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Original Article

Study protocol for periodontal tissue regeneration with a mixture of autologous adipose-derived stem cells and platelet rich plasma: A multicenter, randomized, open-label clinical trial

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ABSTRACT

Introduction: Adipose-derived stem cells (ASCs) secrete various growth factors to promote wound healing and to regenerate various tissues, such as bone, cartilage, and fat tissue. Subcutaneous adipose tissue is a considerable cell source in clinical practice and can be collected relatively easily and safely under local anesthesia. Moreover, platelet-rich plasma (PRP), a plasma component containing many platelets purified by centrifuging the collected blood, also promotes wound healing. PRP can be easily gelled and is therefore attracting attention as a scaffolding material for transplanted cells. The usefulness of a mixture of ASCs and PRP for periodontal tissue regeneration has been in vitro demonstrated in our previous study. The aim of this study is to present the protocol of translation of tissue regeneration with ASCs and PRP into practical use, evaluating its efficacy.

Methods: This study is a multicenter, randomized, open-label comparative clinical trial. Fifteen patients will be randomly assigned to the treatment with mixture of ASCs and PRP or enamel matrix derivate administration into periodontal tissue defects. Increase in height of new alveolar bone in the transplanted area will be evaluated. The evaluation will be performed using dental radiographs after 36 weeks of transplantation. Occurrence of adverse events will be evaluated as secondary outcome.

Results: This clinical study was initiated after meeting the regulations to be complied with, including ethical review and regulatory notifications.

Conclusions: If effective, this cell therapy using autologous mesenchymal stem cells can represent a useful medical technology for regeneration of periodontal defects.

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Abbreviations

AE	adverse event
ASCs	adipose-derived stem cells
CBCT	cone beam computed tomography
CRF	report form
CTCAE	Common Terminology Criteria for Adverse Events
EMD	enamel matrix derivative
IRB	Institutional Review Board
J-TEC	Japan Tissue Engineering Co., Ltd.
MSCs	mesenchymal stem cells
PI	principal investigator
PRP	platelet-rich plasma

1. Introduction

Human adipose tissue is the largest tissue in the human body, accounting for >10% of body weight [1]. It has been reported that human subcutaneous adipose tissue contains a cell population thought to be mesenchymal stem cells [2].

There have been many reports of regeneration in different tissues using adipose-derived stem cells (ASCs). Bone, cartilage, fat, muscle, and nerve tissue regeneration has been proven at the basic research level, together with some practical applications including clinical research [3,4]. The mechanism of action has yet to be elucidated; however, it is thought that cytokines produced by ASCs contribute to various types of tissue regeneration [2].

The advantage of using ASCs for clinical applications is that the physical and mental burden on the patient at the time of collection is very small compared with that of obtaining bone marrow tissue, and the source of ASCs, subcutaneous adipose tissue, can be collected safely and sufficiently [5].

Platelet-rich plasma (PRP), a plasma component containing numerous platelets, is collected and purified from venous blood. When platelets are activated, various growth factors are secreted and are thought to promote wound healing [6]. In Japan, under the Act on the Safety of Regenerative Medicine [7], PRP treatment is frequently performed in private practice, especially in dental and orthopedic disease applications [8]. However, the effectiveness of PRP treatment for each disease is not clearly understood.

Periodontal disease is an inflammatory disease caused by periodontal pathogens in the periodontal tissue, comprising the gingiva, cementum, periodontal ligament, and alveolar bone, and the most common forms of periodontal disease are gingivitis and periodontitis [9]. Periodontal disease not only significantly worsens oral health by destroying periodontal tissue, but also is a risk factor for systemic diseases such as diabetes [10,11], cardiovascular disease [10], and pneumonia [12]. In particular, pneumonia (aspiration pneumonia), one of the leading causes of death, has a strong correlation with periodontal disease [13].

Therefore, strengthening periodontal disease prevention, establishing treatments that regenerate lost periodontal tissue, and improving the overall quality of periodontal disease treatment are important for extending healthy life expectancy. While periodontal tissue regeneration therapies using cytokines and scaffolds are currently used clinically, medical technology capable of recovering from severe periodontal tissue destruction and regenerating this complex structure has not been established, and cell therapy using tissue engineering technology is expected to be developed [14].

