

# 論文内容要旨

## **DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS INTO DENTAL EPITHELIAL-LIKE CELLS**

(人工多能性幹細胞の口腔上皮細胞への分化)

主指導教員：谷本 幸太郎 教授  
(応用生命科学部門 歯科矯正学)

副指導教員：加藤 功一 教授  
(基礎生命科学部門 生体材料学)

副指導教員：國松 亮 助教  
(応用生命科学部門 歯科矯正学)

**AIMI NAIM BINTI ABDULLAH**  
(医歯薬保健学研究科 医歯薬学専攻)

## 論文内容要旨

論文題目 **Differentiation of induced pluripotent stem cells into dental epithelial-like cells**

(人工多能性幹細胞の口腔上皮細胞への分化)

学位申請者 Aimi Naim Binti Abdullah

Tooth regeneration holds a great interest in regenerative dentistry. To date, several research groups have successfully regenerated tooth-like structure by mimicking natural developmental process of tooth organogenesis that involves reciprocal interactions between mesenchyme and epithelial cells followed by differentiation of tooth-specific secretory cells. However, clinical applications of such technologies for tooth regeneration are limited primarily due to the lack of appropriate sources of dental epithelial cells. Indeed, dental epithelial cells are limited in embryos, and epithelial cell-derived ameloblasts are mostly lost during tooth eruption. Therefore, alternative sources of dental epithelial cells are absolutely required in order to establish a practical and reliable method for tooth regeneration.

As a source of dental epithelial cells, induced pluripotent stem (iPS) cells attract our attention due to their unique characteristics, including unlimited self-renewal capability, ultimate ability to differentiate into specific lineages and evasion of ethical issues. After iPS cells were established for the first time by Dr. S. Yamanaka, numerous studies have been made to efficiently differentiate iPS cells into specific cell lineages. However, an appropriate method to induce dental epithelial cells from iPS cells remains to be established.

According to the background described above, this study focused on the potential of iPS cells as a novel source for dental epithelial cells. To gain insights into culture conditions efficient for the induction of dental epithelial cells, several cytokines such as bone morphogenetic protein-2 and -4 and neurotrophic factor-4 (NT-4) were screened for their potential as an inducer by analyzing the enhanced expression of specific marker genes for dental epithelial cells, such as cytokeratin-14 (*ck14*) and *p63*, upon addition of these factors. As a result, NT-4 has been successfully identified as the most effective cytokine among them for promoting the differentiation of mouse iPS cells (iPS-MEF-Ng-20D-17, Riken) into dental epithelial-like cells. Based on this finding, a special attention was paid to NT-4 in the latter study, optimizing the method of adding NT-4 to mouse iPS cell culture.

First, NT-4 was added to a medium used for culturing iPS cells in the form of monolayers or embryoid bodies (EBs). The formation of EBs is considered to trigger the differentiation of iPS cells. It was shown in this study that the significantly higher expression of early dental epithelial marker genes (*ck14* and *p63*) was seen in the case of

EBs compared to the monolayer culture when these cells were subsequently cultured on gelatin-coated dishes. It was further found that the effect of NT-4 was in a concentration-dependent manner. These results indicate that the formation of EBs, as well as the addition of NT-4, is a better approach to induce iPS cells toward dental epithelial-like cells. Under this condition, the addition of NT-4 may initiate an inductive microenvironment hence direct iPS cells toward dental epithelial differentiation instead of spontaneous differentiation triggered by EB formation.

It was further found that the presence of serum in a medium used for culture on a gelatin-coated dish had a significant impact on the differentiation of dental epithelial-like cells. The larger numbers of cobblestone-like cells were seen around EBs in a serum-free media than in a media containing 10% fetal bovine serum (FBS). The serum-free condition resulted in the higher expression of *ck14* and *p63* than the condition with 10% FBS, as shown by real-time polymerase chain reaction. It was further shown by western blot analysis and immunofluorescent staining that the addition of NT-4 to EBs and subculture in a serum-free culture medium is the most effective for the up-regulation of *p63* and *ck14* proteins. In addition, surface marker analysis by flow cytometry revealed that cells obtained from this culture expressed highest CD49f ( $\alpha_6$  integrin), a marker for dental epithelial cells, among the cells obtained under different conditions.

Further study was made to evaluate the cells obtained under different conditions (w/o NT-4 and serum) for their differentiation potential to an ameloblast lineage. The results showed that, after culture on the gelatin-coated dish for 9 days, these cells started to express ameloblast-specific genes, including CD29 (integrin  $\beta_1$ ), cytokeratin 19 (CK19), alkaline phosphatase (ALP), ameloblastin (AMBN), amelogenin (AMEL), dentin sialophosphoprotein (DSPP) and dentin matrix protein-1 (DMP-1), undergoing mineralization as assessed by Alizarin red staining. It should be noted that expression of these markers and calcium deposition were most prominent when NT-4 was added to EB culture and FBS-free in subculture on a gelatin-coated dish compared to cells obtained under different conditions.

Taken together, the present study suggests that NT-4 could have a functional role in the differentiation of iPS cells into dental epithelial-like cells with propensity to differentiate into ameloblasts. Although it is known that NT-4 can be detected in early gestation at oral epithelium, the exact functions of this molecule in the tooth development particularly in ameloblasts formation have not been fully understood. A previous study reported that NT-4 could induce one of enamel matrix protein, ameloblastin, *via* a TrkB signaling pathway. Thus, it may be suggested that, based on the results of this study that NT-4 promotes differentiation of iPS cells into dental epithelial-like cells with a potential to form ameloblasts may also be mediated by this TrkB signaling pathway.

In conclusion, NT-4 is an effective inducer for the differentiation of iPS cells into dental epithelial-like cells. This effect is enhanced when NT-4 is added to iPS cells in the form of EBs instead of monolayers and the EBs were cultured on a gelatin-coated dish in the absence of FBS.