

Interaction of Human Dental Pulp Stem Cells and Ameloblastic Cell In-vitro- A Preclinical Analysis

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ABSTRACT

Background: To report in-vitro interaction between ameloblastic cell and dental pulp stem cell.

Methods: Dental Pulp stem cells were isolated from extracted pre molars and Ameloblastoma primary culture was obtained from the patient. Direct co-culture study was performed to do morphological sorting with trypan blue dye exclusion method and transwell co-culture was performed to study anti-proliferative effects.

Results: The viability count from transwell co-culture plates revealed that in the control wells the AM has higher proliferation rate than DPSC. However when co-cultured with DPSCs, the proliferation level is arrested and decreased cell count was noted. This was confirmed by Trypan blue dye exclusion assay also.

Conclusion: Result demonstrated that dental pulp stem cell can induce anti-proliferation or anti-tumorigenic activity in ameloblastoma cells. This has helped us in using these cells in the management of ameloblastoma cases.

Keywords: Human Dental Pulp Stem Cells, Ameloblastic Cell, In-Vitro

Introduction

Ameloblastoma is a tumour formed by the proliferation of odontogenic epithelial cells or tooth bud. This tumour shows slow growth and local invasion. Ameloblastoma usually presents as cystic expansion to more aggressive local invasion of bone and local recurrence. It needs wide surgical excision as treatment. The mechanism of bone invasion remains unclear. Ameloblastomas are often associated with the presence of unerupted teeth. Symptoms include painless swelling, facial deformity if severe enough, pain if the swelling impinges on other structures, loose teeth, ulcers, and periodontal (gum) disease. Lesions will occur in the mandible and maxilla, although 75% occur in the ascending ramus area and will result in extensive and grotesque deformities of the mandible and maxilla. Histopathology will show cells that have the tendency to move the nucleus away from the basement membrane. This process is referred to as “Reverse Polarization”. The follicular type will have outer arrangement of columnar or palisaded ameloblast like cells and inner zone of triangular shaped cells resembling stellate reticulum in bell stage. The central cells sometimes degenerate to form central microcysts. The plexiform type has epithelium that proliferates in a “Fish Net Pattern”. The plexiform ameloblastoma shows epithelium proliferating in a ‘cord like fashion’, hence the name ‘plexiform’. There are layers

of cells in between the proliferating epithelium with well-formed desmosomal junctions, simulating spindle cell layers. Radiographically, it appears as lucency in the bone of varying size and features—sometimes it is a single, well-demarcated lesion whereas it often demonstrates as a multiloculated “soap bubble” appearance. Resorption of roots of involved teeth can be seen in some cases, but is not unique to ameloblastoma. The disease is most often found in the posterior body and angle of the mandible, but can occur anywhere in either the maxilla or mandible.

It is proposed that Wnt signaling in ameloblastoma induces MMP-9 (matrix metalloproteinase) and could be responsible for bone absorption and local invasion. MMP-2 and MMP-9 are proteolytic enzymes and target genes of Wnt pathways.[1] They degrade various extracellular matrix proteins and are associated with angiogenesis, cell mortality or invasion in cancer.

There has been an effort in understanding interaction between ameloblastic cell and stem cells. This can help to understand therapeutic importance of stem cells in cases of ameloblastoma management. Qiao L et al have demonstrated that human mesenchymal stem cells derived from human bone marrow inhibit tumor functions.[2] Wnt signaling pathway plays an important role for the same. Khakoo A Y et al have also demonstrated that in an in vivo

model of Kaposi's sarcomas, human mesenchymal stem cell travel to sites of tumorigenesis and inhibit tumour growth. [3] Different types of stem cells are isolated from human body, among these dental pulp stem cells are mesenchymal stem cells and can be easily obtained.

In the present study we have used human dental pulp mesenchymal stem cells (MSC) to understand the antitumor effect on ameloblast cells (AM).

Materials and Methods

Human extracted premolars were obtained from an oral-maxillofacial surgical center with written informed consent using the guidelines approved by the Institutional Ethical Committee– KSR Institute of Dental Science & Research (IEC-KSRIDSR). The research protocol was on par with the World Medical Association Declaration of Helsinki. The tissue samples were brought to the laboratory after crown cutting via transport media at 4°C and were immediately processed. (Once the crown is cut, sample has to be processed within 2 hours and tooth can be transported and stored at 4°C for 3 days to attain cultivable viable cells)

Transport Medium (Tooth Sample)

The transport medium used for our study is Phosphate Buffered Saline (PBS, 10010, Gibco® Life Technologies™, NY, USA) with antibiotics 100 U/ml penicillin, 100 µg/ml streptomycin and 25 µg/ml amphotericin B (Gibco® 15240, Life Technologies™, NY, USA). PBS is water based salt solution containing sodium or potassium salts of chloride and phosphates. The osmolarity and ion concentrations of PBS matches the human body, hence the dental pulp or other biological samples can be transported.

