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ABSTRACT OF DISSERTATION

Title	Three-dimensional periodontal tissue regeneration using a bone-ligament complex cell sheet [骨-歯根膜線維の複合組織形成による三次元的な歯周組織再生技術の開発]
Author's Name	Resmi Raju, Masamitsu Oshima, Miho Inoue, Tsuyoshi Morita, Yan Huijiao, Arief Waskitho, Otto Baba, Masahisa Inoue, Yoshizo Matsuka.

[Background]

Various clinical techniques have been utilized for periodontal tissue regeneration, however, a long-term stable clinical outcome has not been attained. Various stem cells, cytokines and growth factors have been well characterised, and the information can be applied to the repair of periodontal tissue components such as cementum, PDL and alveolar bone. However, the limitation of cell transplantation and cytokine therapy includes the difficulty in delivering and stabilizing a sufficient quantity of cells/molecules into the defect area. Cell sheet engineering with a temperature responsive culture dish has various advantages over the regenerative methods using artificial scaffolds. Cells can be harvested as a single sheet without destroying the attachment proteins and extracellular matrix.

[Methodology]

We used 4–5-week-old male Sprague-Dawley (SD) rats for the PDL cell collection. CB17/Icr scid/scid female mice were used for ectopic transplantation and orthotopic transplantation. All animal experiments were approved by the Animal Care Committee of Tokushima University (Approval No. T29-50) (Tokushima, Japan).

Cell sheets were prepared using a temperature responsive culture dish. The complex cell sheet was fabricated by layering PDL cells on top of the MC3T3-E1 cell culture. Single cell sheets were fabricated by either one of the above mentioned cells. Cell sheets were sectioned into 4 pieces and each section was wrapped around the autoclaved mice mandibular first molar roots. All samples were transplanted into the subrenal capsule of immunocompromised mice with each mice receiving 4 transplants.

The periodontal tissue injury was created on the palatal bone overlying the maxillary first molars using a small round carbide burr. The injury was carefully created by removing the bone and PDL adjacent to the maxillary first molar tooth without trimming the root. Cell sheets were trimmed to the appropriate shape and adapted to the

exposed root surface.

Three-dimensional modeling analysis of reconstructed images were carried out and region of interest (ROI) was selected from the palatal bone adjacent to the first molar tooth on right and left side and regenerated bone volume at the injury site was analyzed and quantified using CT analyzer software. For histologic analysis, ectopic and orthotopic transplant sections were stained with H&E or azan staining. Anti-periostin antibody and anti-mouse-OCN antibody were used for immunohistochemistry.

[Results]

After 4 weeks of ectopic transplantation a combination of PDL-like and bone-like tissue formation was evident in the complex cell sheet group. On the other hand, only bone like tissue or irregular fibrous tissue formation was observed in MC3T3-E1 group and PDL cells sheet group respectively. Using azan staining, we observed an attachment of PDL-like fibers to the tooth root and a newly formed bone-like tissue in the complex cell sheet group. Immunohistochemistry analysis of complex cell sheet group revealed positive OCN expression in the bone-like tissue regenerated area and periostin expression in the PDL-like tissue. These results confirm the presence of PDL and bone-like tissue support in the complex cell sheet group.

The three-dimensional images of micro-CT evaluation demonstrated an incomplete regeneration of alveolar bone in the control group and PDL cell sheet group after 8 weeks of orthotopic transplantation. Alternatively, complete regeneration of alveolar bone was observed in the complex cell sheet transplanted group similar to the architecture in normal mouse. Histological images showed PDL-like fibers and bone-like tissue formation in the complex cell sheet group. Azan staining revealed the arrangement and attachment of PDL-like fibers were perpendicular and oblique to the root surface similar to the natural tooth. Finally, we performed immunohistochemistry analysis using anti-periostin and anti-mouse-OCN antibody and confirmed the formation of PDL-like and bone-like tissue formation in complex cell sheet orthotopic transplants.

[Conclusion]

Our study demonstrated the fabrication of a complex cell sheet composed of PDL cells and osteoblast-like cells that anatomically reproduced the bone-ligament structure equivalent to natural periodontal tissue. Furthermore, we demonstrated three-dimensional regeneration of periodontal tissue using a complex cell sheet in a large-scale tissue injury. This study represents the feasibility of three-dimensional tissue engineering/regeneration using an anatomically designed bioengineered complex tissue.