ORIGINAL RESEARCH PAPER

Volume-6 | Issue-12 | December-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 93.98

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

# DEMONSTRATION AND CHARACTERIZATION OF STEM CELLS IN PULP TISSUE OF HUMAN DECIDUOUS TEETH – AN EX - VIVO STUDY

Maxillofacial Surgery

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## ABSTRACT

**Aim:** This study was developed to demonstrate and characterize stem cells in pulp tissue of human deciduous teeth.

**Methods:** 10 Human deciduous teeth were collected and cut around the cementoenamel junction and single cell suspensions were obtained from pulp tissue established by enzyme digestion (3 mg/mL type I colagenase). Demonstration and characterization of stem cells with haematoxylin and eosin (H and E) staining as well as by immunocytochemistry staining using antibodies against CD44, CD133, OCT-4 and SSEA-4 surface receptors.

**Results:** Out of 10 samples all samples fulfilled the criteria for stem cells based on morphological characteristics studied on H and E stained smears. Out of 10 samples 8 samples were positive for cell surface marker CD44, 8 samples were positive for cell surface marker CD133, 8 samples showed nuclear expression of Oct- 4, 8 samples showed cytoplasmic expression of Oct-4 and 5 samples were positive for expression of SSEA-4

**Conclusions:** The findings of this study indicated that Hematoxylin and eosin staining demonstrates two different population of stem cells, which significantly differ in morphological characteristics. One being mesenchymal stem cells and other pluripotent stem cells. Presence of these two populations of stem cells is confirmed by immunocytochemical study with cell surface marker CD44, CD133, OCT-4, SSEA-4. This study provides a description of a stem cell population residing in human deciduous teeth and establishes the foundation for further studies to determine the efficiency of using deciduous teeth in cellular based therapies.

## KEYWORDS

Docetaxel, Adverse Drug Reaction, Risk benefit ratio.

### Introduction

Stem cells are undifferentiated embryonic or adult cells that continuously divide with the fundamental property of self-renewal or the ability to go through numerous cycles of cell division while maintaining the undifferentiated state. 1 Stem cells are the foundation cells for every organ and tissue in our body. The highly specialized cells that make up these tissues originally came from an initial pool of stem cells formed shortly after fertilization. Stem cells from human deciduous teeth are found to be highly proliferative in nature. These cells have been demonstrated to be capable of differentiating into variety of cell types including osteoblasts, neural cells, adipocytes and odontoblasts and inducing dentin and bone formation.

In the field of maxillofacial surgery, Stem cells may have a promising role in regeneration of tissues like bone to correct large craniofacial defects due to cyst enucleation, tumor resections and trauma. 2 Till date the closure of such bony defects are commonly carried out with the transfer of tissue from other autogenous sites which have disadvantages like not being able to restore the unique function of the lost part, donor site morbidity, scarring, infection and loss of graft. 4 Stem cells may also find application in coverage and replacement of soft tissue mucosal or skin defects due to trauma or tumor ablations. 3

### Materials and methods

10 Patients were selected undergoing deciduous teeth extraction. Their consent to participate in the study was obtained from patient's parents and patients were subjected to investigations as deemed necessary for the selection criteria. Study was carried out from December 2013 to August 2015. This study was approved by local institutional review board of MGM dental college and hospital.

Collection and transport of extracted teeth: Deciduous teeth were extracted under strict aseptic conditions using local anaesthesia. 10 samples were selected containing pulp tissue from human deciduous teeth, extracted for therapeutic purposes.

**Retrieval of pulpal tissue -:** Immediately after extraction, the deciduous teeth were washed using gauze soaked in 70% ethanol, followed by a wash with sterile distilled water. A cut was made at CEJ of teeth using a diamond disc (Fig.1) and fracture was made on the same line and pulp tissue was retrieved from the pulp chamber using a sterile endodontic K file no.15 in a laminar flow hood. Tissue retrieved was stored in sterile dappen dish containing PBS.

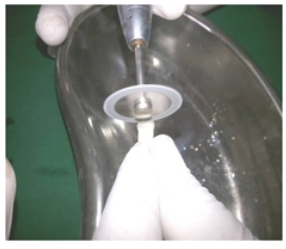

**Fig. 1 Sectioning of deciduous tooth using diamond disc**

Pulp tissue processing -The pulp tissue was stored in 1.5 ml of Eppendoff tube containing Phosphate buffer solution (PBS). Then this tissue was taken and placed in the centre of petri dish which was minced using surgical blade of 200 microns. Cellular separation was completed by digesting the pulp tissue with collagenase type I (3 mg/ml) for 30 minutes at 37 degree centigrade. Collagenase activity was inactivated by adding with PBS up to 10 ml. The suspension was centrifuged for 10 minutes at 1000 rpm. The filtrate was collected in separate tube and again centrifuged at 3000 rpm for 5minutes.The precipitate was washed with PBS twice.

