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THE INFLUENCE OF CISSUS QUADRANGULARIS WITH MESENCHYMAL STEM CELLS FROM DENTAL PULP ON BONE REGENERATION

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Abstract

Background

Recently, Plant Derivatives in Tissue Engineering have demonstrated regenerative potential, but the real effect remains to be examined. This pilot study attempted to isolate dental pulp stem cells from four patients and to evaluate the feasibility and the effect of reconstructing periodontal intrabone defects in each patient using its combination with cissus quadrangularis extract and alone.

Methods

In this split mouth study ,DPSCs-Ps were harvested from four patients with bilateral periodontal intrabone defects with their approval. After discussing the biological characteristics of DPSCs-Ps in each patient, DPSCs-Ps were loaded onto the scaffold material cissus quadrangularis extract and engrafted into the periodontal defect area in the root furcation in one side and another side was filled with DPSCs-P with collagen sponge.

Results

We observed 78±27 % bone fill with DPSC-P /CQ extract complex in comparison to 71±18% in DPSc's –P alone.

Conclusions

In this study, we provide early clinical data as well as experimental evidence to support the efficacy and safety of application of autologous DPSCs-Ps in combination with bioactive cissus quadrangularis related to human periodontitis treatment of bone defect

Background

Periodontitis is a kind of chronic disease prevalent worldwide, featured by a loss of support tissues around the teeth, resulting in damage which continues until the teeth fall out [1]. The final goal of

treatment for periodontitis is to repair the lost periodontal support tissues, especially the bone. In recent years, the rapid development of tissue engineering has shown great potential for applications in reconstruction of periodontal-associated bone defects [2–6]. In particular, the discovery of dental pulp stem cells (DPSCs) and other odontogenic stem cells has furnished new prospects for the repair of periodontal tissue [7, 8].

There have been various examples of plant-derived substances incorporating to scaffolds, which proves they could effectively improve the bioactivity of different biomaterials as well as regulating protein or gene expression responsible for osteogenic differentiation. These phytochemicals could work as growth factors, reducing the need for expensive and difficult biological moieties. The plant derivatives incorporating to these materials would make up for their poor osteoinductive abilities. Recently, The extracts of *Cissus quadrangularis* (CQ) and *Butea monosperma* (BM) incorporated with sulphonated poly aryl ether ketone (SPAEK) sponges is reported to own good biocompatibility. In this study, both SPEAK-CQ and SPEAK-BM remarkably increased the ALP activity, which is an early marker of osteogenic differentiation and mineral deposition [9]. This result incites that the scaffolds with phytochemicals can be applied in cases like regenerating resorbed dental alveolar ridge, to promoting periodontal regeneration. [9]

In the view of aforementioned findings we explored the adjunctive influence of cissus quadrangularis extract on bone regeneration.

Methods

Patient enrollment

Four male patients with bilateral pocket depth from 5 to 6 mm were chosen. Patients were first informed to consent to the entire treatment. The selected patients should be in accordance with the following inclusion criteria: age range from 18 to 40 years without systemic disease, no pregnancy or smoking, and no use of recreational drugs. Patients were excluded if they had undertaken any initial treatment including subgingival scaling or root planing within the previous 6 months. Before this pilot clinical study, approval was obtained from the Institutional Ethical Board.

DPSCs-Ps isolation and cultivation

Pulps from patients were extirpated from third molar deemed for extraction and placed in D-Hank's solution. A routine RCT was performed. Pulps were then quickly placed in culture medium for cell isolation. Each sample was first minced and then digested for 1 hour in a solution of collagenase type I and dispase II (3:4) at 37 °C. Cells were then incubated in Dulbecco's modified Eagle media/Nutrient Mixture F-12 (DMEM/F12 1:1) culture medium with 10 % fetal bovine serum, 2 mmol/L glutamine, 100 μmol/L L-ascorbic acid-2-phosphate, and antibiotics at 37 °C. The colony formation unit-fibroblasts (CFU-Fs) were observed 5 days later.

Cell Counting Kit-8 assay

The Cell Counting Kit-8 (CCK-8) assay was utilized to detect the viability of DPSCs-IPs, 10^3 cells/ml were seeded in 96-well plates, and the absorbance at 450 nm was detected at 1–6 days after seeding.

Preparation and evaluation of the DPSCs-Ps/Cissus Quadrangularis(CQ), extract complex by scanning electron microscope

Scaffold Cissus Quadrangularis extract was placed into dishes when DPSCs-Ps at P3 were at a confluence of 80 %. The medium was generally changed every 3 days. Two weeks later, the complex samples were scraped for scanning electron microscope analysis. They were first put into 2.5 % glutaraldehyde for 2 hours, and then washed with PBS and further fixed with 1 % osmium tetroxide followed by dehydration with ethanol. After displacement, desiccation, and metal spraying, the samples were ready to observe.

