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Original Article

Periodontal regeneration with autologous periodontal ligament-derived cell sheets – A safety and efficacy study in ten patients

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ABSTRACT

Background: Periodontitis results in the destruction of tooth-supporting periodontal tissues and does not have the ability to heal spontaneously. Various approaches have been introduced to regenerate periodontal tissues; however, these approaches have limited efficacy for treating severe defects. Cytotherapies combine stem cell biology and tissue engineering to form a promising approach for overcoming these limitations. In this study, we isolated periodontal ligament (PDL)-derived cells from patients and created cell sheets with "Cell Sheet Engineering Technology", using temperature responsive culture dishes, in which all the cultured cells can be harvested as an intact transplantable cell sheet by reducing the temperature of the culture dish. Subsequently, the safety and efficacy of autologous PDL-derived cell sheets were evaluated in a clinical setting.

Methods: A single-arm and single-institute clinical study was performed to verify the safety and efficacy of autologous PDL-derived cell sheets in patients with periodontitis. Wisdom teeth were extracted from patients diagnosed with chronic periodontitis, ranging in age from 33 to 63 years (mean [±SD], 46 ± 12), and periodontal tissues were scraped for cell sources. Three-layered PDL-derived cell sheets were constructed using temperature-responsive culture dishes and transplanted in an autologous fashion following standard flap surgeries. Bony defects were filled with beta-tricalcium phosphate granules. Clinical variables were evaluated at baseline, 3 months, and 6 months. Cone-beam computed tomography was performed at baseline and 6 months. Additionally, mid-long-term follow-up has been performed with patients' agreements.

Results: Our method was found to be safe and no severe adverse events were identified. All the findings, including reduction of periodontal probing depth (mean ± SD, 3.2 ± 1.9 mm), clinical attachment gain (2.5 ± 2.6 mm), and increase of radiographic bone height (2.3 ± 1.8 mm), were improved in all 10 cases at 6 months after the transplantation. These therapeutic effects were sustained during a mean follow-up period of 55 ± 19 months, and there were no serious adverse events.

Conclusions: The results of this study validate the safety and efficacy of autologous PDL-derived cell sheets in severe periodontal defects, and the stability of this efficacy during mid-long-term follow up. This cytotherapeutic approach, based on cell sheet engineering, offers an innovative strategy to treat the recognized unmet need of treating severe periodontal defects.

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1. Introduction

Periodontitis (gum disease) is a bacteria-induced inflammatory disease that affects the supporting structures of the teeth, including the jawbone, periodontal ligament (PDL), and cementum. Periodontitis not only gives rise to functional and esthetic problems in the oral cavity, but is also associated with systemic diseases, such as diabetes, preterm birth, cardiovascular disease, stroke, and pulmonary disease [1]. Conventional treatments can only delay the progress of the condition, and the therapeutic effect of various resection surgeries is considered minimal. To mitigate these limitations, regenerative therapies have been investigated for almost 100 years [2]. Bone grafts, barrier membranes, and other biological materials have been approved in clinics for the treatment of relatively small size defects; however, there are no appropriate therapies for severe defects, such as one-wall intrabony defects, class III furcation defects, and horizontal defects. The majority of periodontal defects are of these severe defect shapes [3], therefore cytotherapeutic approaches have been investigated during the 21st century based on the development of tissue engineering and stem cell biology [4].

Stem cells were identified from human PDL tissue and suggested as a promising cell source for periodontal regeneration [5,6]. At the same time, our laboratory developed “Cell Sheet Engineering Technology”, using a temperature responsive cell culture surface [7], in which all the cultured cells can be harvested as an intact transplantable cell sheet by reducing the temperature of the culture dish. Several clinical studies using “Cell Sheet Engineering Technology” have been reported, such as corneal reconstruction [8], treating cardiomyopathy [9], endoscopic treatment of esophageal ulceration [10], and middle ear mucosal regeneration [11], and the safety and efficacy of these autologous cell sheet therapies were observed. Our group focused on PDL-derived cells and combined them with this technology to create PDL-derived cell sheets. Previous animal experiments showed the efficacy of PDL-derived cell sheets in several experimental periodontal defect models [12–15]. Additionally, we established the optimal extraction and cultivation methods for human PDL-derived cells [6], and the safety of these cells was confirmed [16,17].

In this study, we examined the safety of autologous PDL-derived cell sheets combined with beta-tricalcium phosphate, and the regenerative potential of this new approach in a clinical setting.