Preparation of smears- The precipitate was filtered through 40 micron strainer and it was loaded onto the slide to make smears, each smear being of 5 microns. Smears were air dried and then treated with 4% Paraformaldehyde (PFA) for 15 minutes followed by PBS wash. 2 washes of PBS each for 5 min were carried out. Slides were air dried and stored at 4 0C. These slides were processed for Haematoxylin & Eosin staining as well as Immunocytochemistry staining.

Immunocytochemistry staining procedure: Heat- induced antigen retrieval were carried out using commercial microwave antigen retrieval system for 10 minutes at 800 watt, 10 minutes at 400 watt and 5 minutes at 320 watt and then washed in PBS saline. The smears were then treated with peroxidase consisting of 3% H2O2 in water for 5 minutes to block the endogenous peroxide activity. This was followed by a power block for 15 minutes to block any non- specific antigenic sites. Incubation for 45 minutes at room temperature with primary antibody, (CD44, CD133, Oct-4, SSEA-4) was then carried out and washed with PBS. Smears were then subjected to a super enhancer for 10 minutes. Smears were then incubated in secondary antibody polymer – (Horse radish Peroxidase) HRP reagent for 20-25 minutes. Visualization was then performed using freshly prepared DAB for

about 7minutes (depending upon acquisition of colour intensity) and washed in PBS saline. The smears were then counterstained with Harris haematoxylin. Smears were then dehydrated in different grades of alcohol, cleared in xylene and mounted using DPX (mountant). Smears were then observed under a light microscope at 40 X magnification. 5

**Results**

Demonstration and characterization of stem cells in pulp tissue of human deciduous teeth – an ex - vivo study was carried out in the department of oral and maxillofacial surgery from December 2013 to August 2015. A total of 10 cases fulfilled the criteria. The selected 10 samples were processed and stained with Haematoxylin & Eosin as well as Immunocytochemistry staining.

The Data was entered into a MS-Excel worksheet and analysed using Statistical Software IBM SPSS 21.0. The data was presented using frequency and percentage followed by graphical presentation (pie chart). Further analysis was done using z-test for proportion. The level of significance was set at 5%. All p-values less than 0.05 were treated as significant.

Out of 10 samples all samples fulfilled the criteria for stem cells based on their morphological characteristics on H and E stained smears. P value was < 0.005 thus was considered as clinically significant (fig.2). Immunocytochemistry staining was performed using CD44, CD133, Oct-4 and SSEA-4 markers. Out of 10 samples 8 samples were positive for cell surface marker CD44, 8 samples were positive for cell surface marker CD133 (fig.3), 8 showed nuclear expression of Oct- 4 (fig.4), 8 showed cytoplasmic expression of Oct-4 (fig.5) and 5 samples were positive for SSEA- 4 (fig.6).

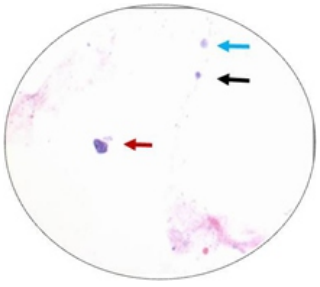

**Fig. 2** Photomicrograph of H and E stained pulp smear obtained from human deciduous tooth showing PSC's(Black arrow), MSC's (Blue arrow) and Somatic cells (Red arrow) [ Haematoxylin and Eosin 400x]

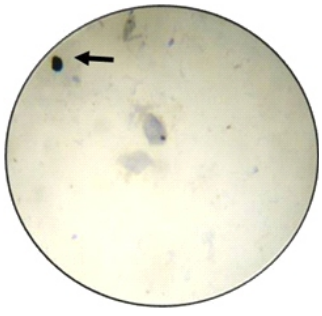

**Fig. 3** Photomicrograph of CD133positive stained smear obtained from human deciduous tooth pulp tissue by Immunocytochemistry, suggestive of MSC's [400x].

