

Combination Use of Autologous Periodontal Ligament-derived Cell Sheets and Beta-tricalcium Phosphate Granules in Periodontal Defects in Humans

Takanori Iwata^{1,2}, Kaoru Washio¹, Toshiyuki Yoshida¹, Kohei Tatsumi¹, Azusa Yamada^{1,3}, Yuka Tsumanuma^{1,3}, Tomohiro Ando², Isao Ishikawa¹, Masayuki Yamato¹, & Teruo Okano¹

¹Institute of Advanced Biomedical Engineering and Science;

²Department of Oral and Maxillofacial Surgery, Tokyo Women's Medical University, Tokyo, Japan;

³Section of Periodontology, Tokyo Medical and Dental University, Tokyo, Japan

Statement of Purpose:

We previously showed that autologous transplantation of periodontal ligament (PDL) cell sheets combined with beta-tricalcium phosphate regenerated the true periodontal tissue in canine models^{1,2}. Translating to a clinical setting, we have optimized the methods for extraction and cultivation of human PDL cells³, and validated both the safety and efficacy of human PDL cell sheets⁴. Finally, we have started the clinical trial named "Autologous transplantation of periodontal ligament cell sheets for periodontal reconstruction", after the approval of the Ministry of Health, Labor and Welfare in Japan. Patient's PDL cells are cultured with autologous serum, and cell sheets are fabricated in temperature-responsive dishes with osteoinductive supplements. Three-layered PDL cell sheets are transplanted to the denuded root surface, and beta-tricalcium phosphate (β TCP) granules are filled in the infrabony defects. In this presentation, current status of this clinical trial will be reported.

Methods:

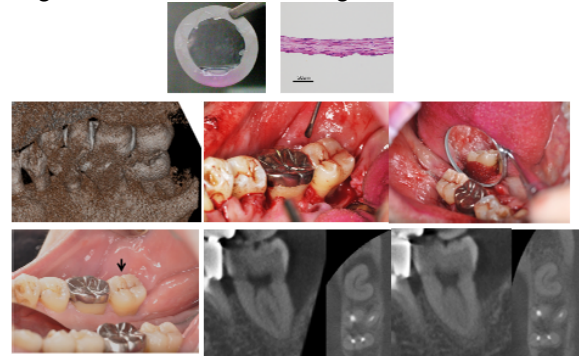
This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Institutional Review Board of Tokyo Women's Medical University Human Subjects Research and the Minister of Health, Labour and Welfare in Japan in accordance with the "Guidelines on clinical research using human stem cells". Autologous PDL tissue in the mid third of extracted tooth was digested with GMP-grade enzymes [0.8 PZ-U/ml collagenase (Serva Electrophoresis, Heidelberg, Germany) and 1200 PU/ml dispase (Sanko Junyaku, Tokyo, Japan)] for 1 h at 37°C with vigorous shaking. All cells which passed through a 70 μ m strainer were spread on a T25 flask. Usually, 10 to 100 colonies were visible a few days later. Cells, which possessed a colony forming activity, were subcultured at the low cell density (less than 5000 cells/cm²). Cells were seeded on temperature-responsive culture dishes (35 mm in diameter, UpCell®, Cell Seed, Tokyo, Japan) at a cell density of 3–5 \times 10⁴ cells/dish and cultured in an osteoinductive medium supplement with 50 μ g/ml ascorbic acid (Wako, Tokyo, Japan), 10 mM β -glycerophosphate (Sigma–Aldrich, St Louis, MO), and 10 nM dexamethasone (DEXART; Fuji Pharma, Toyama) for 2 weeks. For the cell sheet harvest, the temperature was reduced to room temperature before the culture medium was aspirated and a wet sheet of woven polyglycolic acid (PGA) (Neoveil®, PGA Felt-Sheet Type, 0.15 mm in thickness: Gunze, Tokyo, Japan) was placed on the culture dish as a reinforced carrier. Cell sheets attached with PGA were harvested by peeling them from the dishes with forceps and then put on the next culture dish. This procedure was

repeated two more times until three-layered cell sheets were fabricated, and transplanted to the cleaned dental root. After the position of PDL cell sheets was adjusted to the appropriate position, infrabony defect was filled with β TCP (Osferion®, G1, Olympus Terumo Biomaterials, Tokyo, Japan).

Results:

3D image and clinical photographs of a clinical case of "Periodontal Regeneration with Autologous Periodontal Ligament Cell Sheets" is shown below.

A 35-year-old male patient had an infrabony defect in the lingual and distal of the lower right 2nd molar.



Upper panels show the created thick autologous PDL cell sheet. Middle panels show 3D reconstructed image before the transplantation (left), clinical view after the debridement (center), and transplanted 3-layered PDL cell sheets with PGA into the cleaned root surface (right). Lower Panels show the clinical view of 2 month after the transplantation (left), CT view at the baseline (center), and 6 months after the transplantation (right). In this case, bone height was increased 2.71 mm and the pocket depth was decreased from 6 mm to 2 mm. Clinical attachment level was also gained (7 mm to 4 mm).

Conclusions:

We have experienced 4 cases of autologous PDL cell sheets transplantation, and no adverse reaction has been observed. In the finished case, the reduction of pocket probing depth (PPD) and newly formed bone-like structures were observed. Although this clinical trial is still conducting, the safety and efficacy of our clinical trial would be proven in the near future.

References:

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4. Washio K, et al. *Cell Tissue Res*. 2010;341:397-404.