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Protocol for primary cultures of HUVECs

I- Buffers and reagents

- **Fetal calf serum (FCS)** (Biosepra)

- Unfreeze the FCS
- Heat for 30 min. at + 56°C (decomplementation; bottle closed)
- Fractionate (25 ml) in sterile *Falcon* of 50 ml
- Freeze the fractions at – 20°C

- **Phosphate Buffer Saline (PBS)**

- 100 ml of PBS 10X without Ca⁺⁺ and Mg⁺⁺ (Life Technologies)
- 900 ml of distilled water
- 2 g of glucose

- Filtrate (0.22 µ), fractionate (50 ml) in sterile Falcon and freeze at –20 °C

- **Collagenase** (0.2 % in PBS)

- Dissolve 0.2 g of collagenase H from *Clostridium Histolyticum* (Roche) in 100 ml of PBS
- Mix during 10 min. in the dark (aluminium foil)
- Adjust the pH at 7.40 with NaOH 1N
- Filtrate (0.22 µ)
- Fractionate (10 ml) and freeze at –20°C

- **Buffer for conservation and transport of umbilical cords**

- PBS 50 ml
- Colimycine 1M 1 ml
- Penicilline G 1M 1 ml

• Cord manipulation and culture:

- Tidily cut the 2 ends of the cord with scalpel
- Introduce a cannula at each extremity of the vein (the widest vessel) and tightly maintain it with string
- Wash the cord with PBS using the syringe of 50 ml
- Inject the collagenase (0.2 %) at one end of the vein using the syringe of 30 ml
- When leaking out the other end, tightly clamp it with the surgical clip
- Maintain the syringe and protect the cord's ends with "clean" aluminium foil
- Incubate the cord 10 min in the physiologic serum at 37 °C
- Upon the sterile envelope of gloves, gently squeeze the cord
- Fill up a sterile falcon of 50 ml with 10 ml of "full" culture medium
- Collect the cells in this falcon by washing the vein with 40 ml of PBS
- Centrifuge at 900 rpm for 10 min
- Carefully discard the supernatant and suspend cells in ~14 ml of "full" culture medium
- Dissociate the cells (aspiration and repulsing X 3) with the syringe of 30 ml with needle
- Add 2 ml of the cell suspension in each fibronectin-coated Petri dish

Cultures are placed in a humidified 95% air and 5% CO₂ atmosphere at 37° C.

The following day, non-adherent cells are removed by changing the culture medium.

The culture medium is changed every two days and confluency is typically achieved in 6-8 days with "cobblestone appearance" in optical microscopy ($\sim 1 \cdot 10^6$ cells/dish).

