Name：Islam Syed Taufiqul（Department of Pediatric Dentistry, Health Sciences University of Hokkaido）

Title：Analysis the characteristics of Epithelial rests of Malassez cloning cells

The purpose：Cap stage of tooth development includes four different cell layers. The inner layer is called inner enamel epithelium（IEE）, the outer layer is outer enamel epithelium（OEE）. After the bell stage, Hertwig's epithelial root sheath（HERS）is formed at the lower edge of enamel organ during root formation stage. This structure consists of IEE and OEE and is thought to have an ability that decides the shape of tooth root formation.

After root formation, HERS disintegrates and forms epithelial rests of Malassez（ERM）. The characteristics of ERM cells in vitro have been demonstrated as cultured epithelial cells derived from periodontal ligaments.

However, there are two kinds of epithelial cell in ERM. The identity of those cells has yet to be explored. In this study, we established clone cells derived from ERM by single cell limiting dilution method and analyzed the characteristics of ERM cloning cells.

Materials and Methods：

Cloning：ERM cells were isolated from porcine periodontal ligament by outgrowth method, from which a group of cloning ERM cells was obtained through the single cell limiting dilution. Each cloning ERM cell was checked for epithelial marker ck-wide and odontogenic epithelial marker Cytokeratin 19. Growth rate was assessed by proliferation assay.

Real-time RT-PCR：Total RNA was extracted from crude ERM and cloning ERM cells. After cDNA synthesis, expression levels of amelogenin were evaluated by real-time RT-PCR.

The statistical significance of the difference was analyzed using One-way ANOVA and Scheffe's test.

Results and Discussion：

Isolated number of cloning cells from crude ERM was 30. From them, several cloning ERM cells were found with different distinct characteristics. To continue further experiment three type of Cloning ERM cells were selected by visual inspection and proliferation assay. Authenticity of ERM origin were proved by CK-wide and CK-19 positivity. These cloning ERM cells exhibited high and low expression level of amelogenin by Real-time RT-PCR. High amelogenin expressed cells showed low proliferation ratio where else low expressed cells showed high proliferation

