Characterization of enamel and dentin protein expression in bioengineered human tooth tissues

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ABSTRACT

Objectives: Existing therapies to replace missing teeth rely on the use of synthetic materials, such as titanium. Here we have used previously described methods to bioengineer teeth from human dental stem cells (hDSC). **Methods:** Dissociated epithelial and mesenchymal hDSCs, harvested and cultured from an immature human tooth bud, were gently pelleted, wrapped with biodegradable collagen-coated Poly-L-lactate-co-glycolate (PLGA) scaffolding material, and implanted subcutaneously into athymic Lewis rats, where they were allowed to develop for 25 weeks. Micro-CT, X-ray, hematoxilyn-and-eosin (H&E), and immunohistochemical (IHC) analysis were used to characterize the bioengineered dental tissues. **Results:** While most of the bioengineered structures consisted of mineralized osteo-dentin like material, one construct consisted of a well-formed, mineralized structure grossly and microscopically resembling a human tooth ~5mm in diameter and ~5mm in height. Micro-CT analysis revealed a dome-shaped crown with a central pulp chamber, and a distinct dentin enamel junction (DEJ). The bioengineered dental tissues were positive for dentin-expressed dentin sialophosphoprotein (DSPP), enamel-expressed amelogenin, and periodontal ligament (PDL)-expressed periostin. H&E analyses revealed distinct and organized dentin tubules, and PDL tissues with organized Sharpey's fibers. **Conclusions:** We have demonstrated that dissociated hDSC-seeded polymer constructs can form bioengineered teeth that are morphologically similar to natural human teeth at both gross and microscopic levels. We anticipate that further modifications of this approach will eventually result in reliable methods to bioengineer replacement teeth in humans. This work was supported by NIH/NIDCR R01DE016132 (PCY), and R03TW7665 (MTD, SED).

INTRODUCTION

The loss of teeth and oral tissues can occur through dental caries, periodontal disease, general trauma, and cancer. Existing therapies to replace missing teeth with artificial structures, often compromise the remaining oral tissues, and may do little to truly rehabilitate the lost tissue. Consequently, efforts are currently underway to develop alternative methods to regenerate teeth and supporting structures using tissue engineering approaches.

Tooth development, the result of dental epithelial-mesenchymal interactions via a series of reiterative growth factor signaling pathways, culminates in the formation of dental mesenchymal cell derived pulp, dentin, periodontal ligament tissues, and dental epithelial derived enamel, exhibiting characteristic cellular and molecular signatures. Bioengineered dental tissues generated from pig and rat tooth bud cells exhibited dentin and enamel with the appropriate cellular organization.[1,2]

As the next step towards the goal of generating functional bioengineered *human* teeth, this study will determine whether methods used to generate bioengineered pig and rat teeth can be used to bioengineer human teeth from human tooth tissues.

ACKNOWLEDGMENTS

Research was sponsored by NIH/NIDCR grants R03TW007665 and DE016132 (PCY) and the TUSDM Dean's Research Program and Summer Research Grant. (SS).

RESULTS

Figure 1: Micro-CT images of the Bioengineered sample.

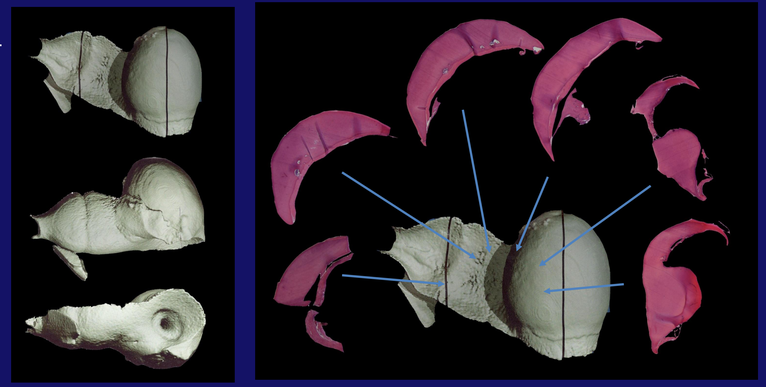

Figure 2: H&E stained sections and their relationship to the 3-dimensional structure of the Bioengineered sample.

