STEM CELLS FROM INFLAMED HUMAN DECIDUOUS DENTAL PULP: ITS CHARACTERISTICS AND POTENTIAL

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Background : Mesenchymal stem cells (MSCs) isolated from healthy deciduous dental pulp (SCD) holds a promising potential in therapeutic usage. Despite this, little knowledge is available on the basic characteristic and therapeutic potential of stem cells isolated from inflamed deciduous pulp

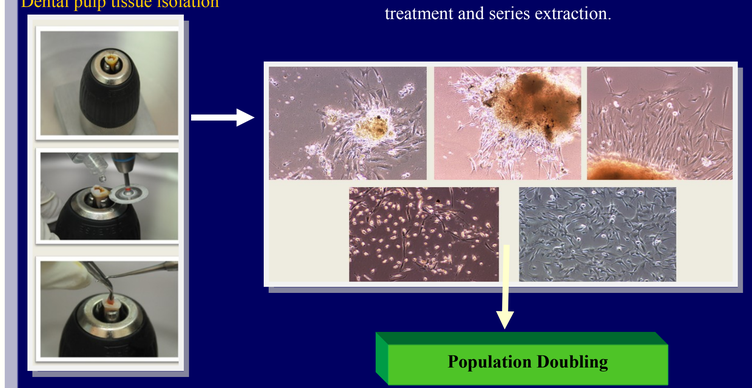
Aim : To determine the characteristic of the stem cells from the inflamed deciduous pulp (SCDIPs)

Materials and Method

Samples: The inflamed pulp tissues (n=3) were obtained from the teeth undergone pulpectomy procedures.

Vital tissues (n=3) from the permanent and deciduous pulp were collected from carious free teeth extracted for an orthodontic treatment and series extraction.

Dental pulp tissue isolation



Isolation and culture of dental derived stem cells

Prior to the commencements of the study, approval from the Medical Ethics Committee, Faculty of Dentistry, University of Malaya was obtained [DF: CD11201/0013 (P)]. The isolation and culturing method was carried out as described previously by Govindasamy *et al*., 2010a. In brief, inflamed extracted teeth (SCD and DPSC) were sterilized externally with Povidone iodine (Sigma Aldrich) before being cut to expose the roof of the pulp chamber. The dental pulp tissue was extirpated from the deciduous teeth (SCD, DPSC and SDCIP) by using barbed broaches and then minced into small fragments before digestion in a solution of 3 mg/mL collagenase type I (Gibco, Grand Island, NY, USA) for 40 min at 37°C. After neutralization with 10% of fetal bovine serum (FBS) (Hyclone; ThermoFisher Scientific Inc., Waltham, MA, USA), the cells were centrifuged and then seeded in culture flasks with culture medium containing DMEM Knock out basal media (Invitrogen, Carlsbad, CA, USA), 0.5% 10,000 mg/mL penicillin/streptomycin (Invitrogen), 1% 1x Glutamax (Invitrogen), and 10% FBS, with humidified atmosphere of 5% CO_2 at 37°C. Cells were trypsinized prior to 80% confluence and processed for subsequent passages. All experiments were conducted in subculture 3.

Mesoderm lineage differentiation

The cultures were initiated at a density of 1000 cells/cm^2 in 6-well plates and were grown to 80% confluence and subjected to differentiation into adipogenic, chondrogenic, and osteogenic lineages. Adipogenic lineage was stimulated by inducing the cells with 10% FBS, 200 mmol/L indomethacin, 0.5 mmol/L 3-isobutyl-1-methyxanthine (IBMX), 10 mg/mL insulin, and 1 mmol/L dexamethasone (all reagents from Sigma Aldrich). For chondrogenesis differentiation, cells were cultured in media supplemented with ITS+1 (Sigma Aldrich), 50 mmol/L L-ascorbic acid-2 phosphates (Sigma Aldrich), 55 mmol/L sodium pyruvate (Invitrogen), 25 mmol/L L-proline (Sigma Aldrich), and 10 ng/mL of transforming growth factor-beta (TGF-b) (Sigma Aldrich). The osteogenic differentiation was stimulated in a 3-week culture in media supplemented with 10% FBS, 10^{-7} mol/L dexamethasone, 10 mmol/L b-glycerol phosphate (Fluka, Buchs, Switzerland), and 100 mmol/L of L-ascorbic acid-2 phosphates.

