Single Enzyme Isolation and Characterization of Human Placenta Mesenchymal Stem Cells

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Introduction: Human placenta is a readily available, highly vascular tissue with abundant sources of mesenchymal stem cells (MSC). HP-MSCs can be used in conjunction with biomaterials to study mechanisms of tissue repair, and have potential for tissue regeneration.

The aim: Isolation and characterisation of human placenta derived MSCs for use in wound repair and identification of the expression pattern of stem cell markers Stro-1, CD29, CD34, CD44, CD90, panCD45, CD73, CD104, CD133, CD166, and previously unreported Sox-2 (SRY-related HMG-box (SOX)) & CD18 (Integrin β-2).

Materials and Methods:

- Full Term human term placentae were donated by Watford general hospital (n=3)
- Placental cotyledons were isolated, washed with Hank’s Buffered Salt Solution (HBSS), finely minced and sieved through 250 µm metal sieve to collect tissue fragments
- Collagenase IV (3000 U/ml) digestion for 45 minutes.
- Neutralisation and removal of enzyme and resuspension of pellet in MCDB 131 media
- The cell suspension was plated onto gelatin (1%) coated culture plate. MCDB 131 media containing 50U/ml Penicillin, 1 µg/ml Hydrocortisone, 50 µm Dibutyryl cyclic adenosine monophosphate, 5ng/ml EGF and 20% human heat inactivated serum media was then added
- Cells were incubated at 37 °C, CO2 incubator to grow
- To investigate the pluripotent nature of the cells osteogenic, adipogenic and chondrogenic differentiation of the cultures were carried out
- 2000 passage 3 cells were seeded on 6 well 1% gelatin coated plates. Once confluent differentiation media was used to encourage cell differentiation
- Alizarin red staining, Oil Red O and Alicant Blue staining were carried out on day 21 to confirm osteoblastic, adipogenic and osteogenic differentiation of the cells
- Immunocytochemical analysis was performed to assess the expression of selected stem cell markers
- Cells were incubated for 2hrs at room temperature with mouse anti human CD29, CD90, CD34, CD45, CD44, CD104, CD18, CD73, CD166, Stro-1 and Sox-2 antibodies (1:200 dilution)

Results:

- Adherent colonies were observed on day 1
- Cell sprouting as early as day 3 and confluent culture wells of spindle like cells by day 14
- Presence of mineralised nodules in osteogenic differentiated group
- Presence of lipid droplets in adipogenic differentiation group
- Chondrocytes’ extra cellular matrix staining in chondrogenic group
- Osteogenic, adipogenic and chondrogenic differentiation of the isolated cells confirmed their stromal nature (Fig 1)
- Immunocytochemistry results were positive for Stro-1, Sox-2, CD133, CD 166, CD104, panCD45, CD90 and CD44 and negative for CD34 and CD18 suggesting an MSC phenotype (Fig 2)

Conclusions: Mesenchymal stem cells can successfully be isolated from placenta using a single enzyme, (collagenase IV).
The isolated cells can be used for differentiation into various cell types for use in tissue regeneration purposes.