Decellularized Tooth Bud Scaffolds for Tooth Regeneration
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Statement of Purpose: Therapies to replace lost teeth with Titanium implants have become astonishingly widespread, due to their documented survival and success rates. Still, the long-term survival of implants is a concern due to bone loss from excessive occlusal load, caused by lack of proprioception and natural periodontal ligament (PDL). We have worked to create living, bioengineered teeth as an alternative therapy to titanium implants. It has recently been shown that decellularized organ scaffolds can support the regeneration of full size organs, such as heart, bladder and lung [1]. Furthermore, eECM scaffolds have recently received FDA approval, and have reached commercialization stage for therapeutic applications in humans [2]. Our preliminary study in a mini-pig jaw implant model showed guided dental tissue formation by dental cell-seeded decellularized porcine tooth bud scaffolds (dTB) [3]. Based on our successful pilot study, we tested revised methods using dTB scaffolds for tooth regeneration in the mini pig.

Methods: Unerupted molar tooth buds (TB) were harvested from discarded 6 month-old pig jaws and decellularized by successive SDS/Triton-X cycles. Three types of replicate tooth implants (n=3) were prepared: 1) dTB seeded with three types of cells (Recell-dTB) including porcine dental epithelial (pDE) cells, human dental pulp (hDP) cells and human Umbilical Vein Endothelial Cells (HUVEC); 2) acellular dTB scaffolds alone; and 3) freshly isolated non-decellularized natural TB (nTB). Replicate implants were implanted in fresh tooth extraction sockets of adult (2Yr) Yucatan mini-pig hosts and grown for 2 or 4 months. Each animal received 2 implants in each hemi-mandible. For each time point, seven minipigs were used, including two empty socket controls. Xylene orange and calcine dyes were injected intravenously to label new calcified tissue formation. Mandibles were fixed in formalin via perfusion and examined using Micro-CT. Imaged mandibles were then either embedded in PMMA for hard tissue sectioning, or decalcified, embedded in paraffin, and sectioned for histological and immunohistochemical analyses.

Results: Full decellularization was achieved after five SDS/Triton-X cycles. After being transplanted for 2 and 4 months, formation of tooth like tissue with surrounding PDL were observed in all types of dTB samples, but mainly in the cell seeded dTB. Newly formed calcified tissues were easily identified by Xylene orange and calcine dyes under fluorescent microscopy.

Figure 1. Regenerated dental tissues. H&E staining of a sectioned Recell-dTB sample showed the formation of calcified tissues closely resembled dentin and cementum. PDL-like tissue structures, with Sharpey’s fibres oriented perpendicular to the surface of newly formed mineralized tissues were also observed. Panels 1-3 are higher magnification images of the boxed areas of lower mag images, respectively. Panels 1’-3’ are polarized light images of respective areas of Panels 1-3. Abbreviations: C, Canine; Ce, Cementum; D, Dentin; PDL, Periodontal Ligament. Scale bar=2 mm (lower mag), and 50 μm (higher mag).

Conclusions: Recell-dTB-ECM scaffolds supported robust formation of tooth-like structures, including PDL tissues. Our results indicate the promise of using dTB as a scaffold material for whole tooth and periodontal tissue regeneration. We are looking to secure funding to support validation in pre-clinical Minipig model, leading to clinical trials in humans.

References: