Generation of mesenchymal cells from periorbital adipose tissue

The periorbital adipose tissue was harvested from the intact eyeballs of patient with advanced stages of familial retinoblastoma and undergone primary enucleation. The human tissue samples were collected with proper informed consent of the patients and with the approval of the Institutional Ethics Committee (IEC). The tissues were aseptically processed to establish adipose-derived mesenchymal cell cultures (ADMCs). Briefly, the adipose tissue was collected in normal saline (0.9% NaCl), rinsed multiple times with 1X DPBS containing double strength antibiotics to surface sterilize and also to remove the blood cells and excess fat in the tissue. Further, the tissue was finely minced, and incubated with Collagenase Type I (0.1%) for 2 hrs at 37°C with intermittent shaking. The digestion was stopped by the addition of equal volume of DMEM/F-12 medium supplemented with 10% fetal bovine serum (FBS) followed by centrifugation at 500 *g* for 5 minutes. The cell pellet along with the partially digested tissues were plated in a T-25 flask containing the fibroblast growth medium (DMEM/F-12 supplemented with 10% FBS, 100 nM Hydrocortisone, 1x Non-Essential Amino Acids, 5 ng/mL of recombinant human bFGF, 10 ng/mL of recombinant human EGF) and cultured in an incubator at 37°C with 5% CO_2 supply. The cultures were left undisturbed for the first two days to support cell adhesion and fresh media change was done on alternate days thereafter. The cultures reached 70-80% confluence in about 7-10 days and are further passaged using TrypLE and expanded at a split ratio of 1:3, up to passage 5. A portion of ADMCs were cryopreserved at every passage and the cells at passage 4 was used in reprogramming experiments.