OsteoCord: Bone from Blood

European Union Project 018999

STANDARD OPERATING PROTOCOL

MSC ISOLATION FROM BONE MARROW

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NB: Put the scoop and scissors on tissue following sterilisation by immersion in 70% IMS and leave them for 10 minutes to dry before removing and mincing the trabecular bone.

Before isolating the mesenchymal stem cells (MSCs), check whether the cells of the femoral head are mycoplasma-free. To do this, take a scoop of marrow, add 1ml PBS, suspend, add 50ul to a slide and make a smear. Fix cells in 100% ethanol, air-dry the slide and add a drop of dapi (1ug/ml in PBS). Cover with coverslip and view under fluorescence.

Isolation procedure of MSCs

1. Remove as much trabecular bone as possible from the femoral head and collect into 10 ml α-Minimum Essential Medium (α-MEM, Invitrogen, cat no. 32561-029) with 1% penicillin/streptomycin (p/s, Invitrogen, cat no. S15140-122).

2. Mince trabecular bone with scissors, allow large bone fragments to settle and transfer the medium containing cells to a new tube. Add fresh medium to the bone fragments and repeat the mincing procedure twice more, collecting the supernatant each time into separate tubes. Finally, add fresh medium to the bone fragments, vortex for 1 minute and collect the medium.

3. Spin tubes containing cell suspension at 450 g for 5 minutes.

4. Resuspend pelleted cells in 16 ml α-MEM + 1% p/s and filter through a 70 μm cell strainer (BD Falcon, cat no. 352350).

5. Lay cells carefully over 12 ml Ficoll-Paque Plus (Amersham Biosciences, cat no. 17-1440-03, density 1.077 g/ml) in a 50 ml Falcon tube and spin at 350 g for 30 minutes. Reduce centrifuge brake to 3.

6. Remove as much of supernatant as possible avoiding the white mononuclear fraction above the Ficoll layer and then collect the white mononuclear layer using a plastic Pasteur pipette and transfer to a new tube.

7. Wash mononuclear cells with 10 ml sterile washing buffer (5 mM EDTA, 0.2% BSA in PBS) with centrifugation at 450 g for 5 minutes.

8. Resuspend cells in 10 ml α-MEM + 1% p/s + 15% batch tested foetal bovine serum (Invitrogen, cat no. 10270-106) and seed in a T75 flask.

9. Leave cells to settle for 3-4 days, then change the medium to remove the non-adherent cells. Change medium every 3-4 days thereafter. After 10 days (or when between 20-50% confluent), harvest the cells and seed at a density of 1000 cells/cm^2.

10. Once confluent, passage cells 1-3 in α-MEM + 1% PIS + 15% batch tested foetal bovine serum.

11. MSCs underwent passage twice prior to FBS batch testing.