Isolation and Culture of Dental Pulp

The tooth samples were processed for isolation of dental pulp derived MSCs as previously described by Perry et al. with some modifications. [4] Briefly, after rinsing in normal saline (0.9% w/v sodium chloride), tooth received several washes in sterile PBS, followed by immersion in 1% povidoneiodine (PVP-I) for 2 min, immersion in 0.1% sodium thiosulfate in PBS for 1 min, and another wash in sterile PBS. The roots of cleaned teeth were separated from the crown to reveal the dental pulp, and the pulp was placed into an enzymatic bath consisting collagenase I (Gibco17100, Life Tech, NY, USA) for dissociation of cells. Pulp was allowed to incubate at 37°C for 40 min to digest the tissue and liberate the cells. The digested tissue (cell suspension) was filtered through a 100 µm, 40 µm cell strainer (BD Falcon) to remove remaining tissue debris. Cells were centrifuged at 400 g for 5 min and seeded onto tissue culture flasks (5 × 10⁵ cells/flask) in MesenPRO RS™ complete medium, consisting of MesenPRO RS™, basal medium supplemented with 20% MesenPRO RS™

growth Supplement (Gibco® Life Technologies™, NY, USA), L-glutamine to a final concentration of 2 mM, 100 U/ml penicillin, 100 µg/ml streptomycin and 25 µg/ml amphotericin B (Gibco® 15240, Life Technologies™, NY, USA).

Culture was maintained at 37°C with 5% CO₂ and 21% O₂ in a humidified atmosphere. For optimal cell growth, medium was changed at the moment of subculture or, if the medium appeared acidic prior to cells reaching 80% confluence, half-medium change was performed.

Isolation and Culture of Ameloblastoma Cells (AM)

The ameloblastoma specimen was obtained from a 14 year male patient, at the Department of Oral and Maxillofacial Surgery, KSR Dental College, Tiruchengode, Tamilnadu, India. This study was performed with written informed consent from his parents under the approval of Institutional Ethical Committee – KSR Institute of Dental Science & Research (IEC-KSRIDSR). Specimen was taken from right mandibular canine region with expanded buccal cortical plate and lining tissue of 1cmx1cm size. It was washed in normal saline and placed in transport media and then sent to the MCRC lab (Mother Cell Regenerative Center, Trichy, TN, India) as per protocol. The tissue was processed as described by Stenman *et al.* [5] with slight modification. After removing extraneous soft connective tissues, the solid part of the specimen was dissected into pieces with an approximate size of 1 mm³ and placed into 6-well plates coated with collagen I, incubating at 37°C in a 5% (v/v) CO₂ atmosphere. Dulbecco's modified Eagle Medium (DMEM, Gibco® Life Technologies™, NY, USA), containing 10% (v/v) fetal bovine serum (FBS10270, Gibco® Life Technologies™, NY, USA) was added after incubation for 5 hrs and used for subculture of the proliferative cells.

Direct Co-Culture and Morphological Sorting

In order to address the question of whether dental pulp stem cells (DPSC) restrict the proliferation of ameloblastoma cells, an initial attempt was made with co-culturing of these two cells. Direct co-cultures were performed with DPSC and AM cells at 1:1 ratio (1 × 10⁶ cells) and 2:1 ratio (2 × 10⁶ cells DPSC & 1 × 10⁶ cells ameloblastoma) in six-well plates (BD) in triplicate. DPSC and AM cells alone were considered as control for morphological sorting.

Transwell Co-Culture and Viability Assay

The anti-proliferative effect of DPSC were determined in transwell co-culture systems (Corning) followed by Trypan blue dye exclusion assay. A total of 2 × 10⁵ of DPSC was seeded on each lower chamber and 2 × 10⁵ of AM cells were loaded in the upper chambers. Both chambers were filled with a complete medium containing Dulbecco's modified Eagle Medium (DMEM, Gibco®),

containing 10% (v/v) fetal bovine serum, L-Glutamine to a final concentration of 2 mM, 100 U/ml penicillin, 100 µg/ml streptomycin and 25 µg/ml amphotericin B (Gibco®). Culture was maintained at 37°C with 5% CO₂ and 21% O₂ in a humidified atmosphere. To measure the cells viability, 0.4% Trypan blue (Gibco®) was used as per protocol of Darlington.[6]

Statistical analysis was performed using SPSS version 20. Statistically significant (p<0.05)

