Supplementary Notes

Analysis of the Development of a Chimeric Bioengineered Tooth Germ in *in Vitro* Organ Culture

We investigated whether our current bioengineered tooth germ is reconstituted by completely dissociated single cells from both epithelial and mesenchymal tissues of incisor tooth germ. We developed a chimeric bioengineered tooth germ analysis using the single cells dissociated from tooth germ of normal and GFP-transgenic mice. Each dissociated cells of epithelial and mesenchymal cells of normal or GFP-transgenic mice were prepared as described (see Supplementary Methods online). At first, we analyzed the development of the chimeric bioengineered tooth germ reconstituted between normal mice-derived epithelial cells and the mixed mesenchymal cells isolated from normal and GFP-transgenic mice with cell compartmentalization at high-cell density. The mesenchymal cells were premixed with normal and GFP-transgenic mice-derived cells at the chimerism of 95% and 5%, respectively (see Supplementary Fig. 1 online). Whereas the green fluorescence could not be detected in epithelial cell-derived ameloblast, the fluorescence was observed and distributed in dental mesenchymal-derived cell types, pulp and odontoblast. The number of GFP-positive cells correlated to the chimerism between normal and GFP transgenic mice-derived cells in this chimeric tooth germ (data not shown). Furthermore, we also examined the chimeric germ reconstituted between epithelial and mesenchymal cells each containing of equally number of normal and GFP-transgenic mice-derived single cells with cell compartmentalization at high-cell density (see Supplementary Fig. 1 online). GFP-positive cells largely distributed in the bioengineered tooth according to the chimerism and detected in the all cell types of ameloblast, pulp and odontoblast.
These results indicate that our current tooth germ method successfully reconstituted by single cell manipulation.

**Multiple Tooth Induction in a Bioengineered Incisor Tooth Primordium by Gene Expressions of Signalling Network**

We analyzed the expression profile of signalling networks that play essential roles both in early tooth development and in morphogenesis\(^1,2\). The expression of the enamel knot marker genes\(^3\), Shh and Wnt10b, were detectable in plural sites and at the boundary surface between epithelial and mesenchymal cells in our bioengineered incisor tooth germ (see **Supplementary Fig. 3** online). Activin \(\beta a\) and Fgf3 transcripts were also mainly detectable in the mesenchyme that had formed in the region adjacent to the epithelial Shh-expression site (see **Supplementary Fig. 3** online). Msx1 transcript was additionally observed throughout the entire mesenchyme, as seen in normal tooth germ (see **Supplementary Fig. 3** online).

Ectodin inhibits the expression of p21, through its antagonistic effects upon BMP4 signalling and is critical for the spatial delineation of enamel knots and cusps\(^4,5,6\). Consistent with this, ectodin and p21 were found to be inversely expressed at the boundary surfaces between the epithelial and mesenchymal cells (see **Supplementary Fig. 3** online). Furthermore, the signalling between ectodysplasin (Eda) and its receptor, Edar, is thought to induce enamel knot formation\(^7\) and the expression pattern of Edar was identical to those of Shh and p21 in the reconstituted tooth germ (see **Supplementary Fig. 3** online).
The Shh signalling network molecules, patched 1 (Ptc1) and growth arrest-specific gene (Gas1) are thought to regulate the regionalization of the odontogenic mesenchyme in the mandibular arch\(^8\). Ptc1 and Gas1 transcripts were detectable in the proximal and distal mesenchyme, respectively, of the boundary surface between the epithelium and mesenchyme, and the strong expression of Ptc1 transcripts could be observed in the regions adjacent to the enamel knot (see Supplementary Fig. 3 online). These observations are clearly indicated the evidences that our current tooth germ model reproduce the interaction between epithelial and mesenchymal cells in early tooth organogenesis.

**Generation of a Reconstituted Whisker from a Bioengineered Follicle**

We investigated whether it would be possible to a bioengineered mouse whisker using our developed bioengineering technology for the reconstitution of artificial primordial organs. Tissues containing whisker follicles were dissected from the cheeks of ED14.5 mice and the epithelial and mesenchymal tissues were completely dissociated to single cells via the same enzymatic method used for tooth germ regeneration (see Supplementary Fig. 1 online). Epithelial and mesenchymal cells were also reconstituted with cell compartmentalization at a high-cell density in a collagen gel, and the bioengineered whisker follicle was transferred to a cell culture insert. After one day of incubation, the explant was then transplanted into a subrenal capsule for 14 days (see Supplementary Fig. 4 online). At post-transplantation day 14, the explants in the subrenal capsule were found to regenerate a whisker at a 100% frequency (20/20; see Supplementary Fig. 4 online). Histological analysis of these explants also revealed the expected tissue morphologies, such as the whisker shaft (ws), inner root sheath (irs) and outer root sheath (ors). Moreover, these tissues were
arranged normally, when compared with a natural whisker (see Supplementary Fig. 4 online).

Reference


