BB05

CHONDROGENIC, NEUROGENIC AND OSTEOGENIC ANALYSIS OF DENTAL PULP STEM CELLS

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INTRODUCTION

The main purpose in stem cell research is to find available source for getting progenitors cells because they able to give rise to another cells. Among these cells, dental pulp, entrapped with the pulp chamber, is a rich site for stem cell collection. These stem cells are called dental pulp stem cells. There are benefits to use dental pulp stem cells (DPSCs) as a new source of stem cell. The first study in dental stem cell showed that stem cells isolated from dental pulp was able to produce ectomesenchymal stem cells from exfoliated deciduous teeth (Arthur et al. 2008; Gronthos et al. 2000; Miura et al. 2003). The purpose of this study was to investigate the neurogenic, chondrogenic and osteogenic capacity of mouse dental pulp cells and identify gene expressions for dental pulp stem cell differentiation.

METHODOLOGY

Dental Pulp Cell Culture Healthy teeth were extracted from 64 mouse aged 6-8 week. Surfaces of the extracted teeth were cleaned with PBS. Healthy Teeth were place in sterile PBS solution containing1% (v/v) penicillin /streptomycin and placed in 4° C. Dental pulp was extracted completely in sterile conditions. The pulp tissue gently separated from the crown and root, and then digested in a solution of 4 unit/ml collagenase type I for 1 h at 37◦C as previously reported. The dental pulp sample from each individual was pooled, and pipeting single –cell suspensions with alpha-modified Eagle's medium (AMEM). The released cells were seeded into T25 culture dishes and cells were treated with α-MEM containing 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin. Followed by incubation at 37°C and 5% CO_2, After 48 hours, the cells washed with PBS to remove other cells.

To induce the cartilage differentiation, dental pulp stem cells ($1x10^5$ cells/each well) were cultured in chondrogenic medium (Zen-Bio, Inc) for 21 days. After 21 days of treatment with the chondrogenic medium, the cells stained with Toluidine blue.

Alkaline phosphatase activity was assayed by enzymology. The DPSCs were seeded at a density of 1×10^5 cells/well in 96-well plates. After 1, 3, 5, 7, 10, and 14 days of cultured with differentiate medium and control medium, the ALP activity of DPSCs was detected using an ALP assay. The control medium and differentiate medium was removed every two days. After washing the cells with PBS the cells were incubated in 0.5 M NaNO3-Na2Co3 buffer (pH 10.0) containing 1%(v/v) Triton X100, 20 mM MgSO4 then 600 mM P-Nitrophenyl Phosphate was added as substrate for 30 minutes at 37°C. 1.5 M NaOH was added to stop the reaction. Absorbance at 405 nm was measured with a spectrophotometer.