O39-ESTABLISHMENT OF *IN VITRO* CULTURE SYSTEM FOR EVALUATION OF THE DENTIN-PULP COMPLEX REGENERATION WITH SPECIAL REFERENCE TO DIFFERENTIATION CAPACITY OF THE BRDU-LABEL-RETAINING DENTAL PULP CELLS.

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Key words
Bromodeoxyuridine, Cell differentiation, Cell proliferation, Dental pulp, Odontoblasts, Stem cells, Mice (Inbred ICR).

Introduction
Our recent study has demonstrated that a pulse of the thymidine analog BrdU given to the prenatal animals revealed the existence of slow-cycling long-term label-retaining cells (LRCs), putative adult stem cells, reside in the pulp tissue. Furthermore, we have proposed the new hypothesis that both progenitors and dental pulp stem cells (DPSCs) are equipped in the dental pulp and that the DPSCs with proliferative capacity play crucial roles in the pulpal healing process following the exogenous stimuli in cooperation with the progenitors (Ishikawa Y *et al*., 2010). This study aims to establish *in vitro* culture system for the evaluation of the dentin-pulp complex regeneration with special reference to differentiation capacity of the LRCs using immunocytochemistry for BrdU, Ki67, nestin, and α-smooth muscle actin (SMA), and to compare the results obtained from *in vitro* system with those obtained from *in vivo* animal models such as tooth replantation/transplantation for the verification of our hypothesis.

Materials and Methods
Three peritoneal injections of BrdU were given to pregnant Crlj:CD1(ICR) mice to map dense LRCs in the mature tissues of born animals. The labeled born animals or GFP-transgenic mice were used for *in vitro* and *in vivo* experiments. The upper-right first molars (M1) of BrdU-labeled or GFP-transgenic mice (3 weeks old) were extracted under anesthesia. For *in vitro* culture, the extracted teeth were divided into the two pieces and cultured for 0, 1, 3, and 7 days using the Trowel's method. For *in vivo* experiments, the extracted teeth were replanted in the original socket or the crown portion without roots were allogenic transplanted in the sublingual region of non-labeled animals.

Results
We succeeded to establish the *in vitro* culture system for the evaluation of the dentin-pulp complex regeneration, where most odontoblasts were occasionally degenerated and lost nestin-immunoreactivity because of the separation of cell bodies from cellular processes in the dentin matrix until the beginning of *in vitro* culture. Numerous dense LRCs were mainly resided in the center of the dental pulp associating with blood vessels throughout the experimental periods. On postoperative Days 1–3, the periphery of pulp tissue including the odontoblast layer showed the degenerative features, although some odontoblasts survived throughout the experimental periods. Until Day 7, nestin-positive odontoblast-like cells were arranged along the pulp-dentin border and dense LRCs were committed in the odontoblast-like cells. These chronological changes in the pulp-dentin border *in vitro* organ culture were similar to the changes in the *in vivo* experimental models.

Conclusion
These results suggest that dense LRCs in the center of the dental pulp associating with blood vessels were supposed to be dental pulp stem cells possessing regenerative capacity for forming newly differentiated odontoblast-like cells.

Acknowledgements
This work was supported in part by Grants-in-Aid for Scientific Research (B) (no. 22390341 to H.O.) and Exploratory Research (no. 20659296 to H.O.) from MEXT and JSPS, and a Grant for Supporting Project for Strategic Research of Nihon Univ Sch Dentistry at Matsudo from MEXT, 2008-2012 (Team: Dental Morphogenesis).

References

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