CHARACTERIZATION OF PERIODONTAL FIBROBLAST SPHEROIDS IN VITRO, INTERACTION WITH 3D MEMBRANES AND POTENTIAL FOR TISSUE REGENERATION

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Characterization of periodontal fibroblast spheroids *in vitro* and their potential for tissue regeneration

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Abstract

Autologous transplantation of periodontal fibroblasts may be a promising technique to induce tissue regeneration in the treatment of periodontal disease. Spheroid culture is a form of three dimensional cell culture that promotes cellmatrix interaction and cellular differentiation without recourse to exogenous growth factors. The aim of this study was to develop 3D spheroidal cultures of periodontal fibroblasts *in vitro* and characterize them with respect to their potential use in periodontal tissue repair and regeneration. In this study commercial normal periodontal fibroblasts were grown in spheroidal form *in vitro* using the liquid overlay technique. The fibroblast spheroids were characterized by histology, DNA quantification, scanning electron microscopy and immunohistochemistry. Over 14 days, periodontal fibroblasts formed viable spheroids which incorporated an extracellular matrix rich in collagen type I and periostin. Production of cementum attachment protein which is a marker of dental hard tissue formation, was not detected. This study shows that periodontal fibroblast spheroids may have the potential to be used as an adjunct for periodontal regeneration.

Keywords: Periodontal fibroblast, spheroid culture, periodontal disease, *in vitro*, periodontal regeneration

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Abstract

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Characterization of periodontal fibroblast spheroids *in vitro*, interaction with 3D membranes and potential for tissue regeneration

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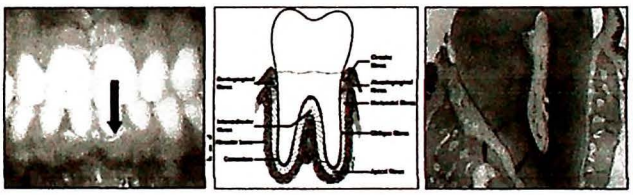
Outline

- Introduction
- Development of periodontal spheroids
- Spheroid characterization
- Potential application of spheroids
- Conclusion

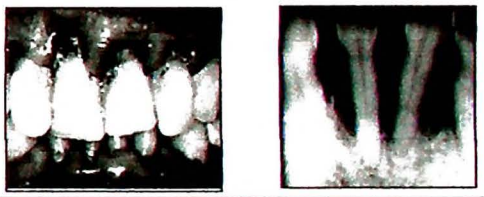
Introduction

- **Periodontium**-comprises cementum, periodontal ligament, alveolar bone and gingiva.



Periodontitis

- Periodontal disease is an inflammation of periodontal tissue due to accumulation of bacterial plaque and tartar.
- Characterized by red, swollen , bleeding gum
- Loss of connective tissue and bone



Periodontitis

- Chronic periodontitis affected 70% of adult population.
- 5 to 20% suffered the advanced form of the disease.
- Destruction of periodontal tissue resulting from periodontal disease is one of the common tooth loss world wide.

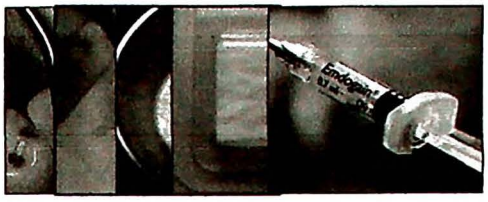
Periodontal defect

- Bone and soft connective tissue loss

Courtesy Dr Akram Hassan, USM

Periodontal treatment

- Current treatment did not provide a reliable outcome



- Recent studies trying to deliver cell into periodontal defect in 3D form

Cell spheroids

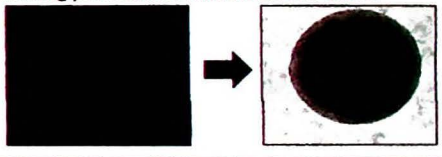
- **Also known as cellular sphere, microsphere, and multicellular spheroids**
- **3D approach conta[illegible] extracellular matrix (ECM)**
- **Variable in size range [illegible]µm to 1mm in diameter depending on the amount of cell *seeded***

