**Appendix A. Immunostaining on Paraffin Sections**

1. Warm slides for 10 minutes at 60 **°**C on slide warmer.

2. Deparaffinize the sections.

a. Soak slides for 2 minutes in histology grade xylene.

b. Move slides to fresh xylene for 2 minutes.

c. Move slides to fresh xylene for 2 minutes.

d. Move slides to fresh xylene for 2 minutes.

3. Rehydrate the sections.

a. Soak slides in 100% ethanol for 2 minutes.

b. Move slides to fresh 100% ethanol for 2 minutes.

c. Move slides to 95% ethanol for 2 minutes.

d. Move slides to 70% ethanol for 2 minutes.

e. Move slides to ddH2O for 2 minutes.

f. Move slides to PBS for 5 minutes.

4. Conduct antigen retrieval

a. Move slides to target retrieval solution.

b. Place the histology cup with slides and target retrieval solution in a water bath at 85**°**C for 2 hours 40 minutes.

5. Remove the histology cup from the water bath and cool for 20 minutes.

6. Wash the slides twice for 5 min in PBS.

7. Block the sections with 20% fetal bovine serum in PBS for 45 minutes at room temperature.

a. For many slides, this can be done in a histology staining cup.

b. For fewer slides, use a hydrophobic pen and apply the solution onto the flat section.

8. Wash the slides twice for 5 min in PBS.

9. Use a hydrophobic pen to draw circles around the tissue sections on the slides if they have not been drawn already.

10. Apply the primary antibodies at the appropriate concentration in PBS-1% BSA to the tissue sections. Use 1:25 for the PECAM-1 antibody and 1:100 for the -SMA antibody.

11. Incubate the slides overnight at 4°C in a closed container with damp towels to ensure drying does not occur.

12. Wash the sections with PBS twice quickly, then three times allowing 5 minutes per wash.

13. Label the sections with secondary antibodies at 1:1000 dilution in PBS-1% BSA with a 1:1000 dilution of DAPI (from a stock solution of 1 mg/ml). Incubate for 75 minutes at room temperature protected from light.

15. Wash the sections with PBS twice quickly, then three times allowing 5 minutes per wash.

16. Put a drop of hard set mounting media without DAPI on each of the sections.

17. Carefully apply a glass coverslip, ensuring bubbles do not form over the section

18. Allow the hard set mounting media to dry overnight.

**Appendix B. Preparation of Cholesterol Diet**

1. Fill a bucket with 9 kg of rabbit chow.

2. Prepare a cholesterol/corn oil/chloroform solution in a chemical fume hood. The recipe below creates a high fat diet with 0.1% cholesterol. For other high fat diets use can be created by changing the amounts of cholesterol and/or corn oil.

a. Weigh out 9 g of cholesterol and put it in a large beaker

b. Add 200 mL of chloroform to the beaker and stir

c. Add 45 mL of corn oil to the beaker

d. Fill the beaker up to the 1000 mL marker with chloroform and stir

*Note: be sure to use appropriate personal protective equipment when handling chloroform. The brand of corn oil we used (see Table 1) worked well for this purpose. Other corn oils may work as well but the results will depend on the fat content of the corn oil.*

3. Spread the food out on a stainless steel tray inside a chemical fume hood.

4. Pour the cholesterol/corn oil/chloroform solution over the food in the hood.

5. Wearing heavy-duty chemical resistant gloves and using a stainless steel spoon, mix the food and the solution until no puddles are present and the food appears an even color.

6. Lower the sash of the hood to leave a two-inch gap and allow the solution to evaporate in the hood for 2 days.