Supplementary data

Epithelial Cell Rests of Malassez Provide a Favorable Microenvironment for Ameliorating the Impaired Osteogenic Potential of Human Periodontal Ligament Stem Cells

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MATERIALS AND METHODS

Flow Cytometry analysis

Approximately 1×10^6 PDLSCs were incubated with PE-conjugated human antibodies against CD90 and CD45 (Biolegend, 1:100), and FITC-conjugated human antibodies against CD29 and CD105 (Biolegend, 1:100). Cells were incubated in a 4 °C dark environment with these antibodies for 1 hour and observed with flow cytometer (Beckman Coulter, USA). For demarcating positive cells, nude cells were used as negative control.

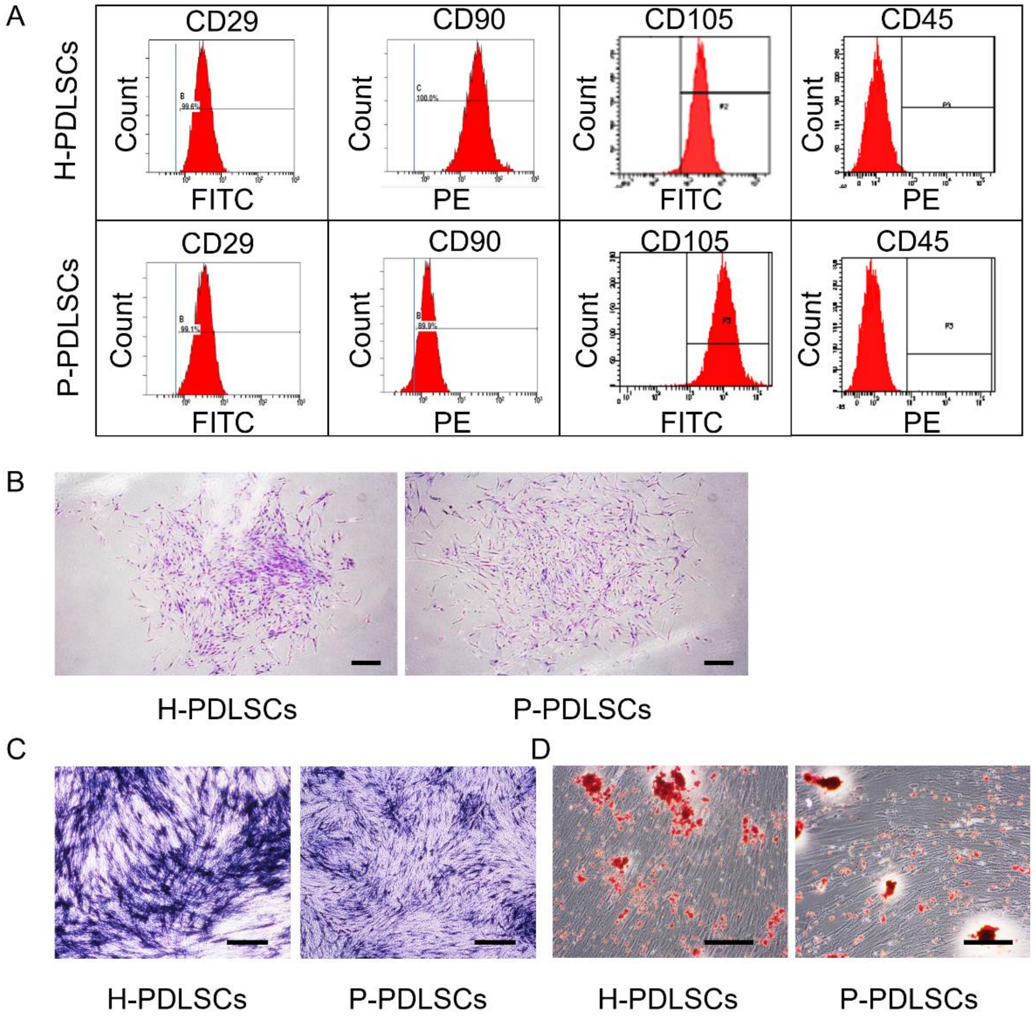
Colony Forming Assays

PDLSCs single-cell suspensions ($1x10^3$ cells) were seeded in 10-cm diameter culture dishes and cultured in α-MEM supplemented with 10% FBS. After 14 days, the sample was fixed in 4% paraformaldehyde and stained with 0.1% toluidine blue.

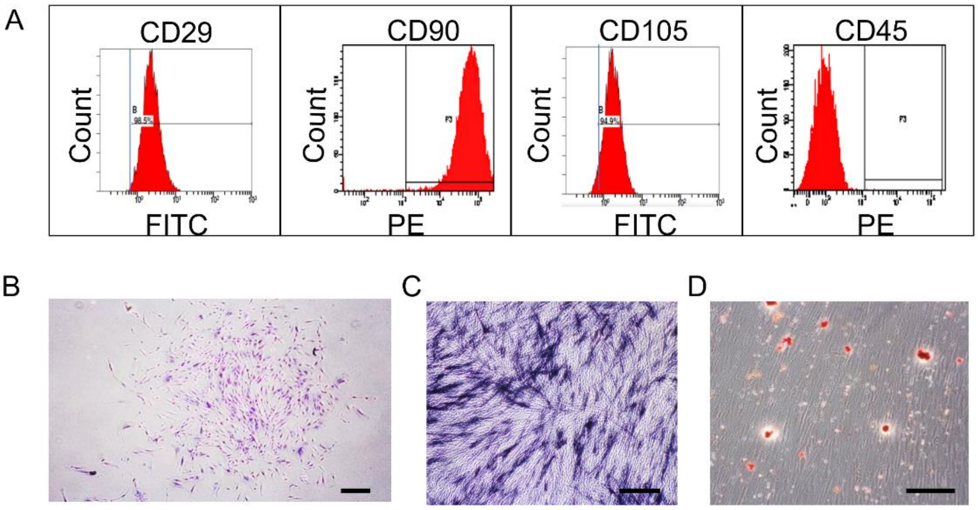
Osteogenic Differentiation Assay

PDLSCs were cultured in α-MEM supplemented with 10% FBS. When the cells reached approximately 90% confluence, the culture medium was changed to osteoinductive medium. After 21 days of osteogenic induction, the samples were fixed for Alizarin red S staining.

FIGURE LEGENDS



SUPPLEMENTARY FIGURE 1 Isolation and identification of H-PDLSCs and P-PDLSCs. (A) Mesenchymal stem cell phenotype examination by flow cytometric analysis. (B) Colony-formation assay of H-PDLSCs and P-PDLSCs observed by microscopy. Scale bar, 200 mm. (C) ALP staining of H-PDLSCs and P-PDLSCs observed by microscopy. Scale bar, 100 mm. (D) Alizarin red S staining shows the osteogenic nodule formation of H-PDLSCs and P-PDLSCs. Scale bar, 200 mm.



SUPPLEMENTARY FIGURE 2 Isolation and identification of A-PDLSCs. (A) Mesenchymal stem cell phenotype examination by flow cytometric analysis. (B) Colony-formation assay of A-PDLSCs observed by microscopy. Scale bar, 200 mm. (C) ALP staining of H-PDLSCs and P-PDLSCs observed by microscopy. Scale bar, 100 mm. (D) Alizarin red S staining shows the osteogenic nodule formation A-PDLSCs. Scale bar, 200 mm.