Introduction

Improved craniofacial and tooth regeneration therapies are needed to treat the increasingly high incidence of genetic craniofacial and skeletal defects, injury to the pulp-dentin complex, and tooth decay or loss caused by periodontitis. Most current dental tissue engineering approaches have used a combination of biodegradable scaffold materials and dissociated single cells to regenerate multiple, small tooth-like structures. In this study we have utilized a novel cell-sheet technology to guide dental epithelial (DE) and dental mesenchymal (DM) cell layer interactions, for the subsequent elaboration of mineralized dentin and enamel in a manner similar to that of natural tooth development.

Materials and Methods

DE and DM cells isolated from immature pig molar tooth buds were cultured on Upcell® culture dishes (Thermo Fisher Scientific, NY), which exploit mild temperature treatment for reversible cell adhesion and detachment. DE and DM cell sheets with intact cell-cell junctions and basal lamina were generated, and combined to form DE-DE (control), DM-DM (control) and DE-DM (experimental) constructs. The cell sheets were combined with basal lamina facing each other to facilitate cell-cell and cell-matrix signaling at the cell layer interface, and layered on collagen I coated plates. Porcine DE and DM cells were labeled with green and red quantum dots respectively, for in vitro visualization of DE/DM cell interactions throughout the 5 week culture period in osteogenic media. Ongoing histological, molecular, and immunohistochemical characterizations of developmentally staged in vitro co-cultured cell sheet constructs are currently underway, including analysis of secreted growth factor(s) which may be used to improve tooth regeneration.

Results

Our preliminary results show early evidence of matrix mineralization and dental tissue organization in DE-DM cell sheet constructs, which are distinct from that of DE/DE and DM/DM constructs.

Discussion and Conclusions

We anticipate that the findings of this study will help to establish a novel method to generate organized DE-DM cell structures to elaborate mineralized dental tissues, and eventually, whole teeth of predetermined size and shape.

References


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