RESULT

The in-vitro culture of DPSC and AM showed proliferation in the control wells. The DPSC processed spindle shaped morphology and the AM cells were polygonal in nature (Fig.1A & 1B). When these cells were subjected to direct co-culture at 1:1 ratio there was reduction in the proliferation of AM compared to DPSC. More spindle

shaped cells were present than the polygonal AM cells, eventually the number of AM cells were decreased as the days of incubation increased (Fig. 1C & 1D). The trypan blue viability staining revealed that AM cells alone take up the dye and appears blue whereas the DPSC were unstained (Fig. 2). Similar result was also found in the next set of wells where the number of DPSCs was doubled (2x10⁶ cell/ml) to AM. The morphology of the AM cells was also noted to be changed in the co-culture set up. The viability counts from trans well co-culture plates revealed that in the control wells the AM has higher proliferation rate than DPSC that is as due to the fact that AM is a benign tumor cells secretes its own growth factors and increases cell proliferation. However, when co-cultured with DPSCs, the proliferation level is arrested and decreased cell count was noted (Fig 3). This could be due to the immune modulatory property of DPSC as reported by Yamaza *et al.*[7].

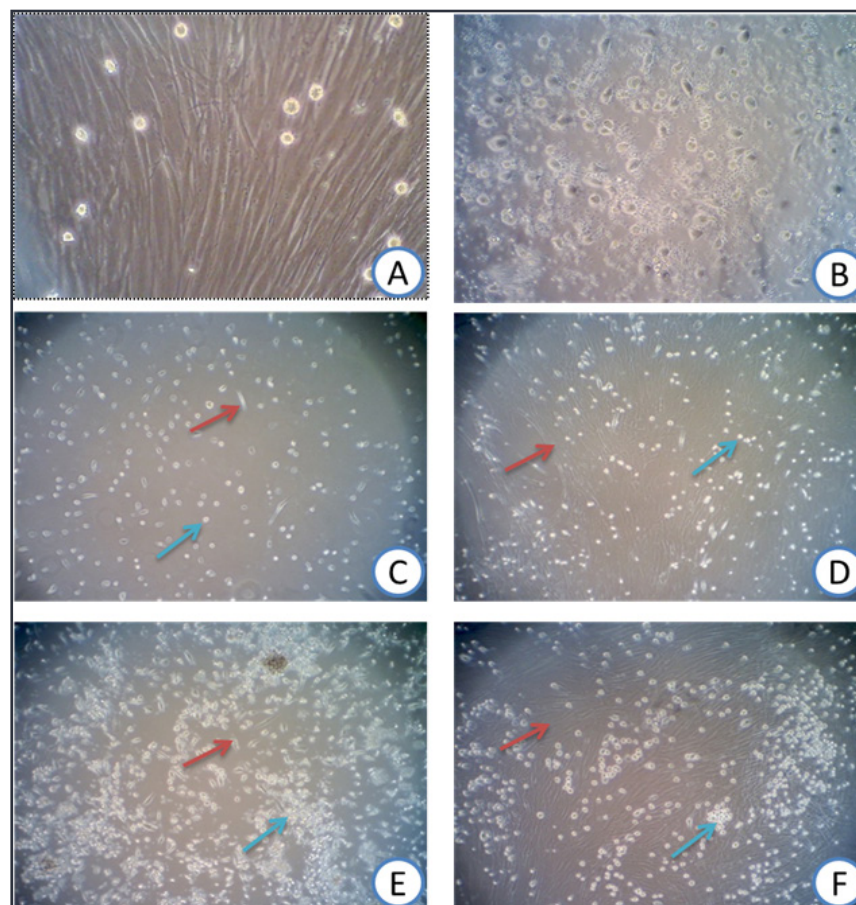


Fig.1: In-vitro culture of Dental Pulp Stem Cells (DPSC) and Ameloblastoma (AM) cells, A. Cultured DPSC for 24 Days showed spindle morphology (x200). B. Cultured Ameloblastoma cells showed polygonal morphology 24 days (x200); C. Co-culture of DPSC (red arrow) and ameloblastoma cells (blue arrow) at 1:1 ration on day 14 (x100); D. Co-culture of DPSC (red arrow) and ameloblastoma cells (blue arrow) at 1:1 ration on day 24 with reduced ameloblastoma cell proliferation (x100); E. Co-culture of DPSC (red arrow) and ameloblastoma cells (blue arrow) at 2:1 ration on day 14 (x100); F. Co-culture of DPSC (red arrow) and ameloblastoma cells (blue arrow) at 2:1 ration on day 24 (x100).

