Video Article

Isolation of Human Umbilical Vein Endothelial Cells (HUVEC)

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Abstract

Angiogenesis is a complex multi-step process, where in response to angiogenic stimuli, new vessels are created from the existing vasculature. These steps include: degradation of the basement membrane, proliferation and migration (sprouting) of endothelial cells (EC) into the extracellular matrix, alignment of EC into cords, lumen formation, anastomosis, and formation of a new basement membrane. Many in vitro assays have been developed to study this process, but most only mimic certain stages of angiogenesis, and morphologically the vessels often do not resemble vessels in vivo. Here we demonstrate an optimized in vitro angiogenesis assay that utilizes human umbilical vein EC and fibroblasts. This model recapitulates all of the key early stages of angiogenesis, and importantly the vessels display patent intercellular lumens surrounded by polarized EC. Vessels can be easily observed by phase-contrast and time-lapse microscopy, and recovered in pure form for downstream applications.

Video Link

The video component of this article can be found at http://www.jove.com/video/183/

Protocol

Procedure

1. Lay cord on clean pad and dab off excess blood. Make fresh cuts on both ends of the cord.
2. **Insert 21 1/2 G needle** with the plastic needle sheath ON, into the vein. (The vein is the largest opening; the 2 smaller ones are arteries)
3. **Clamp the needle** in place with a hemostat and attach the 20cc syringe of Hanks to the needle.
4. **Push the Hanks through** the vein with moderate pressure. Collect the waste in the beaker with bleach. (Holding the needle and cord with one hand while pushing would prevent the needle popping out of the vein.) If there is a lot of blood in vein, wash a second time.
5. Lay cord on pad and clamp the other end of the vein. Fill with a few mL of Hanks to **check for leaks** along the cord. Withdraw the 5 mL and disconnect the bottom clamp.
6. Disconnect the 20cc syringe. Remove the plunger from the 10cc syringe. Attach 10cc syringe to needle, **pour in 10ml collagenase** and replace plunger. Push collagenase into vein until you see the first amount exit the open end. Re-clamp the open end and fill with collagenase until there is **moderate distention of vein**. Too much distention results in smooth muscle contamination.
7. **Massage the cord gently**.
8. Incubate cord (with hemostats, needle and syringe attached) in DPBS at 37°C for 15 min.
9. While incubating, continue steps 1-8 with 2nd cord.
10. After incubation, take cord out of beaker and while holding the cord over the 50mL tube cut the end **above** the bottom clamp. **Be sure to collect everything in the tube**. Push the remaining collagenase through the cord, then attach the 20cc syringe and push Hanks through with moderate pressure.
11. If no smooth muscle cells are needed, discard the cord at this time. Otherwise, place the same cord into a second 50mL tube with ~5ml collagenase and incubate at 37°C for 30-60 min.
12. Keep the tube until all cords are done.
13. **Spin tubes** at ~1200 rpm for 5 min.
14. Aspirate supernatant (except for ~1-2 mL). **Resuspend** the pellet in 5mls PHEC+ and plate in a T25.
15. **Incubate at 37°C** with 5% CO_2 overnight.
16. Next day remove supernatant and replace with fresh media. If there are many RBCs, wash once with M199, then add PHEC+. Continue incubating as usual until the plate is confluent (1-4 days). Split into a gelatinized T75.
17. Once T75 is confluent, split into three T75s. Freeze two vials per flask.

Note: *Endothelial cells (unactivated) have a cobblestone appearance. Endothelial cells are sometimes activated (long and pointy) right after isolation, after a couple of passes they usually return into an unactive state.*

Cleanup

1. Very carefully, dispose of needles in sharps container.
2. Dispose of syringes in big biohazard container.
3. Put all tissue into small biohazard bag. Close bag and freeze at -20° until incineration.
4. Soak all instruments in virucide for at least 10 min.
5. Add incubation media to waste/bleach beaker. Let sit for at least 10 min.
6. Discard the bench pads into biohazard waste.
7. Spray down the inside and outside of beakers, bench top and the water bath lid with vircide. Let sit 10 min, then wipe.
8. Rinse beakers and instruments with warm water and hang on rack to dry (blot off the instruments so they don't rust).
9. Dispose of decontaminated waste down the sink.
10. Return unused media to fridge.
11. Dispose of gloves in biohazard waste.