We have previously conducted basic research on transplantation of ASCs into periodontal defects using PRP as a scaffold material in animal experiments and have confirmed periodontal tissue regeneration by mixed transplantation of ASCs and PRP [14,15].

Therefore, we planned to conduct a clinical trial to evaluate the periodontal tissue regeneration ability of this cell therapy. The main objective of this study is to verify the height of alveolar bone regenerated by ASCs/PRP mixed transplantation. Patients were randomly assigned to receive ASCs/PRP mixture transplantation or enamel matrix derivative (EMD) in the periodontal tissue defects to verify the alveolar bone height after ASCs/PRP mixture transplantation.

2. Methods

2.1. Study design

This clinical study is a multicenter, randomized, open-label comparative clinical trial in accordance with the Declaration of Helsinki and the Act on the Safety of Regenerative Medicine in Japan, transplanting a mixture of autologous ASCs and PRP into a vertical alveolar bone defect caused by moderate or severe periodontitis.

2.2. Ethical approval

The study protocol was approved by the Deliberation Committee for Specific Regenerative Medicine of Gamagori City Hospital (committee number: NA8150012, approval number: 29-569), and has been registered in the Japan Registry of Clinical Trials (https://jrct.niph.go.jp/, registry number: jRCTb030190173).

2.3. Recruitment

The patients will be recruited from the outpatient of Juntendo Hospital, Nihon University School of Dentistry Matsudo Hospital, and Aichi Gakuin University Dental Hospital from March 15, 2019 to March 31, 2022. Eligible patients satisfying the screening of the inclusion and exclusion criteria will be invited to participate in this study by physicians.

2.4. Randomization and concealment

After obtaining informed consent, the 15 patients with periodontal disease recruited will be randomly divided into the ASCs and PRP transplantation group and the EMD group in a 2:1 ratio. In this clinical study, the block randomization method is designed to randomize subjects into groups to an allocation rate of 2:1. This study will include a 36-week follow up time after periodontal surgery (transplantation). The schedule of enrollment, interventions, and assessments is shown in Fig. 1, and the study flow diagram is shown in Fig. 2.

2.5. Eligibility criteria

2.5.1. Inclusion criteria

Patients are required to fulfill all the following criteria for inclusion in this study:

1) A probing depth >5 mm at baseline examination
2) Intrabony defect ≥5 mm in depth and ≥2 mm in width at the interproximal site of the experimental tooth by X-ray examination
3) Having received initial periodontal therapy at screening
4) Mobility of experimental tooth of 0, 1, or 2, and adequate keratinized gingiva for flap surgery
5) Good oral hygiene

Study Period										
	Screening/ Enrollment (weeks)	Hospitalization for Fat tissue harvesting	Baseline (weeks)	Follow-up (weeks)						
Time point			0	1 wk	2 wk	4 wk	8 wk	12 wk	24 wk	36 wk
Enrollment										
Informed consent	○									
Eligibility screen	○									
Clinical examination	○		○							
Allocation	○									
Interventions										
Fat tissue harvesting		○								
Blood collection	○		○							
Cell transplantation or EMD			○							
Evaluations										
Dental x-ray	○		○	○	○	○	○	○	○	○
CBCT	○			○				○	○	○
Probing of depth	○							○	○	○
Gingival index	○							○	○	○
Periodontal index	○							○	○	○
Safety evaluations										
SAE/AE		○	○	○	○	○	○	○	○	○
Vital signs	○	○	○							

Fig. 1. Study periods and data collection: The baseline (ASCs/PRP mixture or EMD transplantation) is followed by a follow-up period defined to be 36 weeks. The figure depicts the data collection time points.

