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 resuspention 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 Alician Blue staining were carried out on day 21 to confirm osteoblastic, adipogenic and osteogenic differentiation of the cells

➢ Immunocytochemical analysis was performed to asses the expression o f selected stem cell markers

➢ Cells were incubated for 2hrs at room temorature 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 diffrentiated group

✓Presence of lipid droplets in adipogeneic differentiation group

✓Chondrocytes' extra cellular matrix staining in chondorgenic 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, pan CD45, CD90 and CD44 and negative for CD34 and CD18 suggesting an MSC phenotype (Fig 2)

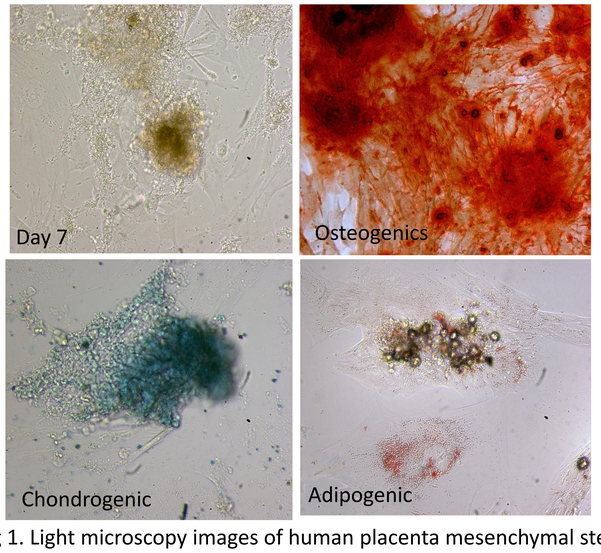

Fig 1. Light microscopy images of human placenta mesenchymal stem cells differentiated into osteoblats, chondrocytes and adipocytes.20x magnification images.

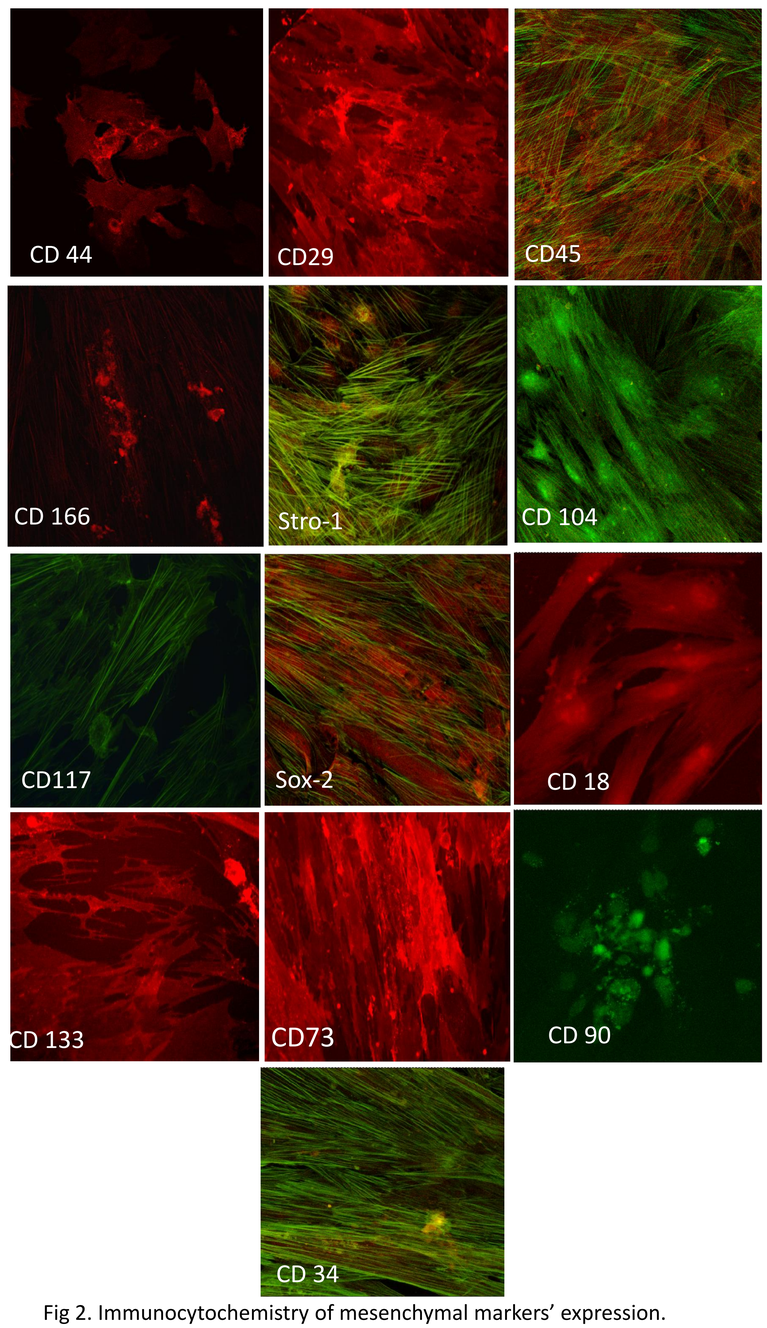

Fig 2. Immunocytochemistry of mesenchymal markers' expression.

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.