Results

Figure 1: The adherent cells of dental origin displayed typical fibroblast-like spindle shaped morphology under microscope at SC3. The morphology were retained throughout.

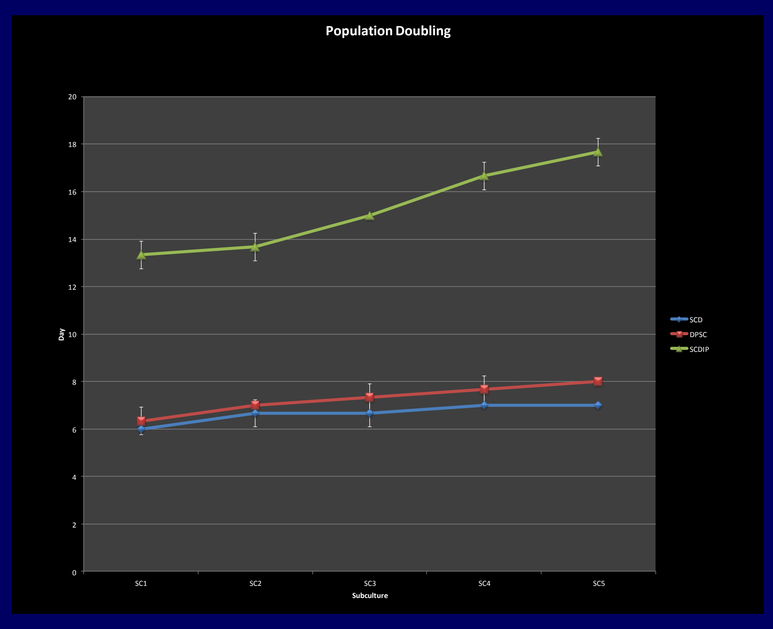


Figure 2: Population doubling of SCD, DPSCs and SCDIPs from subculture 1 to subculture 5. SCDIPs portrays a delayed proliferation in comparison to SCD and DPSCs.

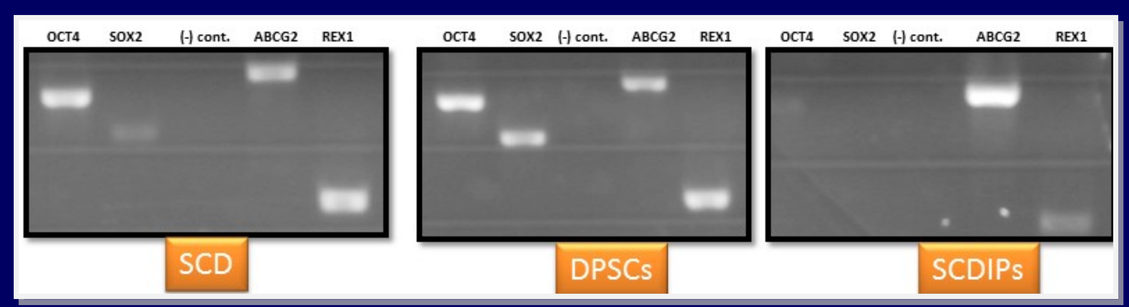


Figure 3: RT-PCR pluripotent gene expression in SCD and DPSCs showed similar gene expression pattern with low expression of SOX2 in SCD. High expression of ABCG2 were observed in SCDIPs and OCT4 and SOX2 were not expressed.

