

ORIGINAL ARTICLE

Stem cells from childrens' teeth

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Keywords

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Abstract The aim of this study was to isolate stem cells from dental pulp of primary molars and incisors to be used as possible source for tissue engineering. Human primary molars and incisors were collected from subjects aged 4-7 year-old under standardized procedures. Within 24 hours, the tooth was cut at the cemento-enamel junction using hard tissue material cutter. The dental pulp tissue was extracted, digested and then cultured in Alpha Modified Eagles's Medium (α -MEM) supplemented with 20% FCS, 100 mM L-ascorbic acid 2-phosphate, 200 mM L-glutamine and 5000 units/ml Penicillin/Streptomycin. The cells were observed daily under the microscope until confluence. Children's tooth pulp- derived progenitor cells were found positive for stem cell markers CD105 and CD166, which are consistent with the finding for mesenchymal stem cells (MSCs) from bone marrow.

Introduction

Stem cells are generally defined as clonogenic cells capable of both self-renewal and multi-lineage differentiation. Post-natal stem cells have been isolated from various tissues, including bone marrow, neural tissue, skin retina and dental epithelium (Gronthos *et al.*, 2000).

Stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells which do not normally replicate themselves, stem cells may replicate many times. When cells replicate themselves over many times it is called proliferation. A starting population of stem cells that proliferates for many months *in vivo* can yield millions of cells.

If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal. Dental pulp cells provide great support for nerve cells lost in Parkinson's disease and could be transplanted directly into the affected parts of the brain (Nosrat *et al.*, 2004).

Thus, this study is aimed to isolate stem cells from dental pulp of primary molars and incisors to be used as possible source for tissue engineering.

Materials and methods

Extraction of teeth

Teeth were extracted from children aged 4-7 year-old under standardized procedures. Ethical approval was obtained from Institutional Review Board (FWA Reg. No: 00007718, IRB Reg. No: 00004494, Period: 06/02/07-29/10/07). The teeth were cut at cemento-enamel junction using hard tissue material cutter. The sectioned teeth were then briefly immersed in 75% ethanol followed by Dulbecco's Phosphate Buffered Saline (DPBS). Later, the teeth were transferred to another beaker containing DPBS before the extraction of the dental pulp tissues was done in a class II safety biocabinet.

Isolation of teeth stem cells

Sterile tissue forceps, sterile tooth forceps and medium size barb roach were used when to extract the dental pulp tissue. The dental pulp tissues were put into a 15ml tube containing PBS and penicillin-streptomycin, and spinned down at 2000 rpm for 5 minutes. The supernatant was discarded and then the dental pulp tissues were digested using digestive solution containing 3mg/ml of Collagenase Type 1 and 4mg/ml of Dispase and incubated in a 5% CO₂ incubator at 37°C for one hour.

A 70 μ m cell strainer was used to disaggregate the digested and undigested pulp tissue. The digested tissue was put into 50ml tube and centrifuged at 2000 rpm for 5 minutes.

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The supernatant was discarded, then 1ml of culture medium (α MEM supplemented with 20% FCS, 100 mM L-ascorbic acid 2-phosphate, 200 mM L-glutamine and 5000 units/ml Penicillin/Streptomycin) was added into the tube, resuspended and then poured into T-25 culture flask.

Culture of stem cells

About 4ml of the culture medium prepared as mentioned above was added to the flask and then incubated in a humidified atmosphere at 37 °C with 5% CO₂. After 24 hours, non-adherent cells were removed. The adherent cells were washed vigorously twice with Phosphate Buffered Saline (PBS) and were shaken to remove adherent debris and fresh complete medium was added. The medium was replaced every 3 to 4 days.

Immunocytochemical analysis

Immunocytochemical staining was used to confirm the expression of antigenic markers (Ikeda *et al.*, 2006). Immunostaining was performed by biotin- streptavidin – horseradish

peroxidase (HRP) complexed antibodies to detect primary antibody. MSCs early passages, positive control of human MSCs and negative control of human breast cancer cell line were plated in 4-well chamber slide with the density of 6×10^4 cells per well. After 80% - 90% confluence, cells were fixed in absolute cold methanol at 0° for 20 minutes and then blocked and incubated over night with primary antibodies. The monoclonal mouse anti-human endoglin CD105 with dilution 1:25 and monoclonal mouse anti human CD166 with dilution 1:50 were used. Primary antibodies were detected by immunoperoxidase diaminobenzidine (DAB) secondary detection system.

Results

The proliferation of cells demonstrated that the adherent cells obtained from the stem cells of children's teeth have a durable and self renewing capability. Characterization of MSC derived from children teeth demonstrated by immunocytochemistry using phenotypic markers CD105 and CD166 antibodies showed positive staining as shown in Figure 1 and 2.

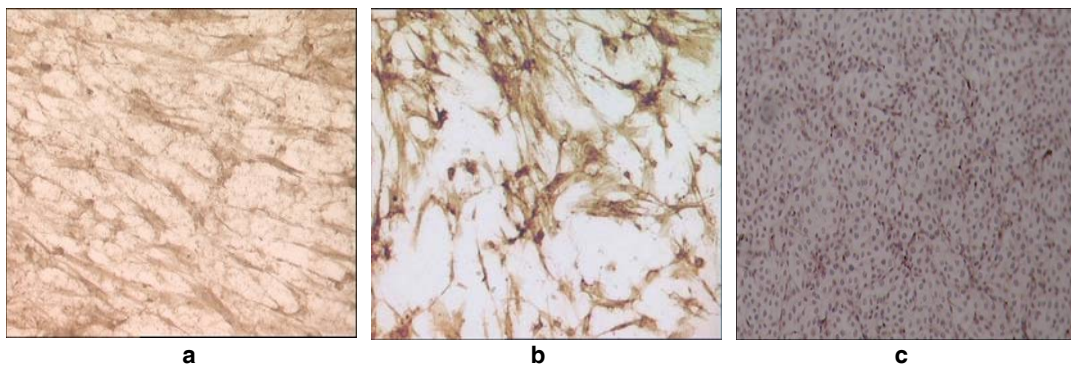


Figure 1 Characterization of childrens' teeth-derived MSCs by immunocytochemistry staining using primary CD105 antibody viewed at 10x. The CD105 was expressed on positive control (A) and children's teeth – derived MSCs (B). No expression on negative control (C).

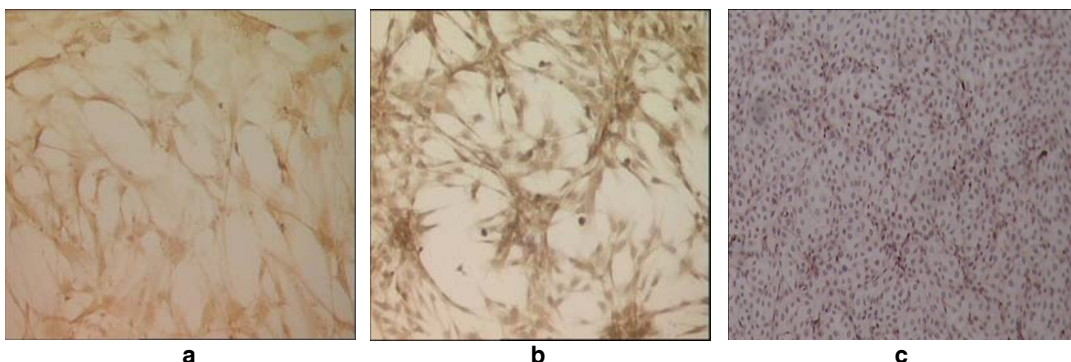


Figure 2 Characterization of childrens' teeth-derived MSCs by immunocytochemistry staining using primary CD166 antibody viewed at 10x. The CD166 was expressed on positive control (A) and children's teeth – derived MSCs (B). No expression on negative control (C).

Discussion

Molar and incisor deciduous teeth used in this study have been collected from very young children with the aim of yielding a highly proliferative cell population that are vigorously ready to differentiate. The most important factor to consider is that the proliferation and differentiation of undifferentiated MSC reduced with age. This study demonstrated that the dental pulp from deciduous teeth contains a living pulp that when cultured under specific conditions can proliferate and showed typical mesenchymal stem cells characteristics. The human deciduous teeth developed intra-uterine and this may contribute to the fact that it contained stem cells that are considered "very young" and is capable to proliferate and differentiate into various cell types. This had been proven by Lee *et al.*, (2004), where they claimed that umbilical cord blood (UCB) may be an excellent alternative source of MSC because of the cells contained in UCB could be considered as "very young". Moreover, D'ippolito *et al.* (1999) demonstrated that the number and the differentiating potential of bone marrow mesenchymal cells decrease with age. Meanwhile, Justesen *et al.* (2002) claimed that MSC differentiation capacity to osteoblasts and adipocytes was maintained irrespective of donor age. Immunocytochemistry was performed to demonstrate the presence of a clonogenic cell population in dental pulp tissue by using cluster of differentiation prefix CD which identifies more than a thousand specific cell membrane molecules that are expressed on cells. In the present study, CD 105 and CD 166 were selected because they act as MSC marker, instead of STRO-1 and CD 45 which only act as

hematopoietic stem cell marker. Thus, the study on stem cells from childrens' teeth holds promise in its differentiation capacity to different tissues in future.

Acknowledgement

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