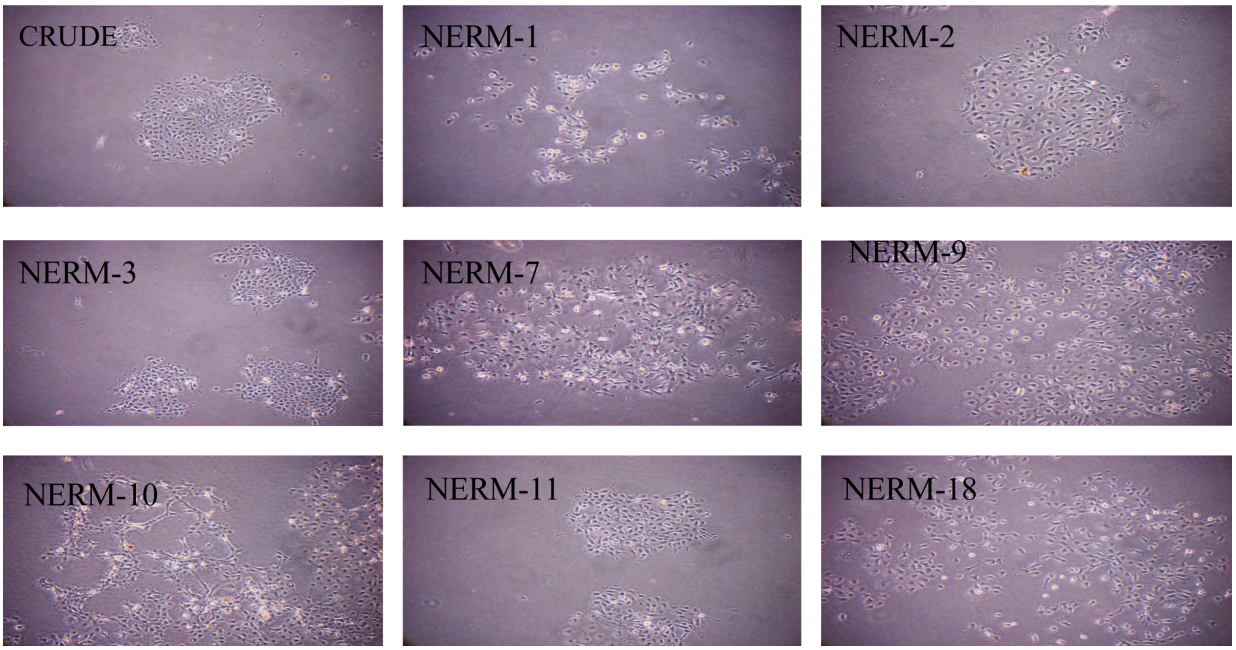

Fig.1, cell morphology

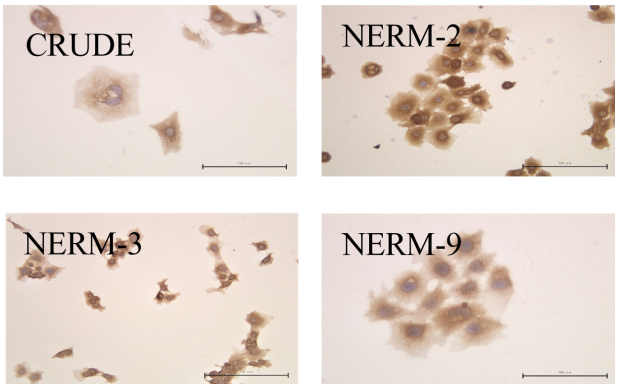

Fig. 2, Immunohistochemical staining CK-wide

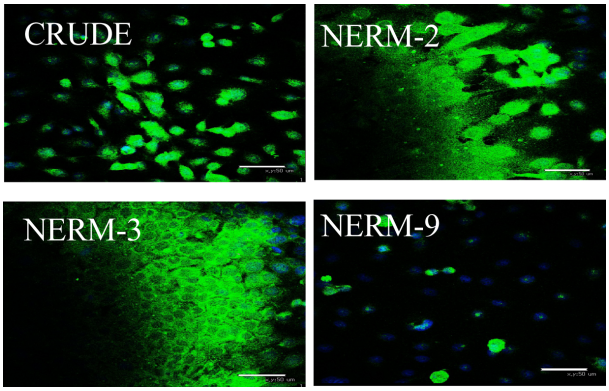

Fig. 3, Immunofluorescence staining CK-19

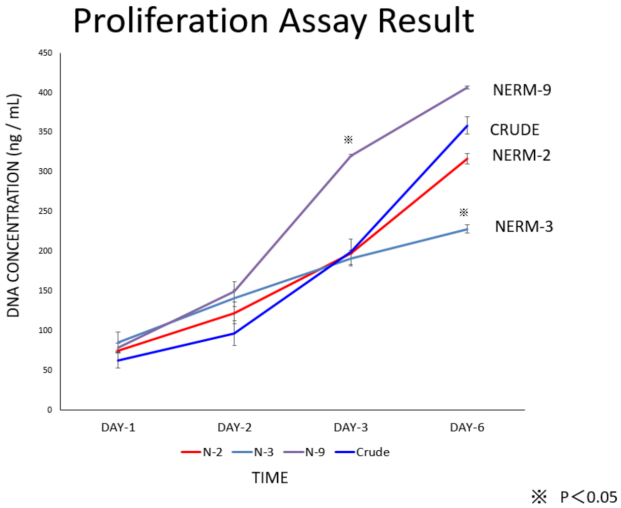

Fig. 4, Proliferation assay

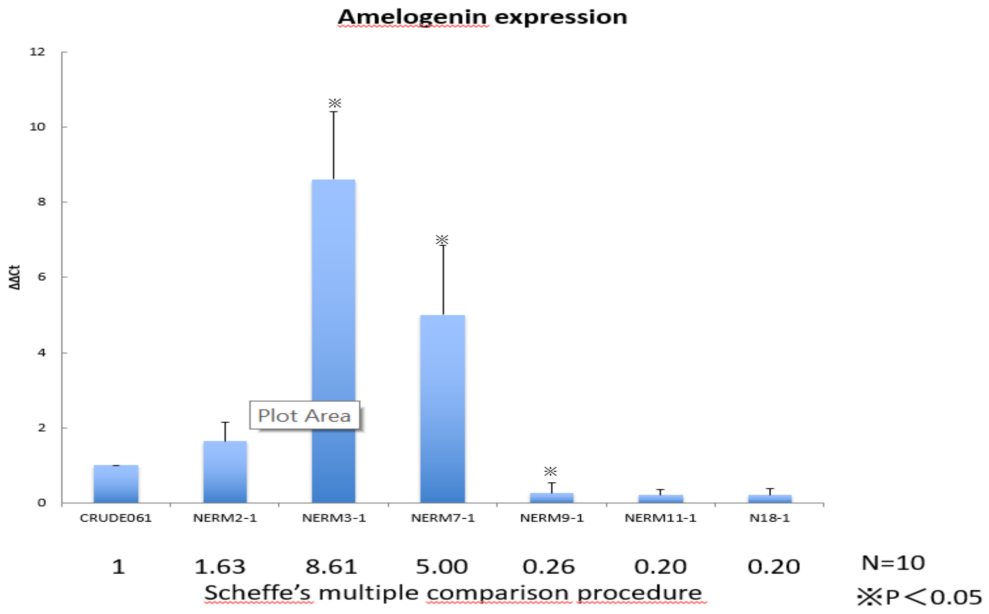

Fig. 4, Expression of Amelogenin by Realtime RT-PCR

Conclusion：

In this experiment we were able establish clone cells from ERM by single cell dilution method. Our next step will be to characterize those clone cells to rule out the identity and possible functional differences.

Presentation：

1. *Syed Taufiqul Islam, Erika Minowa, Daisuke Noro, Yoshihito Kurashige, Masato Saitoh, 'Analysis the characteristics of Epithelial rests of malassez cloning cells' The 37th of Dental Society of Health Sciences university of Hokkaido, March 16, Sapporo, Hokkaido, Japan*