Advantages over 2D system

- *show changes in shape and behaviour as response to* specific cell signals (Weaver et al., 1997)
- gene expression profiles of cells grown in 3D culture show higher correlation in their gene expression profiles with cells grown *in vivo* (Sivaraman et al., 2005)
- interactions of ECM with attaching cells lead to crosstalk between extracellular and intracellular signaling pathways, closely resembling the situation *in vivo* (Abbot et al., 2003)
- ECM serves as a substratum for cell adhesion and promotes cell spreading and cytoskeletal organization

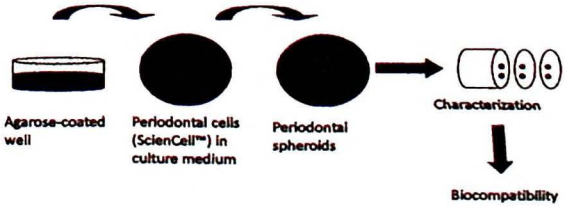
Periodontal spheroids

- Derived from periodontal cells
- Periodontal cells-from periodontal ligament, possess heterogeneity
- Melcher AH (1976),suggests the potential of periodontal ligament cells in periodontal regeneration
- Implanting periodontal cells inside the defect



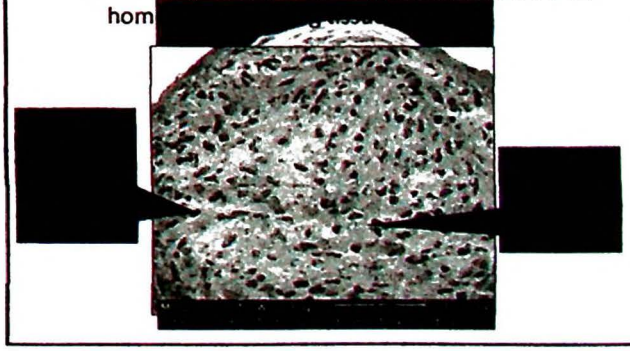
Methods

- Rotation culture
- Hanging drop method (reviewed by Lin et al 2008)
- **Liquid overlay technique** (Carlsson and Yuhas 1984)



Spheroid culture

- *In vitro* spheroid culture appear to resemble cell hom[illegible]



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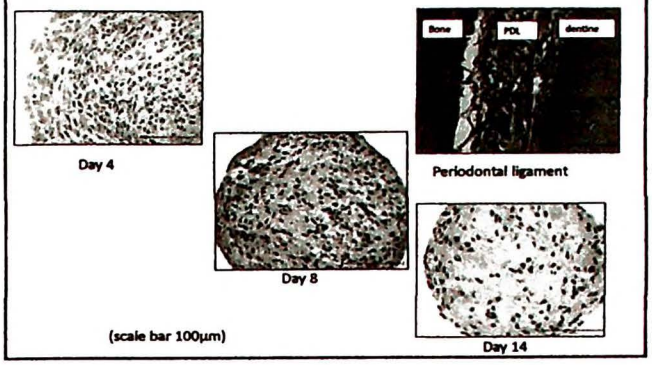
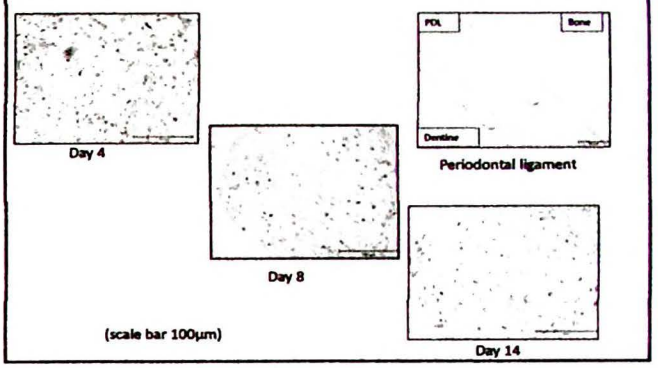


