Protocol for Isolation of Adult Fibroblasts by Digestion

Materials
- 20 mL Krebs Henseleit Buffer (KHB) cold
- 40 mL KHB warm
- PBS warm
- Collagen Gel Media warm
- Liberase warm (4.15 mg aliquot)
- 10 50 mL centrifuge tubes
- 5 T75 flasks
- small petri dish
- mincing tools:
  - scissors
  - tweezers
- 400 mL waste beaker
- sacrificing tools:

Procedure
1. Warm Collagen Gel Media and Liberase in water bath.
2. Wipe down hood with 70% alcohol.
3. Spray mincing and sacrificing tools with 70% alcohol. Allow to dry in hood.
4. Wipe down incubator and rotator with 70% alcohol.
5. Label a 50 mL centrifuge tube with "20 mL KHB cold" and aliquot 20 mL KHB cold. Warm KHB in water bath.
6. Label centrifuge tubes:
   - 1 tube "40 mL KHB warm"
   - 2 tubes "30 mL Liberase/KHB warm"
   - 1 tube "DIGESTION" (for initial digestion)
   - 5 tubes "CELLS #2", "CELLS #3", …, "CELLS #6" (for 5 additional digestions)
7. Label flasks:
   - what type of cell (ACF)
   - today’s date
   - which digestion (2, 3, 4, 5, 6)
   - optional: times first plating cells
8. Aliquot 40 mL KHB warm into appropriate centrifuge tube.
9. Prepare two 30 mL aliquots of Liberase/KHB solution:
   - Add 9.2 mL KHB to original Liberase tube containing 829 uL Liberase for a total volume of approximately 10 mL.
   - Aliquot 5 mL of this initial solution each into the appropriate 50 mL centrifuge tubes.
   - Add 25 mL KHB to each tube.
   - Record date and initials of Liberase.
11. Place heart in petri dish with 10 mL KHB warm. Remove unwanted parts:
• atria (lighter colored pink)
• valve tissue (white)

12. Mince heart with scissors until very fine. Place minced tissue into 50 mL centrifuge tube labeled “DIGESTION” using a 10 mL pipette. Remove as much liquid as possible.
13. Wash minced heart tissue with 10 mL KHB warm and remove as much liquid as possible.
14. Wash two more times by repeating step 13 two additional times.
15. Digest heart tissue by adding 10 mL of Liberase/KHB solution to the centrifuge tube.
16. Wrap tube with parafilm and rotate in 37°C incubator for 15 minutes.
17. Vortex tube for 30 seconds after removing from rotator and incubator.
18a. First digestion: Remove as much of the first supernatant as possible with a 10 mL pipette and discard. Repeat from step 15.
18b. Second through sixth digestion: Remove as much supernatant as possible with a 10 mL pipette and put supernatant in appropriate tube (2, 3, 4, 5, or 6). Centrifuge supernatant at 400g for 10 minutes.

For second through sixth digestions:
20. Discard supernatant from centrifuging. Resuspend pellet in 20 mL Fibro Media. Plate on appropriate flask.
21. Rinse and refeed plated cells after first 4 hours, then every two days. Watch carefully for signs of infection.
22. Passage cells after one week. From healthy adult rats expect 20 – 30 million cells at one week.

Modified 1-4-13 TLB