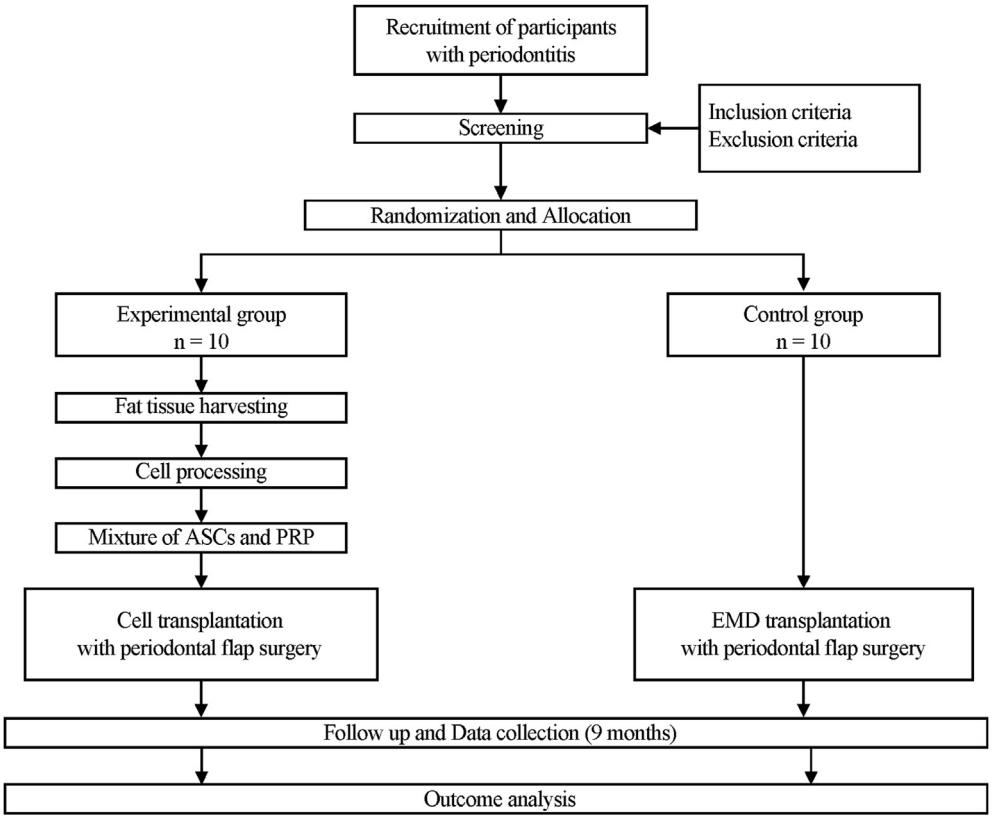

Fig. 2. Flow chart: This figure illustrates the study design. A total of 15 patients will be randomized to cell processed product transplantation or standard treatment implementation.

6) Subcutaneous fat tissue can be harvested normally and safely
7) ≥20 years old
8) Signed informed consent

2.5.2. Exclusion criteria

Patients will be excluded if they meet any of the following criteria:

1) The clinical attachment level of the experimental tooth cannot be measured
2) History of complicated malignant tumors
3) Suspected oral malignant tumor or precancerous lesion
4) History of bisphosphonate usage, if necessary
5) Surgical, restorative, or root canal treatment on the experimental tooth within 36 weeks after transplant
6) Pregnancy, lactating, or possible pregnancy
7) Kidney, liver and/or blood disease
8) Hemoglobin A1c >6.8% at the time of screening
9) Active infectious diseases
10) Alcoholism or drug dependence
11) Mental or consciousness disorder
12) Positive HCV antibody, HBs antigen, ATLA virus antibody, or HIV antibody
13) Smoking >10 cigarettes per day
14) Other criteria which the investigator believes makes him/her unsuitable for participation in the study

2.6. Termination and withdrawal criteria

A patient may be discontinued from the study at any time if the patient or the investigator feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Screening Failure
- Patient withdrawal of consent
- Patient is not compliant with study procedures
- Adverse event that, in the opinion of the investigator, would result in discontinuation being in the best interest of the subject
- Protocol violation requiring discontinuation

All patients are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. Reasonable attempts will be made by the investigator to provide a reason for patient withdrawals. The reason for the patient's withdrawal from the study will be specified in the patient's source documents and the case report form (CRF). If a patient is withdrawn from treatment due to an adverse event (AE), the subject will be followed up and treated by the investigator until the abnormal parameter or symptom has resolved or stabilized. The investigator must make every effort to contact subjects who are lost to follow-up. Attempts to contact such patients must be documented in the subject's records (e.g., times and dates of attempted telephone contact, receipt for sending a registered letter, and others).