Alcian blue-Glycosaminoglycans



Proteins of periodontal ligament

Proteins that are associated with periodontal ligament - osteogenic in nature

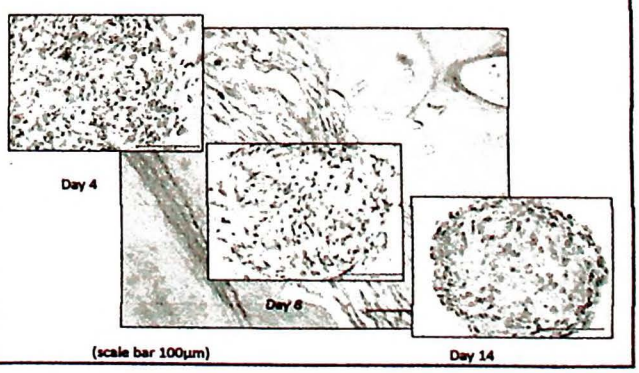
- Collagen type I- main protein in periodontal ligament
- Periostin- known to be marker of periodontal ligament
- Osteocalcin -bone specific marker

A cell line with characteristics of the periodontal ligament fibroblasts is negatively regulated for mineralization and Runx2/Cbfa1/Osf2 activity, part of which can be overcome by bone morphogenetic protein-2. Saito et al., J Cell Sci. 2002 Nov 1;115(Pt 21):4191-200.

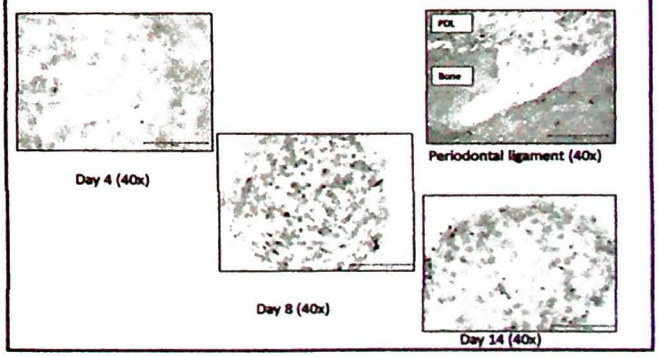
Collagen type I

Somerman MJ, Archer SY, Imm GR, Foster RA. A comparative study of human periodontal ligament cells and gingival fibroblasts in vitro. J Dent Res 1988;67:66–70.