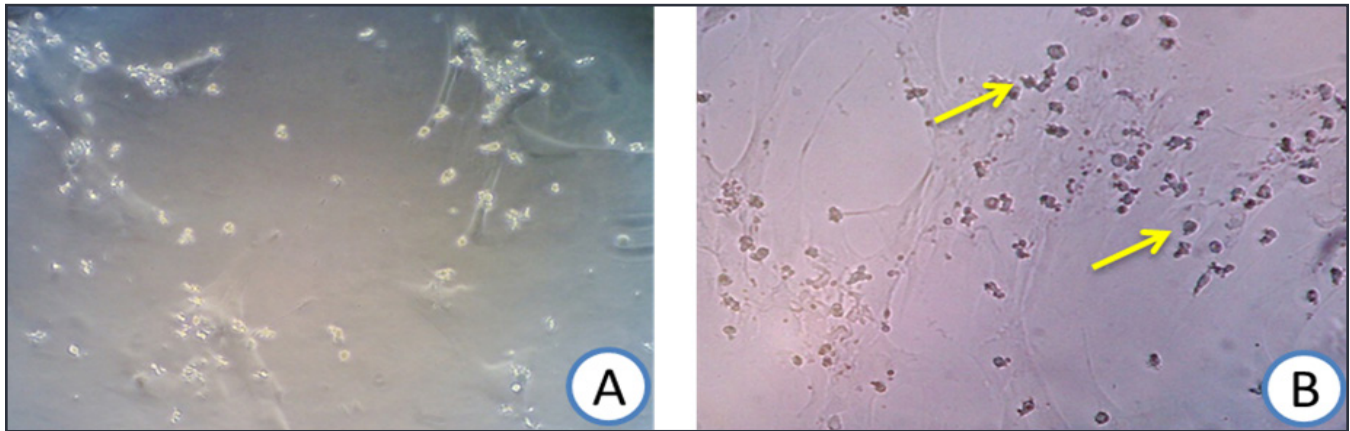


Fig. 2: Trypan Blue staining of culture of Dental Pulp Stem Cells (DPSC) and Ameloblastoma (AM) cells demonstrating viable DPSC and stained (dead) AM cells (A) before staining (B) after staining (Arrows) (x200).

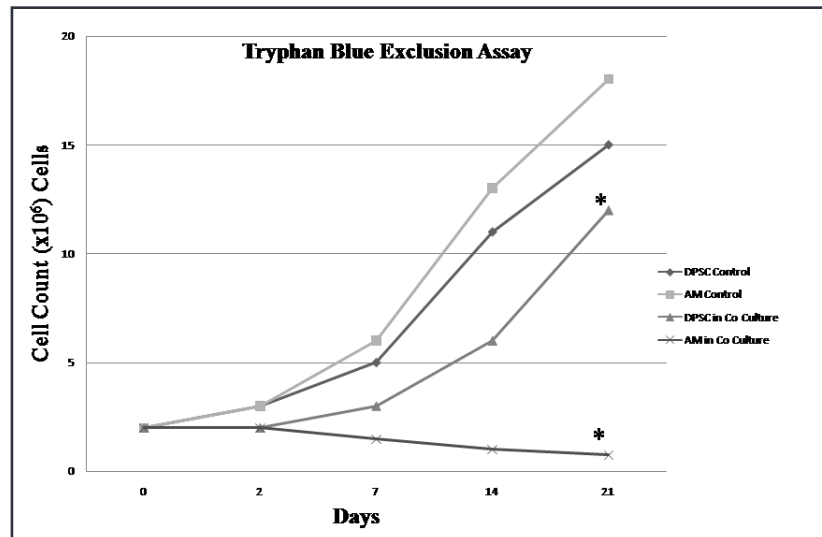


Fig. 3: Trypan blue viability staining showed Proliferation of Dental Pulp Stem Cells (DPSC) and Ameloblastoma (AM) cells in the control wells but in the co culture trans wells Proliferation of DPSC alone was noted and the AM cells showed decreased population as the days increases.

Discussion:

Mesenchymal stem cells (MSCs) have the capacity to self-renew and differentiate into all three cell lineages, including mesodermal, endodermal, and ectodermal cells. MSCs have been reported to be isolated from various adult tissues like bone marrow, umbilical cord, adipose tissue etc. [8] Dental pulp also serves to be a potent source of MSCs. [9] Apart from the abilities of self-renewal and multipotent differentiation, MSCs are nonimmunogenic and more importantly, display profound immunomodulatory and anti-inflammatory capabilities.[10,11] This experiment has helped us in understanding of antitumor capabilities of human pulp stem cells and prompted us to use in the management of Ameloblastoma case successfully without recurrence till date.

Conclusion:

This study suggests that human dental pulp stem cells possess intrinsic antineoplastic properties and that this stem cell population might be of particular utility for treating odontogenic tumors.

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