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# ABSTRACT OF DISSERTATION

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| Title | Deciphering defective amelogenesis using *in vitro* culture systems (*in vitro* 細胞培養系によるアメロジェネシス不全メカニズムの解読) |
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**[Background]** The conventional two-dimensional (2D) *in vitro* culture system is often used to analyze the gene expression with or without extracellular signals. However, the cells derived from primary culture and cell lines frequently deviate the gene expression profile compared to the corresponding *in vivo* samples, which sometimes misleads the actual gene regulation *in vivo*. To overcome this gap, I developed the 2D and 3D *in vitro* culture systems and applied them to the genetic study of Amelogenesis imperfecta (AI) as a model. Amelogenesis imperfecta is an inherited enamel disorder caused by alteration of gene expressions involved in enamel formation and mineralization. Previously, it was reported that a novel mutation in the specificity protein 6 (Sp6) caused AI-phenotype in SHRSP rat called AMI rat, and then AMI-derived ARE-B30 and wild type cells, G5 were established in our laboratory. However, the role of Sp6 is remains unclear.

**[Objective]** To analyze the molecular basis for the genotype and phenotype correlation in an *in vitro* AI model with Sp6 mutation.

**[Materials and methods]** Two-D and 3D culture systems were established *in vitro* to mimic the *in vivo* condition, and the comparative phenotypic screening was performed to identify defective amelogenesis in AI model. First, two types of 2D culture systems were prepared. G5 or ARE-B30 was seeded on a 35 mm plastic culture dish or a collagen membrane (CM-6). Second, the 3D culture system was maintained with two different volumes of culture medium. 3D separated culture medium; G5 or ARE-B30 was co-culture with RPC-C2A (rat pulp cell lines) and the culture medium for the RPC-C2A cells was added into each well of the culture plate, while the media for the G5 or ARE-B30 cells were added separately into the CM-6 device. 3D mixed culture

medium; the culture medium was added into the well of the culture plate until it connected to the culture medium inside the CM-6 device. The cells were seeded with 6.0 x $10^5$ cells/well, which reached 100% confluency by day 1, and cultured for 1 to 14 days. The mRNA expression levels of differentiation marker genes were analyzed by semi-quantitative RT-PCR.

**[Results]** Comparative analysis of amelogenesis-related gene expression in ARE-B30 and G5 using our 2D and 3D *in vitro* systems revealed distinct expression profiles, showing the possible causative outcomes. *Bone morphogenetic protein 2* and *follistatin* were reciprocally expressed in G5, but not in ARE-B30 cells. All-or-none expression of *amelotin*, *kallikrein-related peptidase 4,* and *nerve growth factor receptor* was observed in both cell types. Additionally, *Amtn* was enhanced in cell density- and time-dependent manner, while *Klk4* showed limited enhancement.

**[Discussion]** Phenotypic screening using 2D and 3D culture systems identified stage-specific defects in amelogenesis by demonstrating aberrant gene responsiveness in the ARE-B30 cells. Furthermore, these defects were well correlated with the *in vivo* AI phenotypes of AMI rat. The aberrant expression of differentiation marker genes in ARE-B30 cells suggests that Sp6 may play a dual role in cell fate decision and regulation of gene expression.

**[Conclusion]** Our *in vitro* culture systems demonstrated the phenotypical differences in the expression of the stage-specific amelogenesis-related genes. Parallel analysis with 2D and 3D culture systems may provide a platform to understand the molecular basis for defective amelogenesis caused by *Sp6* mutation, and that these findings shed light on the tooth development and contribute for tooth regeneration.