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Working

Isolation of endothelial cells from umbillical vessels

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Epigenetic control of the angiotensin-converting enzyme in endothelial cells during inflammation PLOS ONE

Reagents

1

- Hank's Balanced Salt Solution (HBSS) (H6648-500ml, Sigma-Aldrich)
- Enzymatic digestion with Dispase II (29585300, Roche) (2.4 U/ml) in Ham's F12
- Ham's F12 with 10% fetal calf serum (FCS), penicillin (50 U/ml) and streptomycin (50 µg/ml) for centrifugation
- Endothelial cell culture medium: MCDB131 (10372-019, Gibco) containing 10% FCS, L-glutamine (10mM), penicillin (50 U/ml), streptomycin (50 µg/ml), epidermal growth factor (0.1 ng/ml), basic fibroblast growth factor (1 ng/ml) and endothelial growth supplement with Heparin (0.4%)

Endothelial cell isolation

2

1. umbilical cord is cut at one end (about 1 cm) to identify the arteries and the vein
2. the vein or artery is then cannulated with a button cannula connected to a three-way stop cock. The cannula is fixed with a cable connector to the cord
3. The cord is the rinsed with HBSS until the HBSS remains clear and no blood is washed out any longer
4. Repeat steps 1-3 on the other end
5. Now both ends are cannulated and the vessel is filled with Dispase II solution
6. The cord is incubated for **00:50:00 min** at **37 °C**
7. Discard the Dispase II solution and replace with Ham's F12 containing 10 % FCS
8. Carefully massage the cord to loosen the endothelial cells
9. Collect the medium in a suitable centrifugation tube and centrifuge for **00:05:00** at 100 x g
10. Discard the supernatant and resuspend pellet in endothelial cell culture medium and distribute on a fibronectin-coated cell culture dish. Coating with fibronectin should be performed **00:30:00 min** to **01:00:00 min** before use. Place the cell culture dish containing your cells in a cell incubator at **37 °C** in 5% CO_2.
11. After **04:00:00 hr** cells are washed with cell culture medium.
12. **48:00:00 hr** to **96:00:00 hr** after isolation the cells should have formed a confluent cell layer

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