2.7. Intervention

2.7.1. Treatment plan

This study is a comparative study between the cell transplantation group and the standard treatment group. The standard treatment group was established as the group receiving EMD. Patients assigned to the cell transplantation group will have approximately 50 mL of subcutaneous adipose tissue aspirated from the abdomen or buttocks under local anesthesia.

The collected subcutaneous adipose tissue will be transported to Japan Tissue Engineering Co., Ltd. (J-TEC) for ASCs' manufacturing using a common mesenchymal stem cell culture method [14,15]. On the day before transplantation, ASCs will be transported from J-TEC to the medical institution performing the regenerative medicine procedure.

On the day of transplantation, PRP will be manufactured at the cell processing center of each medical institution; ASCs will be delivered to the cell processing center, mixing the two to prepare the final processed cells. Just before transplantation, the mixture of 1.5 × 10E7 of ASCs and 1 mL of PRPs will be gelatinized by the addition of 2% $CaCl_2$ equal to 1/10 of the total amount of the mixture.

Then, the final processed cells or EMD will be transplanted into the periodontal tissue defect when performing periodontal flap surgery on a patient diagnosed with moderate or severe periodontitis who has completed initial periodontal therapy. In both groups, periodontal flap surgery will be followed by oral antibiotic therapy.

2.7.2. Study endpoints

The primary endpoint is the increase in height of new alveolar bone in the transplanted area. The evaluation will be performed using dental radiographs after 36 weeks of transplantation.

The safety-related secondary endpoint is the occurrence of adverse events. The secondary endpoints related to efficacy are: (1) width of new alveolar bone at the transplanted area evaluated using dental radiographs and (2) increase in height and width of new alveolar bone at the transplanted area assessed by cone beam computed tomography (CBCT).

The secondary endpoint regarding efficacy will be evaluated by the analysis population based on the distance from the cementoenamel junction to the bottom of the alveolar bone defect and width of the top of the alveolar bone defect by CBCT at the time of registration, the date of transplantation, and at 12, 24, and 36 weeks after transplantation. The differences between pre- and post-transplantation will be compared among the cell-transplanted and EMD transplanted groups.

In addition to the time of registration, periodontal tissue examination (attachment level, probing depth, tooth mobility, bleeding on probing, and gingival index) will be conducted at 12, 24, and 36 weeks after transplantation. The difference between pre- and post-transplantation will be compared between groups.

2.7.3. Safety assessment

After establishing the manufacturing process for clinical research, the ASCs were subjected to karyotype analysis as a non-clinical safety test, and it was confirmed that chromosomal abnormalities did not occur during the culture period (data not shown).

2.7.4. Adverse events

The principal investigator (PI) must record all adverse events in the medical records. The Common Terminology Criteria for Adverse Events (CTCAE ver. 4.0) will be used to grade each event. All severe adverse events must be reported to the Institutional Review Board (IRB) of each site and to the Gamagori City Hospital, Deliberation Committee for Specific Regenerative Medicine, in compliance with the Act on the Safety of Regenerative Medicine.

2.8. Data management and quality control

All records in the CRF will be filled out and collected by trained and qualified researchers. After the CRFs are filled out completely, the original data recorded will not be altered, even if any changes

are made. The clinical examiner will review all the completed CRFs. In this study, J-TEC will support data management and quality control, and a medical statistician of J-TEC will guide data entry and management. Once the established database is reviewed, it will be locked by key researchers and statistical analysts. The locked data will not be changed, and will be submitted to the statistics division of J-TEC for statistics and analysis.

