OsteoCord: Bone from Blood

European Union Project 018999

STANDARD OPERATING PROTOCOL

MSC ISOLATION FROM FRESH UMBILICAL CORD BLOOD

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(derived from Bieback et al. Stem Cells 2003)

Material plus reagents

- PBS w/o Ca/Mg (#L1820 from Biochrom AG)
- EDTA from Sigma
- Ficoll-Hypaque Plus solution #17-1440-03 from GE-Helathcare
- MSCGM single quots (#PT-4105 (PT-4106E MCGS; PT-4107E L-Glutamine; PT-4108E Pen/Strep) from Cambrex used to complete DMEM)
- DMEM with 1g/l glucose without L-Glut (#BE12-707F from Cambrex)
- Fetal bovine serum for flask precoating
- 75cm2 tissue culture flasksNunc surface (#156499 from Nunc)
- Trypsin/EDTA 0.05%/0.02% in D-PBS (#L11-004 from PAA Laboratories)

Collection of UCB

UCB units from full term deliveries are collected from the unborn placenta with informed consent of the mothers [13]. A bag system containing 17 ml of CPD (citrate phosphate dextrose) anticoagulant within the collection bag is used (Cord Blood Collection System, Eltest, Bonn, Germany). The units are stored at 22 ± 4℃ prior to processing.

Isolation and culture of adherent cells from UCB

To isolate mononuclear cells (MNC), each UCB unit is diluted 1:1 with PBS/2mM EDTA and carefully loaded onto Ficoll-Hypaque solution.

In the meantime, tissue culture flasks are coated with FBS (1ml-T25; 2ml-T75 and 5ml- T175 flask): FBS is added and evenly distributed on the flask bottom. Flasks are incubated at room temperature until use (they can be stored at 4℃ af ter removing the FBS).

After density gradient centrifugation at 435 x g for 30 min at room temperature, MNC are removed from the interphase and washed two to three times with PBS/EDTA. Nucleated cell counts are

performed using an automated cell analyzer (Cell-Dyn 3200, Abbott, Wiesbaden, Germany, www.abbott.de).

UCB derived MNC are set in culture at a density of 1 x 106/cm^2 in MSCGM medium (DMEM + MSCGM single quots).

After overnight incubation at 37℃ in humidified at mosphere containing 5% CO_2, non-adherent cells are removed and fresh medium is added to the wells. Cultures are maintained and remaining non-adherent cells are removed by complete exchange of culture medium every seven days. Culture wells are screened continuously to get hold of developing colonies of adherent cells. Colonies of fibroblastoid cells occur after 16 to 20 days after initial plating are recovered by using Trypsin/EDTA.

Passaging of cells

Complete medium is removed from the flasks. The cells are washed once with 5-10ml PBS. Trypsin/EDTA is added (1ml-T25; 2ml-T75 and 5ml- T175 flask) and incubated for 2-10 minutes until all cells detach.

An equal volume of MSCGM is added to neutralize Trypsin. An aliquot is used for manual cell counting with trypan blue dye exclusion to determine vitality.

Recovered cells were replated at a density of 200 cells/cm^2 as passage 1 (P1) cells and thereafter.