

Research Article:

The Effects of Dental Pulp Stem Cell Conditioned Media on the Proliferation of Peripheral Blood Mononuclear Cells

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ABSTRACT

Background: Dental Pulp Stem Cells (DPSCs) are multipotent mesenchymal stem cells. DPSCs can renew themselves and differentiate into various cell types such as adipocytes, osteocytes, neurons, etc. DPSCs possess immunomodulatory properties and can inhibit Peripheral Blood Mononuclear Cell (PBMC) proliferation. Recent studies showed that conditioned-medium mesenchymal stem cells also had immunosuppressive activity. The ability of DPSC conditioned medium to suppress proliferation of allogeneic PBMC determined using BrdU (5-bromo-2'-deoxyuridine) proliferation assay.

Materials and Methods: Dental pulp stem cells were extracted from a wisdom tooth. These cells are characterized for differentiation potential to adipogenic and osteogenic lineage and expression of mesenchymal stem cells markers, including CD105, CD73, CD90, CD14, CD-34, and CD45. The characterized DPSCs were cultured, and the Conditioned Medium (CM) got isolated. Stimulated and non-stimulated PBMCs from the allogeneic donor were cultured with DPSC-CM for 24, 48, and 72 hours. The proliferation of PBMCs was measured with BrdU assay.

Results: The BrdU test results showed that DPSC-CM reduced allogeneic PBMC proliferation at different time points. DPSC-CM could inhibit stimulated and non-stimulated PBMC in 48 and 72 hours after incubation.

Conclusion: This study demonstrated that DPSC-CM had an immunomodulatory effect on the proliferation of allogeneic cells.

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Introduction

Dental pulp extracted from teeth may provide an abundant supply of highly proliferative, multipotent Mesenchymal Stem Cells (MSCs). Dental Pulp Stem Cells (DPSCs) are multipotent mesenchymal stem cells that have self-renewal ability. They can differentiate into various cell types, including osteoblasts, chondroblasts, adipocytes, neurons, and so on [1-6]. These cells express mesenchymal lineage surface antigens such as CD105, CD90, CD73 while they do not express endothelial-hematopoietic antigens, like CD33, CD31, CD34, and CD11b [7, 8]. MSCs can inhibit innate and adaptive immune response through producing anti-inflammatory mediators. Recent studies revealed that MSCs-Conditioned Medium (CM) had the essential features of mesenchymal stem cells. MSCs-CM possess immunomodulatory properties and can suppress immune reactions [9-12]. MSC-CM contains anti-inflammatory cytokines, growth factors, and soluble mediators, which can lead to the inhibition of immune cells proliferation, including Peripheral Blood Mononuclear Cell (PBMC) [13, 14]. In the present study, we analyzed DPSCs-CM immunosuppressive effects on the proliferation of allogeneic PBMCs.

Materials and Methods

Isolation of dental pulp stem cells and cell culture

A wisdom tooth was extracted from a healthy donor during routine dental extraction with the approval of the ethical board of the Department of Oral and Maxillofacial Surgery, Dental School of Shahid Beheshti, Tehran, Iran. The pulp tissue was extracted and digested in a solution of 3 mg/mL collagenase type I (Sigma, Germany) for 40 min at 37°C. The cells were centrifuged and seeded in culture flasks. Cells cultured in α -MEM supplemented with (15%) Fetal Bovine Serum (FBS; Gibco), 100 U/mL penicillin-G and 100 mg/mL streptomycin (Biosera, Germany), and cultured at 37°C.

Flow cytometry analysis

DPSCs at passage 3 were used for surface markers analyses. Flow cytometry analysis was performed using the following Monoclonal Antibodies (mAbs); anti-CD105, anti-CD73, anti-CD90, anti-CD14, anti-CD-34, and anti-CD45.

In vitro differentiation

For osteogenic differentiation, 3×10^3 cells were cultured in 24-well plates. Osteogenic medium consisting of low-glucose DMEM (Biosera, Germany) with (10%) FBS, 100 nM dexamethasone, 0.2mM-ascorbic acid-2-phosphate, 10mM β -glycerophosphate (all from Sigma) and 100 U/mL penicillin with 1000 U/mL streptomycin. After 21 days of culture, the cells were stained with Alizarin Red to the determined mineralized matrix.

DPSC conditioned medium preparation

For collecting the conditioned medium, DPSCs at passage 3 were incubated in serum-deprived DMEM for 8 hours in 37°C and (5%) CO₂. MSCs-CM were sucked and centrifuged at 1600 rpm for 5 minutes to remove detached cells.