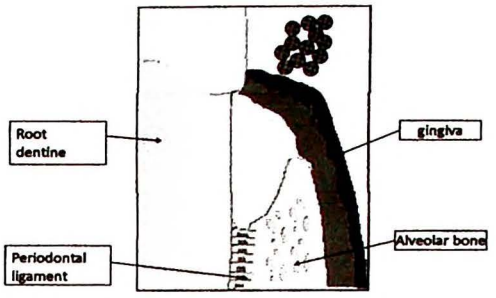
Periostin



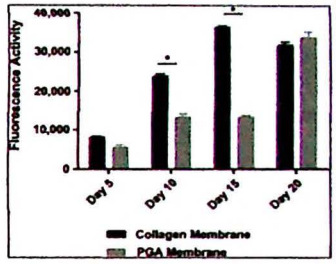
Osteocalcin



Potential for cell seeding

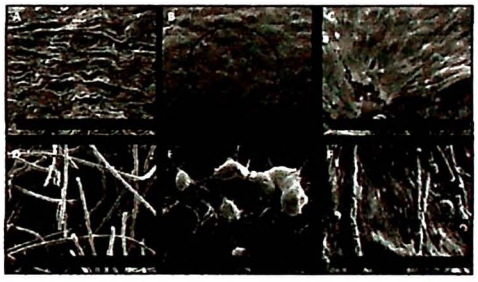


Spheroids interaction on membrane



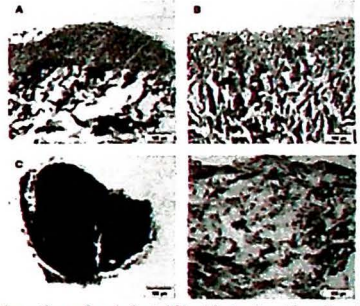
Quantification of the viability of periodontal spheroids cultured on PGA and collagen membranes over 20 days as measured using the Alamar blue assay. * Highly significant difference (P <0.001). J Periodontol. 2011 May;82(5):790-7

Collagen vs synthetic membranes



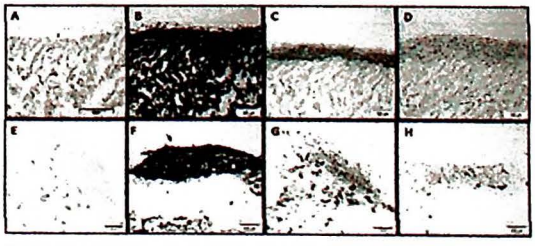
Scanning electron micrographs of membranes pre- and post-spheroid culture. (A) collagen membrane without cells; (B) spheroids on collagen membrane at day 5 (red circle); (C) spheroids on collagen membrane at day 20; (D) PGA without cells; (E) spheroids on PGA at day 5; and (F) spheroids on PGA membrane at day 20. Red arrow shows degradation areas of PGA fibers. J Periodontol. 2011 May;82(5):790-7

Collagen vs synthetic membrane



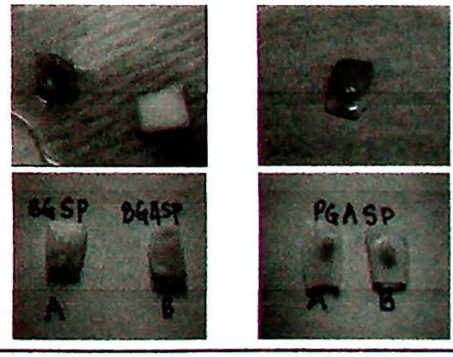
Histologic sections of periodontal fibroblast spheroids cultured on collagen membrane at (A) day 5 and (B) day 20, and on PGA at (C) day 5 and (D) day 20. Scale bar = 100 mm.

Protein expression



Protein expression by periodontal fibroblast spheroids cultured on collagen membrane on day 15 post seeding: (A) negative control; (B) collagen type I; (C) Periostin; and (D) Runx2. Protein expression by periodontal fibroblast spheroids cultured on PGA membrane on day 15 post seeding: (E) negative control; (F) collagen type I; (G) Periostin; and (H) Runx2. Original magnification x 20 (Scale bar = 100μm).

Cells construct on dentine



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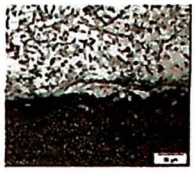
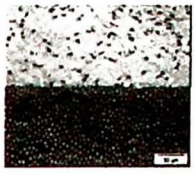


spheroid-Bio-Gide dentine complex



spheroid-PGA-dentine complex

Conclusions

- Periodontal cells are able to **differentiate** in a spheroid system without any exogenous factors. The presence of extra cellular matrix in the spheroid seems to suppress cell division and promotes cell differentiation.
- They **produce proteins** known to be markers of periodontal ligament fibroblasts.
- Cells are able to **migrate out** the spheroids and **proliferate** on both hard and soft tissue surfaces.
- Periodontal spheroids have the potential to be used as a **biological adjunct** to current periodontal regenerative techniques.

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Thank you

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