

## A Pilot Study of In-Vitro Gingival Fibroblast Differentiation in Spheroid Culture

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**INTRODUCTION:** Periodontal diseases are a group of related inflammatory conditions affecting the tooth supporting tissues. Currently available treatments aimed at the regeneration of lost tooth support have limited clinical success, and there is a need to develop further methods. However, the regeneration of periodontal tissue is a complex process involving multiple cells and extra cellular matrix. Traditional two dimensional models for cell culture have limited application in this area of research. Spheroid culture is a form of three dimensional cells culture that promotes cells matrix interaction which could recapitulate the aspect of cell homeostasis in vivo<sup>1</sup>. While this technique has been widely used in cancer research, it has not been used to date in order to evaluate the application to in-vitro research on periodontal tissue regeneration.

**METHODS:** Normal cell lines of gingival fibroblasts were seeded in tissue culture flasks until confluent. Then they were trypsinized and cultured in multi well plates by liquid overlay technique. Each plate was divided into three sections, each section containing a different concentration of cells; 25 000, 50 000, 100 000 cells. The spheroids were fixed and processed for histological examination at interval of 2, 14 and 30 days.

**RESULTS:** Gingival fibroblasts formed spheroids in a period between 12 and 48 hours. After 2 weeks an inner dead cell zone, an outer live cell zone and a mixed cell zone could be observed in spheroids of different ages and different cell number. No fibril formation can be detected from the histological sections. Alcian blue staining showed the present of glycosaminoglycan at the centre of spheroids at 14 and 30 days.

**DISCUSSION & CONCLUSIONS:** This study concluded that cell line gingival fibroblasts can be grown consistently in a spheroid form. However the cells appeared to differentiate along a chondrogenic rather than fibrous connective tissue lineage. This result may be used for further investigation of gingival fibroblast cells in this 3-dimensional model.

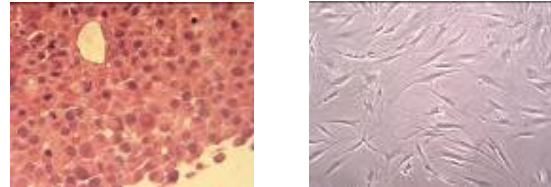


Figure 1: Section of gingival fibroblast in spheroid culture (left) versus gingival fibroblast in 2-dimensional culture (right).

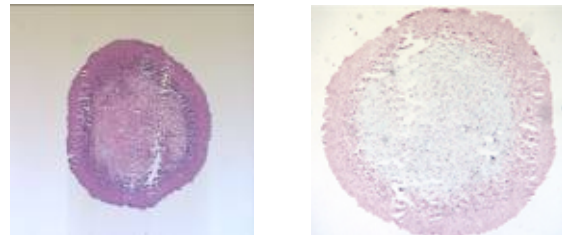


Figure 2: Gingival fibroblast stained with H&E showed three different zones (left) and Alcian Blue showed glycosaminoglycan (right).

**REFERENCES:** <sup>1</sup>Carlsson J and Yuhas JM (1984). *Liquid overlay culture of cellular spheroids*. In: *Spheroid in Cancer Research*, Springer-Verlag Berlin.