10 ml syringe, also filled with Solution 1 to flush heart. Pre-tie suture around cannula.

Rabbit Preparation:

- Obtain New Zealand rabbit (male, 2.2-2.6kg) from Buhl Rm 3922 or 3916 (3-1,4).  
- Place rabbit in holding cage and prep ear for cannulation with alcohol pad. Cannulate ear with butterfly needle and slowly inject 1ml heparin.  
- Administer nembutal at 65mg/kg, and wait until rabbit is unresponsive (via reflex tests).  
- Lay rabbit on back and soak chest with EtOH.  
- Wearing sterile gloves, excise skin and pull back sternum.  
- Carefully cut through diaphragm, revealing the heart.  
- Gently open pericardium, retract heart anteriorly and cut pulmonary veins and aorta.
- Rinse heart in ice-cold Solution 1, gently squeezing out excess blood. Trim away excess tissue from aorta.
- Mount aorta on cannula/syringe, tie with suture, and flush with buffer (avoid air bubbles).
- Transfer cannula with heart to start perfusion.

**Heart Perfusion:**

- Perfuse 3-5 min with Solution 2 to clear blood.
- Perfuse 2 min with Solution 3. Add collagenase/hyaluronidase, and perfuse for approximately 10 min. Keep heart immersed in beaker with enzyme solution, allowing for recirculation of solution.
- Add protease and digest for an additional 15 min. Heart will be swollen and extremely digested.
- Take heart down from perfusion apparatus; remove aorta and atria.
- Gently cut the ventricular myocardium into small pieces to facilitate mechanical digestion. Add ~50ml incubation solution and begin gently swirling with trituration (at room temp) for several min, as tissue begins to fall apart. Pass through cell collector, collecting ~ 50ml solution into 15ml conical tubes.
- Pellet solutions by centrifugation, about a 15 sec spin on #5.
- Slowly bring calcium level up to 1.75 mM by adding 14.6µl 100 mM CaCl₂ every 5 min for 1 h.
- Pellet cells one more time, resuspend in serum-free DMEM, and proceed to plating.

**Plating Cells:**

**Sterilize plates:**
1. Place 1 (1-18mm) cover slip in each well of the 6-well plate and UV (uncovered) for 10 min.
2. Coat each cover slip with 100 µl laminin (40 µg/ml) and UV 10 min.

**Count cells:**
1. Resuspend cells in serum-free DMEM (5-8 ml, depending on pellet size).
2. Transfer a small amount of resuspended cells to hemocytometer using a pasteur pipette.
3. Count live vs. dead cells for each of 9 fields. Calculate percent viability, expecting 65-70% rod-shaped cells.
4. Calculate the concentration of cells: concentration = # of cells in 1 field x 10⁴.
5. Dilute cells to conc. of 1 x 10⁵ rod-shaped cells/ml in DMEM + 5% FBS + P/S.
6. Plate 200 µl/cover slip, which equals 2 x 10⁴ cells/cover slip.

**Incubate cells** for 2 hr, aspirate serum-containing DMEM, then add back 2 mls serum-free DMEM + P/S (or proceed to viral transfection with 1 hr incubation, then add back 2 mls DMEM).
### Solutions:

1. **50/50 Joklik’s-HBSS 15mM HEPES**
   - 50% Joklik’s MMM (Sigma M-8028) 250ml
   - 50% HBSS (Gibco 14170-112) 250ml
   - 15 mM HEPES (Gibco 15630-080, 1M) 7.5ml
   - Pen/Strep 5.0ml

2. **Joklik’s 15mM HEPES**
   - Joklik’s 250ml
   - 15mM HEPES 3.75ml
   - Pen/Strep 2.50ml

3. **Joklik’s 10mM HEPES**
   - Joklik’s 500ml
   - 10mM HEPES 5.0ml
   - Pen/Strep 5.0ml

4. **Incubation Solution**
   - Joklik’s 200ml
   - 10mM HEPES 2ml
   - BSA 0.50g
   - Pen/Strep 2ml
   - CaCl\(_2\) (100mM stock) 25µl

5. **Enzyme Solutions**
   - **a)** Add 70 mg collagenase (Worthington Type 2) +10 mg hyaluronidase (Sigma H-3506) + 17.5µl of 100 mM CaCl\(_2\) to 50ml Solution 3 (bleed right syringe to 10 ml before adding).
   - **B)** Add 22 mg protease (Sigma P-5147) to 5ml Solution 3.

### For plating:
- DMEM + P/S
- DMEM + P/S + 10% FBS