Transplantation of autologous DPSCs-Ps/CQ into patients

Patients underwent initial periodontal therapy before the DPSCs-Ps/CQ treatment. During transplantation surgery, infiltration anesthesia was used first, and then inflammatory tissues were removed. DPSCs-Ps/CQ was transplanted into the periodontal defect areas and sutured carefully.One side was filled with DPSCs-Ps/CQ while other side it was filled with DPSCs-Ps with collagen sponge.

Clinical evaluation

The plaque index (PLI), bleeding index (BI), probing depth (PD), gingival recession (GR), clinical attachment level (AL), and tooth mobility were recorded before surgery and post DPSCs-Ps/CQ transplantation from 1 to 9 months. All measurements were done with a periodontal probe by blinded examiners.

Statistical analysis

Student's *t* test and ANOVA test was used. $P < 0.05$ was considered a significant difference.

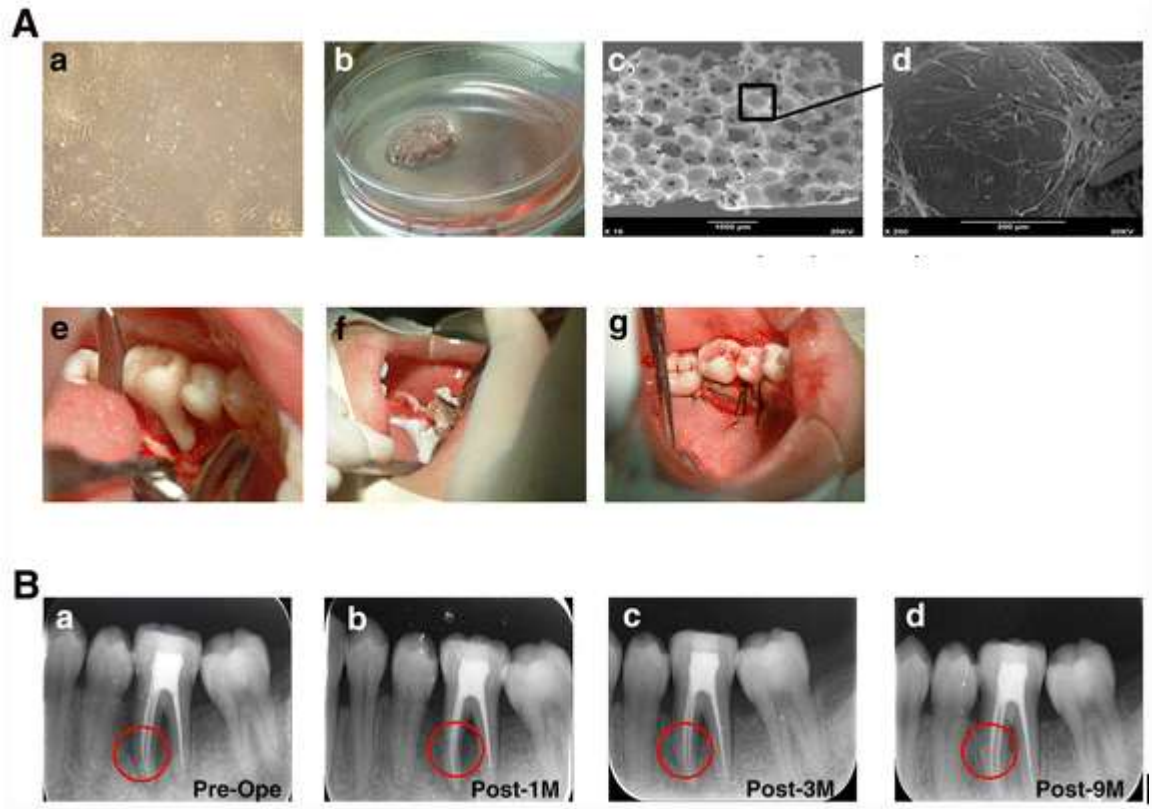
Results

DPSCs-Ps/ CQ transplantation

Figure 2 shows the protocol of a procedure for using DPSCs-Ps from patients to treat periodontal bone defeats. All procedures were done with the patient's agreement and her knowledge. To prepare the DPSCs-Ps/CQ extract complex, DPSCs-Ps were cultured in a 100-mm dish for 3 days, and 40 mg CQ extract particles were added to the dishes; 2 weeks later, the complex samples were ready (Fig. 2Ab). We used scanning electron microscopy to detect the DPSCs-Ps/CQ complex

(Fig. 2Ac, Ad). After removing infectious periodontal tissues, the DPSCs-Ps complex was applied to the periodontal bone defective areas (Fig. 2Ae–g).

