

## THE DEVELOPMENT AND *IN VIVO* TRANSPLANTATION OF AN ARTIFICIAL TOOTH GERM RECONSTITUTED BY THE BIOENGINEERED ORGAN GERM METHOD

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**INTRODUCTION:** The ultimate goal of regenerative therapy is to develop bioengineered tissue that can replace lost or damaged organs following disease, injury or aging. The development of three-dimensionally reconstructed bioengineered organs from dissociated single cells *in vitro* is therefore a goal of this technology. In this study, we developed a novel method for the reconstitution of three-dimensionally organ germ, using completely dissociated epithelial and mesenchymal cells.

**METHODS:** The bioengineered tooth germ was reconstituted with the cell compartmentalization between epithelium- and mesenchyme-derived single cells isolated from incisor tooth germ at cap stage from the lower jaw in ED14.5 mice at high-cell density ( $5 \times 10^8$  cells/ml) within a collagen gel drop. The development of the bioengineered tooth germ was analysed by the transplantation into a subrenal capsule or a tooth cavity after the extraction of a mandibular incisor in an 8-week-old adult mouse.

**RESULTS:** The bioengineered tooth germ generates a structurally correct tooth following both transplantations under a subrenal capsule *in vivo* and also in *in vitro* organ cultures with a high frequency. Plural teeth containing natural tooth materials and correct cell placement were observed to develop from each primordium, give rise from the periphery of the boundary surface between epithelial and mesenchymal cells after 2-3 days growth. By *in situ* hybridization analysis, it was indicated that our current tooth germ model reproduced the interaction between epithelial and mesenchymal cells in early tooth organogenesis. Treatments of both dissociated epithelial and mesenchymal cells by neutralizing anti-CD29 monoclonal antibodies inhibited the tooth formation in a subrenal capsule assay at the frequency of 90% (9/10) and the mRNA expressions of DSP and amelogenin could not be detected. We additionally generated a tooth cavity by extraction of a mandibular incisor in adult mice and find that a complete tooth structure, showing penetration of nerve fibers, can be

successfully developed following the engraftment of bioengineered primordium isolated from cultured bioengineered tooth germ. The lengths of the bioengineered tooth after the transplantation for 14, 51 and 73 days in a tooth cavity developed from a single primordium were found to be  $2.0 \pm 0.5$  mm (8.0-fold increase in length),  $3.6 \pm 0.5$  mm (14.4-fold increase in length) and  $4.4 \pm 0.6$  mm (17.6-fold increase in length), respectively. Periodontal ligaments could be detected in the areas around the dentin in the explants after 73 days transplantation.

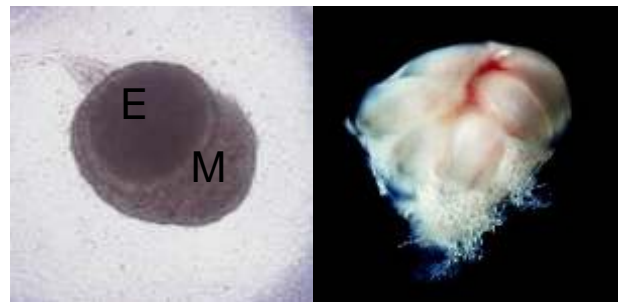


Fig. 1: Representative phase contrast images showing a bioengineered tooth germ (left) and teeth (right) developed in a subrenal capsule for 14 days.

**DISCUSSION & CONCLUSIONS:** Our data suggests that the replacement of biological and functional teeth would be possible by reconstitution in the tooth cavity of adult animal. These results also represent a significant advance in the development of bioengineered organ replacement strategies and regenerative therapies.

**REFERENCES:** Nakao K., Morita R., Saji Y., Ishida K., Tomita Y., Ogawa M., Saitoh M., Tomooka Y. & Tsuji T. (2007) *Nature Method* **4**: 227-230.

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