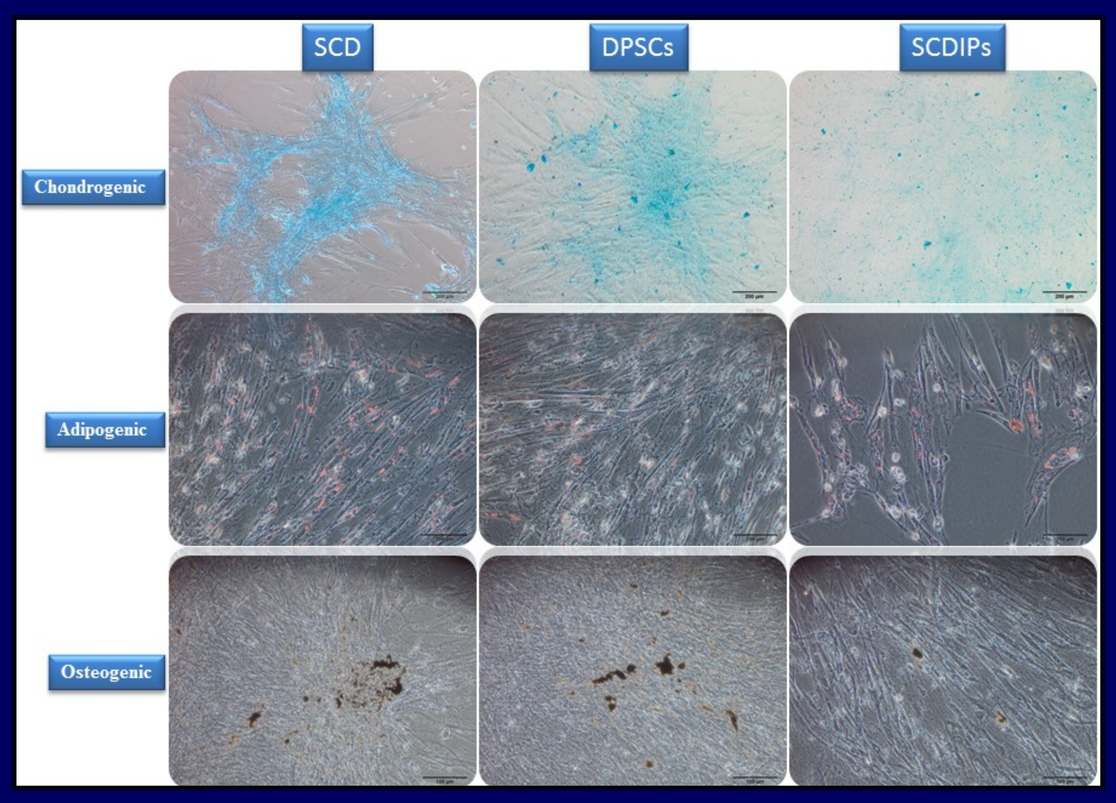


Figure 4: *In vitro* mesoderm differential potentiality of SCD, DPSCs and SCDIPs. Chondrogenesis was detected by the presence of proteoglycans stained with alcian blue at day 21. Adipogenesis was detected by neutral oil droplet formation stained with oil red O at day 21. Osteogenesis was confirmed by mineralized matrix deposition stained with von Kossa staining at day 21. All experiments were conducted at subculture 3. Results represent average of 3 culture replicates.

Discussion

In this study, stem cells from inflamed pulp (SCDIPs) were compared with the stem cells isolated from vital pulp tissues of permanent (DPSCs) and deciduous (SCD) teeth. All cell lines displayed a typical fibroblastic characteristics similar to those of bone marrow mesenchymal stem cells. Nevertheless, the proliferation of cells were higher in SCD and DPSCs. This is in an agreement with previous study that reported a lower success rate in establishing cell culture from inflamed DPSC in comparison to normal DPSC (Pereira *et al*., 2012). The lower proliferation rate most likely was influenced by upregulation of pro-inflammatory cytokines TNF-α and IL-1β in the inflamed site in which they will cause premature senesce of stem cells (Alongi *et al*., 2010).

Further, the expression of several pluripotent markers such as OCT 4 and SOX 2 were not detected in the inflamed pulp indicating that SCDIPs lost its stemness. Huang et al 2008 also have reported that SCDIPs does not express OCT4 and SOX2 in which there will be some limitation in maintaining the pluripotency of the cell in late subcultures. Surprisingly, we noticed the expression of ABCG2 in SCDIPs. It has been reported that, ABCG2 expression in mouse embryonic stem cells (ESCs) reduces accumulation of DNA-damaging metabolites in the cells, which helps prevent cell differentiation (Zeng et al., 2009) and hence we speculate that ABCG2 multifaceted function in SCDIPs which needed further investigations. Although SCDIPs may have appeared to lose some stem cell properties, it is able to undergo chondrogenic, osteogenic and adipogenic differentiation in which it still retains its multipotent differentiation capability.

Conclusion

SCDIPs could be an alternative for DPSC and SCD for future regenerative therapeutic usage with adequate *in vitro* and *in vivo* characterisation work conducted due to minimal ethical issues. However, further studies need to be carried out to understand the biological process of SCDIPs before using these cells as a potential source in regenerative medicine.

References

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