Fig. 2



DPSCs-IPs/β-TCP transplantation and its therapeutic effect. (*a*) Third passage of DPSCs-Ps from Patient (*b*) Generation of DPSCs-Ps/CQ complex. DPSCs-Ps were cultured in 100-mm culture dishes with 40 mg CQ particles. (*c*, *d*) Scanning electron microscopy of DPSCs-Ps/CQ complex. (*e*) Lingual view of periodontitis lesion. (*f*, *g*) Transplantation of the DPSCs-Ps/CQ complex generated from Patient the periodontal lesion. **b** Therapeutic effect of DPSCs-Ps/CQ (*a*) Bone defeats before the operation (*red circle* in *Pre-Op*). (*b*) Therapeutic effect 1 month after the operation (*red circle* in *Post-1 M*). (*c*) Therapeutic effect 3 months after the transplantation (*red circle* in *Post-3 M*). (*d*) Therapeutic effect 9 months after the operation (*red circle* in *Post-9 M*) by X-ray analysis. *DPSCs-Ps* dental pulp stem cells isolated from dental pulp tissues, *CQ-Cissus quadrangularis extract*

After transplanting in-vitro expanded DPSCs-Ps/CQ into the intrabone defects of deep periodontal pockets in Patients following the standard surgical debridement, the patient was monitored carefully and followed up at 1, 3, and 9 months. Routine clinical evaluations including PD, AL, and GR were examined and X-ray scans were taken at 1, 3, and 9 months after surgery (Table 2 and Fig. 2B).

Bone changes

Bone changes were measured on scanned radiographs (Table 1). The two groups gained a substantial amount of bone at 1 year as compared with baseline. The percentage bone fill of the intra-bony component was 78± 27% in the DPSC/CQ group, 71± 18% in the DPSC alone group. Differences among groups were not statistically significant.

	DPSC group (N 4)	DPSC+CQ (N=4)	Significance (p)
CEJ-BD 0 (mm)	9.3 + 2.0	10.5 + 2.1	0.102
INFRA (mm)	4.7 + 1.3	4.5 + 1.3	0.128
CEJ-BD 1 (mm)	6.0 + 1.5	7.2 + 2.1	0.025*
Bone gain (mm)	3.3 + 1.2	3.3 + 1.1	0.815
Bone fill (%)	71 + 18	78 + 27	0.0403*

Discussion

Previous studies have shown that although they lose some of the properties of stem cells, DPSCs-IPs retain the potential for tissue regeneration [9, 10, 11].

In the present study, DPSCs-Ps +Cissus Quadrangularis extract were transplanted into the patients' periodontal bone defects for the first time and the effective repairing effect was observed.

Bone regeneration by autologous transplantation of DPSCs-Ps in patients. We objectively evaluated the characteristics of DPSCs-Ps in each patient first. The study found that inflammatory dental pulp tissues in both patients to a certain extent retain the properties of DPSCs: they can differentiate into osteogenic cells, and they express certain surface markers of mesenchymal stem cells. The expression levels in CD44 and CD90 are highly positive, and the levels in CD34 and CD45 are negative, which is in line with the characterization of mesenchymal stem cells. But the levels in CD105 and CD271 are weak, which slightly differs from previous reports [12-14]. However, the underlying reason remains unclear.

The property of stem cell markers in different species or organs indeed differs in some cases [9]. Using the expression levels in CD44, CD90, CD34, and CD45, however, the stem cell properties of DPSCs-Ps can be determined. The following discusses the therapeutic effect of DPSCs-IPs from many aspects. We have provided evidence here that the dental clinical condition was improved obviously 9 months after transplantation of the DPSCs-Ps/CQ complex. As observed in the clinic, the color of the gum is pink, and its quality is tough and elastic. Although there is only an inconspicuous improvement in GR, the PD was evidently shallow, the gingival BI decreased from 3 to 1, clinical hemorrhage disappeared, the root bifurcation lesions reduced to degree II–I

compared with degree III before treatment, and the treatment effect was obvious from the current clinical symptoms.

Generally speaking, the DPSCs-Ps/CQ dramatically improved the clinical symptoms of periodontitis. Our results provide evidence that DPSCs-Ps/CQ compounds may have a certain repair effect on periodontal hard tissue defects caused by periodontitis and may be a new source for oral tissue regeneration to provide a potential way of being used in future clinical applications.

The petroleum ether extract of Cissus Quadrangulari[CQ] was shown stimulate the differentiation of mesenchymal stem cells into osteoblasts in a dose-dependent manner even in the absence of osteogenic conditioning media. This activity of the plant extract is increased further in the presence of osteogenic media. The plant extract also facilitated extracellular matrix mineralization, which was more pronounced in the presence of osteogenic media.[15]

In the view of safety in the process of transplantation, no patients showed any systemic disorders related to the transplantations or adverse reactions during the process, so the procedures used in this study can benefit DPSCs-IPs clinical studies in the future.

The increased osteogenic ability of DPSCs, the ability to differentiate into osteogenic, adipogenic, and chondrogenic cells proved the characteristics of stem cells and suggests the potential of mesenchymal stem cells to repair defeats.

Conclusions

In this study, we provide early clinical data as well as experimental evidence to support the efficacy and safety of application of autologous DPSCs-Ps in combination with bioactive cissus quadrangularis related to human periodontitis treatment of bone defect. We also speculate that this complex will perform excellent effects in the treatment of periodontal regeneration. We hope to design a clinical trial in the future with a large number of patients to provide further information about this treatment.

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