Report of "Research Award of Oral Sciences"

Major: Oral Sciences
Grade: 4th Grade
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Title: Dentin-Pulp Complex Tissue Regeneration via Three-dimensional Cell Sheet Layering

1. Aim of research and results obtained

The dentin-pulp complex is a unique structure in teeth that contains both hard and soft tissues. Generally, deep caries and trauma cause damage to the dentin-pulp complex, and if left untreated, this damage will progress to irreversible pulpitis. In recent years, tissue regeneration therapy using cell sheet engineering technology has been developed and is being investigated from the regeneration of periodontal tissue in the dental field as well. However, it is difficult to regenerate the dentin-pulp complex as a functional unit of teeth composed of multiple types of cells in a cell sheet constructed of a single cell. The aim of this study was to fabricate a layered cell sheet composed of rat dental pulp (DP) cells and odontogenic differentiation of pulp (OD) cells and to investigate the ability to regenerate the dentin-pulp complex in a scaffold tooth.

In this study, we fabricated two single cell sheets composed of DP cells (DP cell sheet) or OD cells (OD cell sheet) and a layered cell sheet made by layering both cells. The characteristics of the fabricated cell sheets were analyzed using light microscopy, scanning electron microscopy (SEM), hematoxylin-eosin (HE) staining, and immunohistochemistry (IHC). Furthermore, the cell sheets were transplanted into the subrenal capsule of immunocompromised mice for 8 weeks. Following this, the regenerative capacity to form dentin-like tissue was evaluated using micro-computed tomography (Micro-CT), HE staining, and IHC. The findings of SEM and IHC confirmed that layered cell sheets fabricated by stacking OD cells and DP cells maintained their cytological characteristics. Micro-CT of layered cell sheet transplants revealed a mineralized capping of the access cavity in the crown area, similar to that of natural dentin. In contrast, the OD cell sheet group demonstrated the formation of irregular fragments of mineralized tissue in the pulp cavity, and the DP cell sheet did not develop any hard tissue. Moreover, bone volume/tissue volume (BV/TV) showed a significant increase in hard tissue formation in the layered cell sheet group compared to that in the single cell sheet group (BV / TV, $n = 5$, * $p < 0.05$, ** $p < 0.001$). HE staining also showed a combination of soft and hard tissue formation in

the layered cell sheet group. Furthermore, IHC confirmed that the dentin-like tissue generated from the layered cell sheet expressed characteristic markers of dentin but not bone equivalent to that of a natural tooth.

In conclusion, our study represents the feasibility of complex tissue regeneration using a bioengineered tissue designed to simulate the anatomical structure.

2. Self-evaluation of research achievement:

In the research, I successful layered two types of cells in a complex cell sheet and generated dentin-pulp like tissue in vivo. This layered cell sheet enables to reconstruct complex tissues with only one type of cell when the target cells are derived from the same cell lineage which is great merit of this study. But I couldn't establish an injury model in oral cavity, so I think for subsequent experiments I will improve the orthotopic transplantation in large animals.

3. Meeting presentation:

① **Yan H**, Dentin-pulp complex tissue regeneration by three-dimensional tissue engineering technology. (oral) , Bioscience retreat, 徳島, online. 2021-9-17

② **Yan H**, Dentin-pulp complex tissue regeneration by three-dimensional cell sheet engineering. (poster), The 69th Annual Meeting of Japanese Association for Dental Research. 九州大学, online, 2021-10-24/25.

③ **Yan H**, Dentin-pulp complex tissue regeneration via three-dimensional cell sheet layering. (oral), 発生・再生・遺伝クラスター・ミニリトリート,徳島, online. 2020-12-21

4. Journal publication:

Dentin-pulp complex tissue regeneration via three-dimensional cell sheet layering. Tissue Engineering Part C: Methods. Vol 27/No.10/559-570. Published Online 15/10/2021. **Yan H**, Oshima M, Raju R, Raman S, Sekine K, Waskitho A, Inoue MH, Inoue M, Baba O, Morita T, Miyagi M, Matsuka Y.