BrdU Proliferation assay

PBMCs were separated from the fresh blood of a healthy donor with Ficoll (Biosera, Germany). Around 1×10^5 PBMCs were seeded in 96-cell culture plates (SPL, Korea). The cells were stimulated with (1.5%) PHA (Phytohaemagglutinin, Gibco, Germany) and cultured with (50%) DPSC-CM and (50%) RPMI 1640 (Biosera, Germany) supplemented with (10%) FBS for 24, 48, and 72 hours. After incubation, BrdU assay (Roche, Germany) was performed to determine the proliferation of PBMCs.

Statistical analysis

All study experiments were performed three times. All statistics were done using SPSS V. 25 (SPSS Inc.). For multiple comparisons, one-way ANOVA was used. Values of ($P < 0.05$) were classified as statistically significant. The obtained data were presented as Mean \pm SD.

Results

Cell surface marker characteristics of DPSCs

The cell surface markers of DPSCs were characterized by flow cytometry analysis (Figure 1). DPSCs expressed mesenchymal stem cells surface markers. They were strongly positive for CD105, CD90, and CD73. DPSCs were negative for CD34, CD14, and CD45.

Differentiation potential of DPSCs

To determine the differentiation potential of DPSCs, the cells were incubated in osteogenic and adipogenic

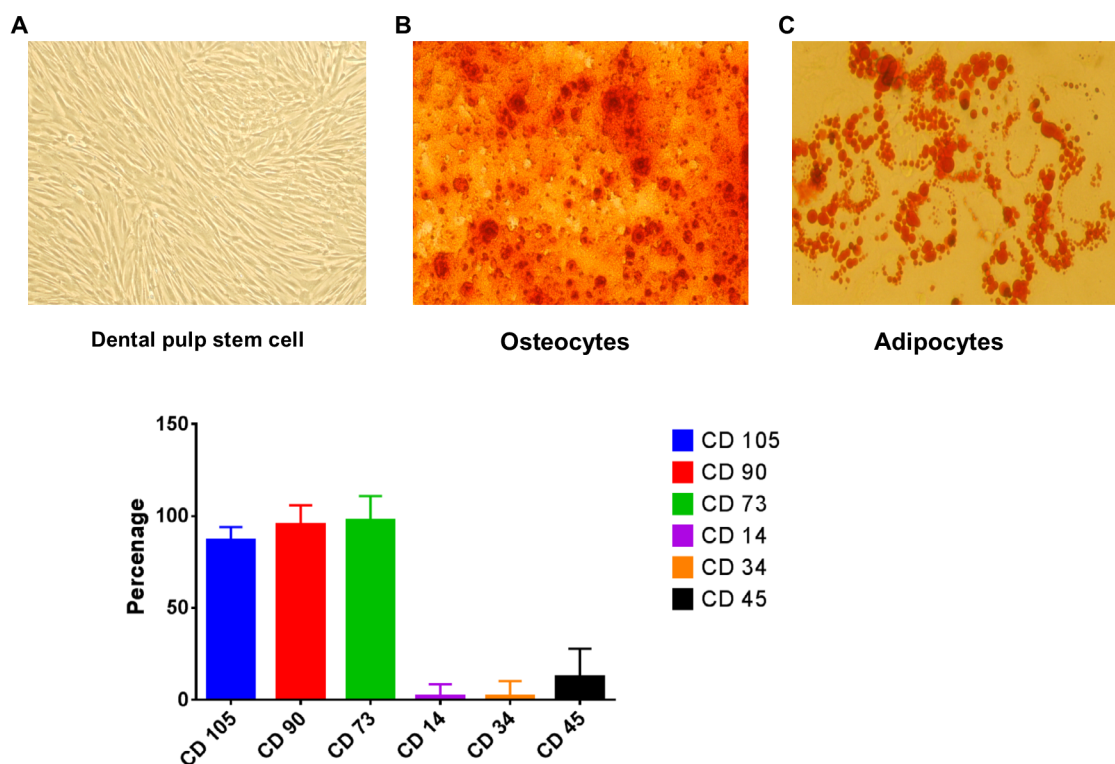


Figure 1. Characterization of dental pulp stem cells

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A: Dental pulp stem cells; B: Osteogenic differentiated dental pulp stem cells; C: Adipogenic differentiated dental pulp stem cells; D: Flow cytometry analysis of dental pulp stem cells

culture mediums. Following 3 weeks of culture, the cells were stained and observed under a microscope.