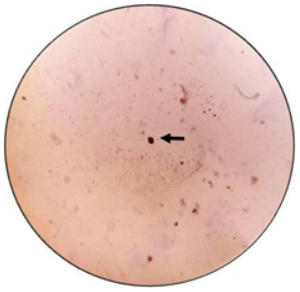

**Fig. 4** Photomicrograph of oct-4 nucleus positive stained smear obtained from human deciduous tooth pulp tissue by Immunocytochemistry, suggestive of PSC's [400x].

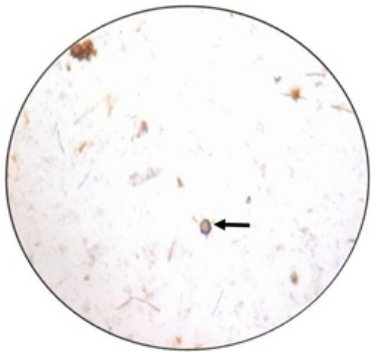

**Fig. 5** Photomicrograph of Oct-4 cytoplasm positive stained smear obtained from the deciduous tooth pulp tissues by Immunoc ytochemistry, suggestive of MSC's [400x].

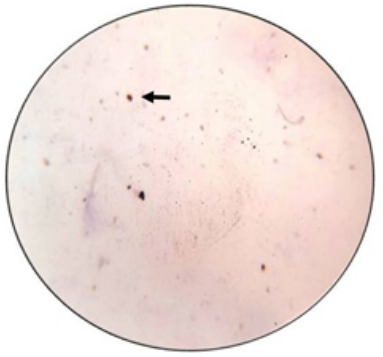

**Fig. 6** Photomicrograph of SSEA- 4 positive stained smear obtained from the human deciduous tooth pulp tissue by Immunocytochemistry suggestive of PSC's [400x].

**Discussion**

Human deciduous teeth have been considered to be a promising source for regenerative therapy because they contain unique postnatal stem cells with self-renewal capacity, multipotency and immunomodu latory function. 6 Various techniques have been used for harvesting adult stem cells such as bone marrow aspiration technique which is an invasive and painful procedure for the donor while in this technique deciduous teeth was obtained noninvasively without any ethical concerns.7 Various techniques have been used by various authors to harvest pulp tissues from deciduous teeth, like retrieval of pulp tissue from apical foramina with the use of nerve puller, use of carbide burs to cut at the amelo-cemento junction and then removal of the pulp tissue with the dental excavator and some advised to give a longitudinal cut through the tooth to approach the pulp tissue. 8 In this study, a diamond disc was used to cut the tooth at cemento-enamel junction in a laminar flow hood under constant irrigation with distilled water in order to avoid heat generation. The cut was made at distance just short of pulp chamber and then with a light force by hand the tooth was fractured at the cut site, after which pulp tissue was extirpated with the help of endodontic file. This manoeuvre prevents heat generation in the pulp tissue and necrosis of cells. Carrying out procedure in a laminar flow hood ensures sterile conditions.

Hanks Balanced Salt Solution 8 has been used as a transport media in many studies but in this study, phosphate buffer saline (PBS) was used as a transport media and viability of cells was maintained in all the samples studied. PBS is an isotonic solution with a pH of 7.4 and is considered in many studies as diluting solution as well as transport medium. Very few studies have previously identified the presence of stem cell populations with embryonic-like phenotypes in human dental pulp at a single-cell level. In this study two different populations of stem cells have been demonstrated i.e. mesenchymal stem cells (MSC's) and pluripotent like stem cells (PSC's), based on their morphological characteristics by haematoxylin and eosin staining. Mesenchymal stem cells have large nucleus and pluripotent like stem

cells have small, spherical and darkly stained nucleus, also PSC's show thin rim of pale cytoplasm around nucleus. Presence of two different populations of stem cells was then confirmed by definitive technique of immunocytochemistry staining using the specific markers for these two different types of stem cells.

## Conclusion

Deciduous teeth are an accessible source of mesenchymal stem cells as well as pleuripotent stem cells which can be easily obtained without contamination. This study provides a description of a stem cell population residing in human deciduous teeth and establishes the foundation for further studies to determine the efficiency of using deciduous teeth in cellular based therapies. Hematoxylin and eosin staining demonstrates two different population of stem cells, which significantly differ in morphological characteristics. Presence of these two populations of stem cells is confirmed by immunocytochemical study. Mesenchymal stem cells are positive for CD44, CD133 and Oct-4 in the cytoplasm, whereas pluripotent stem cells express positivity for SSEA-4 and Oct-4 in nucleus.

Methods to isolate and characterize stem cell population are a preliminary step of any research, and are very important for the development of novel therapies of regenerative medicine and tissue engineering based on stem cell regeneration.

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