2.9. Statistical analyses

Considering the implementation period and the number of participating medical institutions in this study, the target number of patients was set to 15 in total, with 10 cases of cell processed product transplantation and 5 cases of standard treatment implementation. To consider the number of cases required to assess efficacy, we calculated the number of cases in which the height of the new alveolar bone at the transplantation site of the cell transplant group was significantly different from that of the EMD transplant group using a dental radiograph. Based on the results of prior basic research, the height of new alveolar bone was estimated to be 2.5 mm in the cell-grafted group. However, based on a report by Heijl et al. [16], the average height of the new alveolar bone in the EMD group was assumed to be 0.9 mm, and the standard deviation in both groups assumed as 0.6 mm. We calculated the number of cases required to detect the difference between the two groups using Student's t-test with a significance level of 5% on both sides and a power of 90%. As a result, there were 5 cases per group. Therefore, considering the implementation period and feasibility of this study, the target number of cases was set at 10 per group, but it is judged that the efficacy could be assessed precisely.

The modified intent-to-treat population for the efficacy evaluation will include those cases in which consent has been obtained, excluding the following criteria:

1) Cases of violation of the narrowly defined donation plan, such as tissue harvesting outside the contract period and implantation of the relevant processed cells or EMD
2) Cases in which the transplantation of the relevant processed cells or EMD was not performed
3) Cases in which observation for efficacy endpoints was never conducted after implantation of the relevant processed cells or EMD.

To evaluate the primary endpoint, the height of new alveolar bone at the graft site as measured by dental radiographs at 36 weeks after transplantation, the difference in the distance from the base before and after implantation was calculated, and a Student's t-test used to compare these values in the cell-processed material implantation group and the EMD implantation group. In addition, the Holm method was used to adjust for multiplicity, and a Student's t-test was performed at each evaluation time point.

Regarding the secondary endpoints, the safety evaluation tabulated the number of cases in which AEs and serious AEs occurred and calculated a two-sided 95% confidence interval for the incidence rate (%). The handling of protocol exceptions is described in Appendix 1. All analyses will be performed using SAS software (version 9.4; Boston Biomedical Associates, LLC, Boston MA).

2.10. Trial status

This study has been registered at the Clinical Trial Registry (www.umin.ac.jp/ctr/), UMIN000036232. The study was started in February 2019. The plan is to register a total of 15 cases in 2 years.

3. Expected results

This randomized clinical study has been initiated in compliance with the relevant laws and after completing the prescribed procedures. The findings will subsequently be reported consistent with the Consolidated Standards of Reporting Trials (CONSORT) statement. Contributions to knowledge and understanding of novel periodontal tissue regeneration therapy using the mixture of ASCs and PRP are expected.

4. Discussion

Medical technology for regenerating periodontal tissue, comprised of alveolar bone, cementum, periodontal ligament, and gingiva, has not yet been established. Accordingly, it is necessary to develop stem cell technology for the regeneration of periodontal tissue in cases of complicated and widespread periodontal tissue defects [9,17].

Periodontal tissue regeneration is challenging, because it is difficult to hold the periodontal defect in a resting state for a long period of time, and the site where tissue regeneration is performed in a state of chronic inflammation. Therefore, it may be necessary to actively aim for early tissue regeneration.

The stem cells used in this clinical trial can be collected from subcutaneous adipose tissue under local anesthesia, and it is considered an ideal place for collecting raw materials with a view to the actual clinical site. The expected mechanism of action is through the induction of a paracrine effect by the transplanted undifferentiated mesenchymal stem cells (MSCs), in other words, without having undergo osteoblast differentiation [2]. Furthermore, in recent years, it has been reported that MSCs have an anti-inflammatory effect [18] and are considered beneficial in stem cell therapy for periodontitis, a form of chronic inflammation.

In this clinical study, autologous PRP will be used as a scaffold material and mixed with ASCs, then the cells are transplanted after gelation to maintain the stagnation at the transplantation site.

From a safety standpoint, it is important that all cell processed products to be transplanted are autologous-derived. Furthermore, thus far in basic research, it has been suggested that various growth factors produced from PRP significantly improve the paracrine effect from ASCs [19], and we believe that it may be a more effective cell therapy.

However, the mechanism of periodontal tissue regeneration using MSCs, including ASCs, has not been fully elucidated, and further basic research is required. Additionally, in cell therapy using autologous cell processed products, it is extremely important to search for Responders/Non-responders. Therefore, it will be important to explore the clinical significance of cell therapy through both basic and clinical research. In this clinical study, we plan to evaluate the correlation between therapeutic effect and cell characteristics and search for Responders/Non-responders.

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Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2022.09.008.

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