Supplementary material

Adipocytic and chondrogenic differentiation

To promote chondrogenic differentiation, 1×10^6 mesenchymal cells were centrifuged to form a pelleted mass and the cells were cultured without serum and with 10 ng/ml transforming growth factor-β3 (TGF- β3) (Peprotech EC Ltd, London, United Kingdom). Adipogenic differentiation was induced in the expanded mesenchymal cell cultures by treating 30 000 cells in 48 well plates with 0.5 mM 1-methyl-3-isobutylxanthine (Sigma Aldrich), 1μM dexamethasone (Sigma Aldrich), and 10μg/ml insulin (Sigma Aldrich). The MSCs were also phenotypically identified by flow cytometry using CD29 (Abcam, Cambridge, United Kingdom), CD90, CD34 and CD45 markers (Affymetrix, Santa Clara, California).

Cell culture and characterisation of bone marrow-derived cells

The isolated rat MSCs differentiated to multiple lineages and they positively differentiated to osteoblasts, adipocytes and chondrocytes. Osteogenic differentiation was evident by calcium deposits which stained with Alizarin Red (Sigma Aldrich). Under adipogenic conditions for three weeks, the formation of intracellular micro-droplets stained positive for Oil Red O. After 21 days in chondrogenic media, the cells were positively differentiated to chondrocytes, which was confirmed by Alcian Blue (Sigma Aldrich) staining for cartilage. They were also shown to positively express CD29 and CD90, and negatively express CD34 and CD45 (Fig. a).

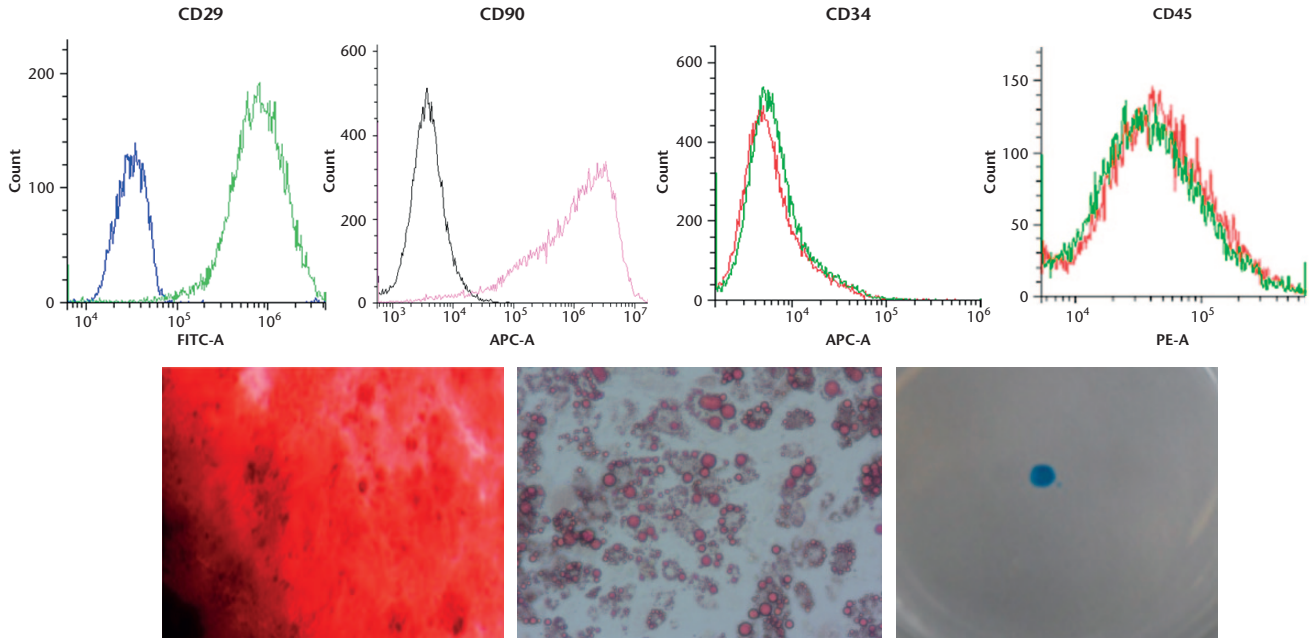

Fig. a

Characterisation of bone marrow mesenchymal stem cells from young rats using flow cytometry to show positive expression of CD29 and CD90, negative expression of CD34 and CD45 (top row) and positive stain for Alizarin Red for osteogenic differentiation (bottom left). Oil Red O stain for adipogenic differentiation (bottom centre) and Alcian Blue stain to show chondrogenic differentiation (bottom right).