



A Detailed Method For The Production Of Bone Marrow Derived Mesenchymal Stem Cells For Clinical Use.



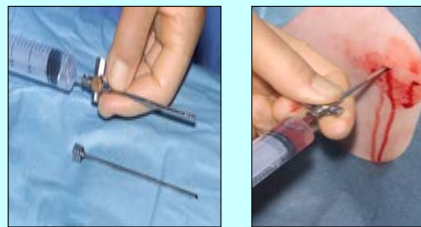
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Introduction.

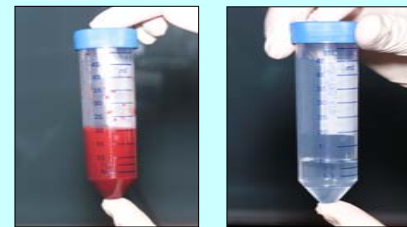
- Traditional ACI as described by Brittberg et al remains a hotly debated technique some eleven years after the first report.
- Recent variations include the replacement of the periosteal patch with a collagen membrane and the implantation of cells seeded within a matrix.
- One general criticism of ACI has been the unavoidable requirement to harvest macroscopically normal cartilage.
- It has been suggested that culture expanded, bone marrow derived, mesenchymal stem cells could provide an alternative to culture expanded chondrocytes. (*Redman et al 2005)
- The Oswestry MSC culture technique was developed during a research phase where bone marrow aspirates were harvested from the iliac crests of consenting patients undergoing spinal fusion.
- The initial work focussed on optimising the centrifugation speed, duration of spin and loading techniques for the Lymphoprep density gradient.
- The seeding density, critical first "feed" step and subsequent sub-culturing regime were then investigated.
- The perfected technique is now described.

Methods.

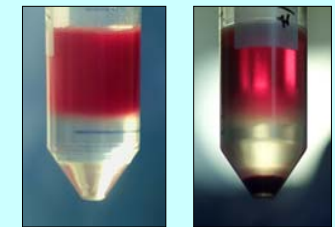
Bone marrow is typically harvested from the posterior iliac crest using a Wolff needle attached to a 20 ml syringe containing 2 mls of heparin (5000 units per ml). No more than 2mls of bone marrow are aspirated from each of 5 adjacent sites.



Once harvested, the aspirate is thoroughly mixed with 10mls sterile Phosphate Buffered Saline in a Blue Max centrifuge tube. With as little delay as possible, the diluted aspirate is split in to two equal volumes which are then carefully layered over pre-prepared 10ml aliquots of Lymphoprep.

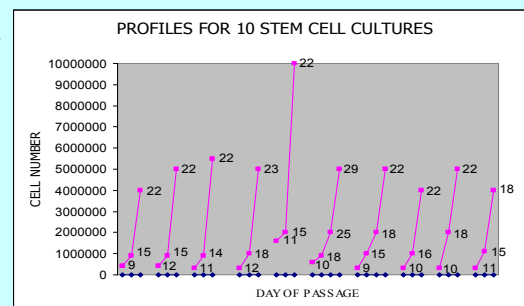


The tubes are centrifuged at 900g for 20 minutes, after which time a "buffy coat" layer should be seen in each tube, these are harvested and pooled using a 5ml syringe and 19 gauge needle. The pooled buffy coat layers are added to 10mls of DMEM/F12 culture medium and centrifuged at 750g for 10 minutes.



The cells are plated out into T75 tissue culture flasks in 15 ml DMEM/F12/15% autologous serum at a seeding density of 20 million cells per flask. The flasks are incubated for twenty four hours at 37 °C, 5% CO₂ and 90% humidity. After this time the non adherent cells are removed and discarded, the adherent cells are washed twice with 10mls PBS and fresh culture medium (DMEM/F12/15% serum) is added. The flasks are again incubated as described. Three days later, the flasks are "fed" with fresh medium and are examined with an inverted microscope, the presence of fibroblast like cells should be noted and at this stage it is usual to also observe adherent white blood cells. The flasks are fed and examined every three to four days until the fibroblastic cells have proliferated sufficiently to cover 60-70% of the flask base, they require passaging at this stage.

Results and Conclusions.



Ten consecutive stem cell cultures have been successfully established using the described technique. Our initial experience has been with bone marrow harvested from patients with persistent non-union fractures. Implantation of the culture expanded MSCs has demonstrated no adverse effects coupled with enhanced osteogenesis in these difficult to treat patients.

We are now planning a trial using culture expanded chondrocytes vs. culture expanded MSCs in patients with osteochondral knee defects.