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Editorial

Chemical Induction of Human under Various Differentiation Conditions Coronado

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INTRODUCTION

Mechanical isolation of adipose derived mesenchymal stromal/stem cells Adipose tissues obtained from human liposuction were processed using Lipogems, which is a class II, single-use medical device for the processing of aspirated adipose tissue. The unit consists of a transparentplastic cylinder with filters and beads for the micro fracturing of adipose tissue. Lipoaspirates from healthy individuals donated by Dr. Lindsey Coombs were received and added into the Lipogems device. Permission for the collection of tissue and subsequent research was unnecessary in Los Angeles because fat was collected under an institutional review board exemption as medical waste. The device further processed the lipoaspirate by washing and mechanically disrupting it into micronized adipose tissue. The resulting micronized adipose tissue was collected and seeded in tissue culture treated plastic-ware at proportions of 2 mL per 25 cm2 and placed in a 5% CO2 humidified incubator at 37°C for 3 hours, then 3 mL of Mesempro RS® media was added. Fresh media was added (3 mL) every 3 days without replacing the old media until day 9. On day 9 a total media replacement was performed without disturbing the attached pieces of micro-fat. On day 12, complete media replacement was repeated after removing non-adhered pieces of microfat leaving only attached ASCs in the flask. The cells were trypsinized (0.25%Trypsin-EDTA Gibco Cat.#25200056) when they reached confluency, washed, and sub-cultured for further analysis. Characterization of ASCs by flow cytometry Collected cells were washed in running buffer and centrifuged at 200 × g, 4°C, for 5 minutes and re- suspended in BlockAid (Thermo Cat. # B10710) × 10 for a final concentration of 15 cells/test/100 µL. Cells were incubated in BlockAid for 15 minutes at 4°C, followed by addition of primary-conjugated antibodies

according to manufacturer's instructions, and incubated at 4°C, in the dark, for 1 hour. Stained samples were washed with 1 mL running buffer (200 × g for 5 minutes at 4°C) twice; then reconstituted in 200 µL/sample running buffer before measurements were taken. Flow cytometry analysis was performed with a CytoFLEX 2-L (Beckman-Coulter). Commercially available adipose derived stem cells from lipoaspirates (Thermo Cat. # R7788115) were used as positive controls. Results were quantitated by CytExpert software (Beckman-Coulter). Antibodies used for flow cytometry: Anti-HLA-DR-FITC- Class II (Molecular Probes #MHLDR01), CD44-FITC (Thermo #MEM85), CD271-VioBright FITC (MACS #130-110-115), CD166-PE (MACS #130-106- 618), CD105-PE (StemCell Technologies #60039PE), CD90-PerCPCy5.5 (Molecular Probes #A16425), CD19-PE (StemCell Technologies #60005PE), CD11b-PE (StemCell Technologies #60040PE), CD34-PE (StemCell Technologies #60013PE), CD45-PE (StemCell Technologies #60018PE), CD73-PE (StemCell Technologies #60044PE). Trilineage differentiation Multipotency of isolated mesenchymal stem cells from adipose tissue aspirates was confirmed by trilineage differentiation adipocyte, osteocytes, and chondrocytes), phenotypic expression, and by flow cytometry. In order to differentiate mesenchymal stem cells into chondrocytes, it was necessary to generate micromass cultures, by seeding 5-µL droplets × 10of cell solution (1.6 7 cells/mL) in a 6 well plate (approximately 10-15 droplets per well) and culturing for 2 hours under standard culture conditions. Prewarmed StemPro Chondrogenesis Differentiation Kit (Thermo Cat.#A1007101) media was added to the culture vessels and incubated at 37°C, 5% CO2.

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