Successful isolation, in vitro expansion and characterization of stem cells from Human Dental Pulp

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BACKGROUND

Recent studies have shown that mesenchymal stem cells isolated from post natal human dental pulp, (Dental pulp stem cells-DPSCs) which is from permanent teeth and SHED (stem cells from human exfoliated deciduous teeth), the Periodontal ligament stem cells (PDLSC) and Stem cells from root Apical papilla(SCAP)have the potential to differentiate into cells of a variety of tissues including heart, muscle, cartilage, bone, nerve, salivary glands, teeth etc1,2,3,4. This multipotential ability of DPSCs is being researched for clinical application for treating a variety of diseases like myocardial infarction, muscular dystrophy, neuro-degenerative disorders, cartilage replacement, tooth regeneration and for repair of bone defects to mention a few. Moreover, the isolation of stem cells from teeth is minimally invasive, readily accessible and the non immunogenic characteristic of dental stem cells has paved the way for efforts to store the exfoliated deciduous teeth or milk teeth which is usually discarded, for use in the future. In this study we have isolated and expanded in vitro, the cells obtained from human dental pulp.

METHODS AND RESULTS

After obtaining written informed consent, 24 teeth that were extracted for therapeutic or cosmetic reasons from 16 patients were used in this study. The specimens were transported from the clinic to NCRM lab taking 6 to 48 Hrs. For removal of the pulp tissue, the teeth were split obliquely at the Cementoenamel junction and the pulp tissue was isolated using brooches. The extracted pulp tissues were subjected to digestion using Collagenase type-I and type II at 37°C for 15-30 minutes. The digested cells were filtered with 70µm filter and centrifuged at 1800 rpm for 10 minutes. The pellet was then suspended in Dulbecco’s modified Eagle’s medium (DMEM)/Ham’s F12 supplemented with 15% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, and 2 mM nonessential amino acids. Cell counting was done by Trypan Blue dye exclusion method and the cells were seeded in 6 well culture plates. The plates with cells were incubated at
37°C with 5% CO2 for varying periods from 14 days-28 days. The cells were observed daily and media change was done every three days.

RESULTS

Viable Dental Pulp tissue-cells were obtained after transportation of up to 48 hrs and the in vitro growth of cells was initially slow but colonies were identified from the 10th day onwards. The cells were harvested at different intervals of 14-28 days for each sample based on their growth and subjected to H & E staining. The H & E staining of the cultured cells of all the samples showed positive results.

CONCLUSION:

We are able to transport extracted teeth and derive viable dental pulp tissue cells after enzymatic digestion and multiply them in culture after a maximum of 48 hrs after transportation. The cells could be grown in culture with a morphology resembling dental pulp stem cells while in culture expansion and in H&E studies. Further characterization of the cells is necessary to confirm their Stemness.

REFERENCES


