General information for Isolation of Endothelial Cells

Procedure

1. Tissues were placed inside a culture hood.
2. Tissue slices were prepared, washed, and suspended in HBSS.
3. Excess HBSS was aspirated, and the tissue slices were minced and transferred to a sterile tube.
4. The minced tissues were digested with collagenase I.
5. Digested tissues are homogenized and spin down digested tissues.
6. Resuspend digested tissues in culture medium.
7. Seed digested tissues onto T25 or T75 flask for certain times.
8. Collect unattached cells and repeat several times.
9. Unattached cells are spinned down.
10. Resuspend cell pellets with endothelial growth medium (VEGF/EGF/FGF/Heparin/FBS etc.).
11. Expand purified endothelial cells with endothelial growth medium.
12. Primary Endothelial Cells are characterized by immunofluorescence staining with antibodies of CD31/PECAM-1, VE-Cadherin, or use of fluorescence-labeled acetylated low-density lipoprotein (DiI-Ac-LDL) uptake, a functional marker for endothelial cells.

THIS IS A PROPRIETARY METHOD.