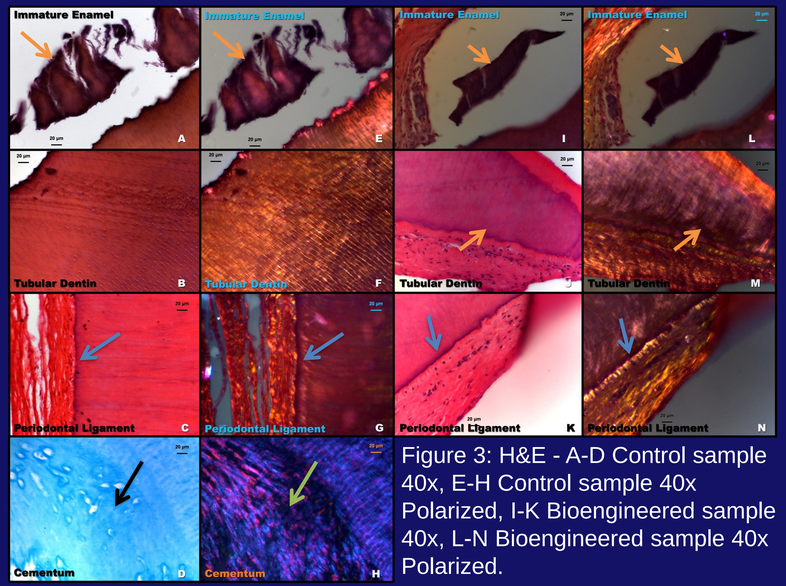

Figure 3: H&E - A-D Control sample 40x, E-H Control sample 40x Polarized, I-K Bioengineered sample 40x, L-N Bioengineered sample 40x Polarized.

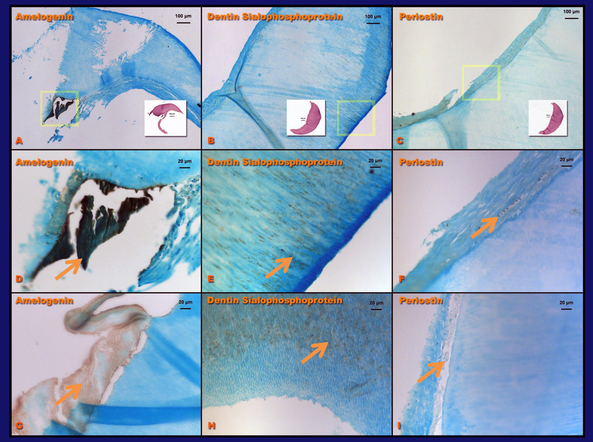

Figure 4: Immunohistochemical Analysis - A-C Bioengineered sample 10x, D-F Bioengineered Sample 40x, G-I Control Sample 40x

METHODS

Collagen-coated Poly-L-lactate-co-glycolate (PLGA) tooth scaffolds were prepared using molds in the shape of human incisors and molars. Unerupted human third molar tooth bud cells were isolated and prepared. The scaffolds were collagen-coated, washed, seeded with the isolated human tooth bud cells, and implanted in the omentum of athymic Lewis rats. 16-21 week old human tooth buds were also implanted as controls. After 25 weeks, implants were removed and imaged with microCT and X-Ray.

Control and experimental implants were fixed in paraformaldehyde, decalcified, embedded in paraffin, serially thin-sectioned (6 microns), mounted onto slides, and selected samples were stained with hematoxylin and eosin. Tooth expressed proteins analyzed using IHC analysis using the Vectastain ABC kit include: amelogenin (AM), dentin sialophosphoprotein (DSPP), periostin (PER), and osteocalcin (OCN). All of the antibodies used are mouse-anti-human, except for amelogenin, which is rat-anti-human. Sectioned and stained specimens are being examined using Leica DMRE compound and Zeiss Axiophot microscopes and digital Zeiss Axiocam camera.

DISCUSSION & CONCLUSIONS

A notable experimental implant was a hard, mineralized structure, grossly and microscopically resembling a human tooth of ~5mm in diameter and 5mm in height. Micro-CT analysis revealed a dome-shaped crown with a central pulp chamber, and a distinct dentin enamel junction (DEJ) (Fig. 1). Histological analysis of the H&E stained sections (Fig. 2,3) revealed tubular dentin with tubules (3J,M), immature enamel (3I,L), and periodontal ligament tissues (3K,N), very similar to those observed in a human tooth bud control implant (A-H). Other possible structures seen included cementum, and osteoblasts and/or odontoblasts. The morphology and staining of structures present within the bioengineered human tooth indicated that the majority of the sample was composed of tubular dentin, surrounded by periodontal ligament. IHC analysis (Fig. 4) showed that the structures and tissues identified to date are dental tissues, confirming the presence of amelogenin (enamel), dentin sialophosphoprotien (tubular dentin), and periostin (periodontal ligament).

The described methods can be used to generate sizeable human bioengineered teeth from human dental stem cells.

The large size of the bioengineered human tooth is a major advancement. We are currently generating and analyzing additional samples, and investigating alternative approaches to facilitate generating full-sized bioengineered teeth of specified size and shape.

REFERENCES

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