Under osteogenic differentiation, the calcified deposits are formed (Figure 1). DPSCs were also cultured in adipogenic medium. After staining with Oil Red O, the lipid vacuoles were observed (Figure 1).

Effects of DPSCs conditioned medium on the proliferation of PBMC

BrdU results showed that proliferation of stimulated and non-stimulated PBMCs reduced significantly ($P < 0.05$) 48 hours after incubation. DPSC-CM could also inhibit the proliferation of stimulated and non-stimulated PBMCs 72 hours after incubation ($P < 0.05$) (Figure 2).

Discussion

In the current study, we examined the immunomodulatory effect of conditioned-medium dental pulp stem cells on the proliferation of PBMC. We observed that DPSC-CM could inhibit stimulated and non-stimulated PBMCs proliferation after 48 and 72 hours. There was no change in the proliferation of PBMCs after 24 hours.

In our previous study, we showed that DPSCs could inhibit the proliferation of PBMCs after 72 hours [15]. Tang et al. also reported that DPSCs had inhibitory effects on the proliferation of PBMCs [16]. Some studies reported that the conditioned-medium mesenchymal stem cells had anti-inflammatory effects [17, 18]. Yamaguchi and colleagues showed that DPSCs-CM could reduce cardiac injury [19]. Moreover, MSCs-CM has some advantages in comparison with MSCs, like unchallenging production and delivery. Importantly, MSCs-CM are not rejected by the immune system.

On the whole, these data showed that the conditioned-medium mesenchymal stem cells have anti-inflammatory and anti-proliferative effects and could be a potential therapeutic tool in regenerative medicine.

Ethical Considerations

Compliance with ethical guidelines

There was no ethical considerations to be considered in this research.

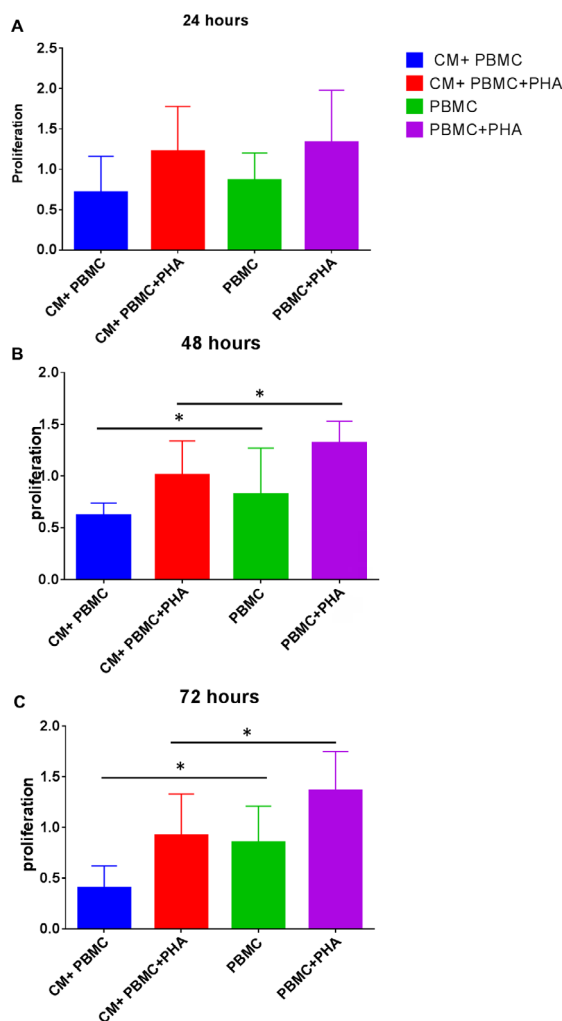


Figure 2. The proliferation of stimulated and non-stimulated PBMCs after culture with DPSC conditioned medium. A. The proliferation of stimulated and non-stimulated PBMCs 24h after culture; B. The proliferation of stimulated and non-stimulated PBMCs 48h after culture; C. The proliferation of stimulated and non-stimulated PBMCs 72h after culture. The results are expressed as the Mean \pm SD
PBMC: Peripheral Blood Mononuclear; DPSC: Dental Pulp Stem Cell; OD: Optical Density; *P<0.05

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Authors' contributions

Methodology, formal analysis, investigation, writing review and editing: Nikoo Hossein-Khannazer; Supervision, software: Mandana Sattari; Supervision, funding

acquisition: Arash Khojasteh; Methodology: Seye Mahmoud Hashemi; Supervision: Saeed Namaki.

Conflicts of interest

The authors declared no conflict of interest.

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