2. Experimental methods

This study was conducted according to the principles expressed in the Declaration of Helsinki and approved by both the Institutional Review Board of Tokyo Women's Medical University (TWMU) Human Subjects Research and the Japanese Minister of Health, Labour and Welfare in accordance with the “Guidelines on clinical research using human stem cells”. All samples were processed and cultured in the cell processing center of TWMU in accordance with the good manufacture practice (GMP) guidelines. This clinical study was registered with the UMIN Clinical Trials Registry, number UMIN000005027 and monitored by a contract research organization. The overall design of this clinical study to regenerate periodontal tissue is presented in Fig. 1.

2.1. Patients

Ten patients, ranging in age from 33 to 63 years (mean [±SD], 46 ± 12), received autologous PDL-derived cell sheet transplantations from November 2011 to May 2014 (Table 1). All patients gave oral and written informed consent at each invasive event, for a total of 4 times. Patients who had periodontitis with probing depths of more than 4 mm after the initial therapy were eligible for inclusion. Other inclusion criteria included age >20 years old and existence of a redundant tooth which retained healthy periodontal tissue for a cell source. Exclusion criteria included relevant medical conditions contraindicating surgical interventions (e.g., diabetes mellitus, cardiovascular, kidney, liver, or lung diseases, or compromised immune system), pregnancy or lactation, severe tobacco smoking (more than 11 cigarettes a day), or positive results for hepatitis B, hepatitis C, HIV, HTLV, or syphilis in the initial blood examination. The schedule before transplantation is written in Supplemental Fig. 1.

2.2. Preparation of autologous sera

Peripheral blood of each patient was tested to confirm negative results for hepatitis B, hepatitis C, HIV, HTLV, and syphilis. For obtaining autologous serum, 100–125 mL of peripheral blood was collected before the tooth extraction and transported to the cell processing center (CPC) of Tokyo Women's Medical University. Blood was then transferred to 50 mL centrifuge tubes and incubated at 37 °C for 1 h. Centrifugation was performed, and the supernatant was collected. The supernatant was again centrifuged, and the supernatant was filtered and then used as autologous serum in cell cultures.

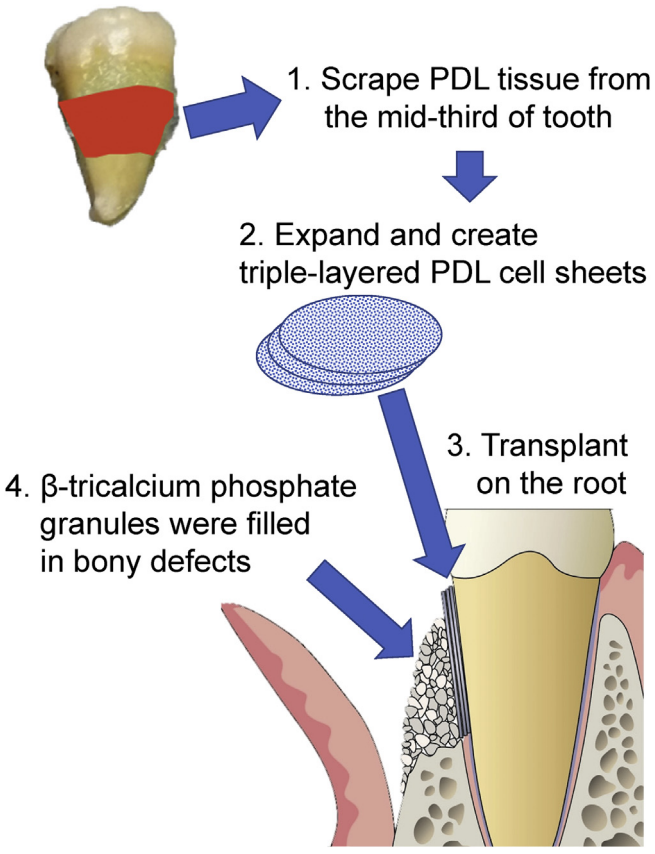

Fig. 1. The procedure of Periodontal regeneration with autologous PDL-derived cell sheets combined with β-tricalcium phosphate granules. 1. Patients' own redundant tooth was extracted, and PDL tissue was scraped and enzymatically digested to single cells. 2. After expansion, PDL-derived cells were spread on temperature-responsive culture dishes, then triple layered PDL-derived cell sheets were created. 3. Triple layered PDL-derived cell sheets with PGA mesh were trimmed to the defect size and transplanted on the root surface. 4. β-tricalcium phosphate granules were filled into bony defects.