

### **1. Isolation and culture of DFSCs**

Briefly, the DFSCs were obtained from un-erupted molars of swine. The dental follicles were aseptically dissected and placed in phosphate buffered solution (PBS). The tissue blocks were incubated in DMEM supplemented with 15% fetal bovine serum (FBS)(Hyclone, USA) in a humidified atmosphere at 37°C and 5% CO<sub>2</sub>. After being cultured for about 7 days, the DFSCs were visualized under a phase-contrast inverted microscope (Nikon, Japan). Cell culture medium was renewed every 3 days and cells from passages 3-5 were used for the subsequent experiments.

### **2. Osteogenic differentiation**

A total of  $1 \times 10^5$  sDFSCs were seeded into each well of a six-well plate. At 80% confluence, sDFSCs were cultured with osteogenic medium containing 10% FBS, 5 mM L-glycerophosphate (Sigma, USA), 100 nM dexamethasone and 50 mg/ml ascorbic acid (Sigma, USA) for 15 days. The control group was cultured in DMEM with 10% FBS only. The medium was renewed every two days. After being cultured for 15 days, cells were fixed in 4% paraformaldehyde for 10 min, washed twice with PBS and then incubated in 0.1% alizarin red solution (Sigma, USA) in Tris-HCl (pH 8.3) at 37°C for 30 min. After being washed twice in PBS, cells were routinely visualized and photographed under a light microscope (Nikon, Japan).

### **3. Adipogenic differentiation**

Adipogenic medium was consisted of DMEM supplemented with 10% FBS, 2 mM glutamine(Sigma, USA), 100 U/ml penicillin/streptomycin (Hyclone, USA), 100 mM ascorbic acid, 0.5 mMmethylisobutylxantine, 0.5 mM hydrocortisone and 60

mMindomethacin (all from Sigma, USA). The cells were treated as described above. After being cultured for four weeks, the cells growing under adipogenic conditions were washed twice with PBS and fixed with 70% ethanol for 15 min. Oil red O (Sigma, USA) staining was performed.

#### **4. Neurogenic differentiation**

At 80% confluence, sDFSCs were cultured in neurogenic medium containing 2% dimethyl sulphoxide(DMSO), 200 mMbutylatedhydroxyanisole, 2 mMvalporic acid, 10 mMforskolin, 1 mMhydroxycortisone 2 mM L-glutamine (all from Sigma, USA), 5 mg/mL insulin (Gibco, USA) and 25 mMKCl (Kelong, China). After 4 h, cells were analyzed by immunocytofluorescence for expression of the neural cell marker,  $\beta$ III-tubulin (Abcam, USA). Images were fixed and analyzed under a fluorescence microscope (Leica Optical, Germany).