

Supplementary materials

Characterization of AuNPs

Four different sizes AuNPs were acquired from BBI. The final concentration of AuNPs was adjusted with α -MEM medium. The AuNPs were characterized using UV-vis spectroscopy (Fig. s1 A), Zeta sizer (Fig. s1B) and potential (Fig. s1 C, D). The results indicated the particle size distribution was uniform.

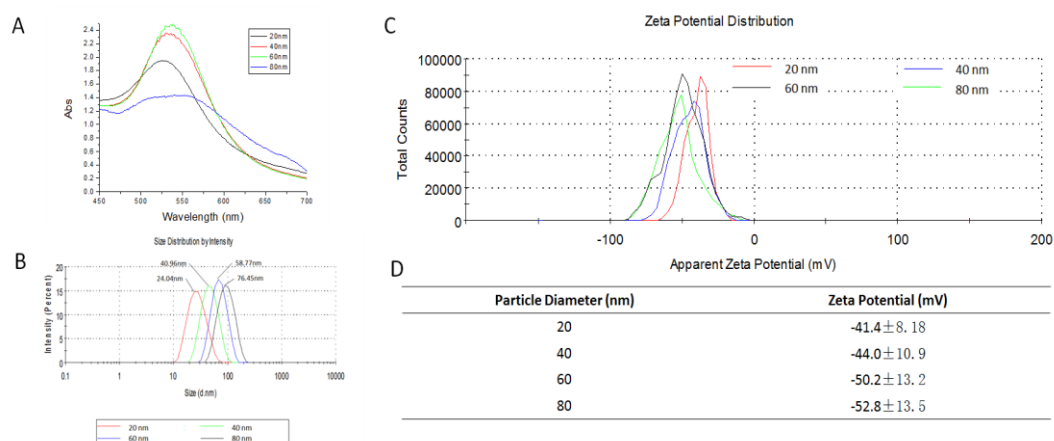


Fig. s1. UV absorption spectra of AuNPs 20 nm (black), 40 nm (red), 60 nm (green) and 80 nm (blue). The absorption peaks at wavelengths 524 nm, 529 nm, 538 nm and 541 nm for AuNPs 20 nm, 40 nm, 60 nm and 80 nm are consistent with their different NPs diameters (A). The diameters of four sizes AuNPs particles were 24.04 ± 10.62 nm, 40.96 ± 16.47 nm, 58.77 ± 22.65 nm, 76.46 ± 32.78 nm (B). The Zeta potentials of 20, 40, 60, 80 nm AuNPs were -41.4 ± 8.18 mV, -44.0 ± 10.9 mV, -50.2 ± 13.2 mV, -52.8 ± 13.5 mV, respectively (C, D). The Zeta potential of AuNPs was increasing as the size of AuNPs was enlarging.

Culture and identification of human periodontal ligament cell

hPDL contains several cell types, including fibroblasts, which are the most numerous, epithelial cells, endothelial cells, inflammatory cells, and neural and lymphatic cells. hPDLs were obtained by enzyme digestion method which could yield enough cells in a short time. hPDLs were fibroblast-like cells (Fig. s2 A, B). Immunofluorescence result showed that hPDLs were vimentin positive (Fig. s2 C, D, E) and cytokeratin negative (Fig. s2 F, G, H), proving that the cells were originated from mesoderm.

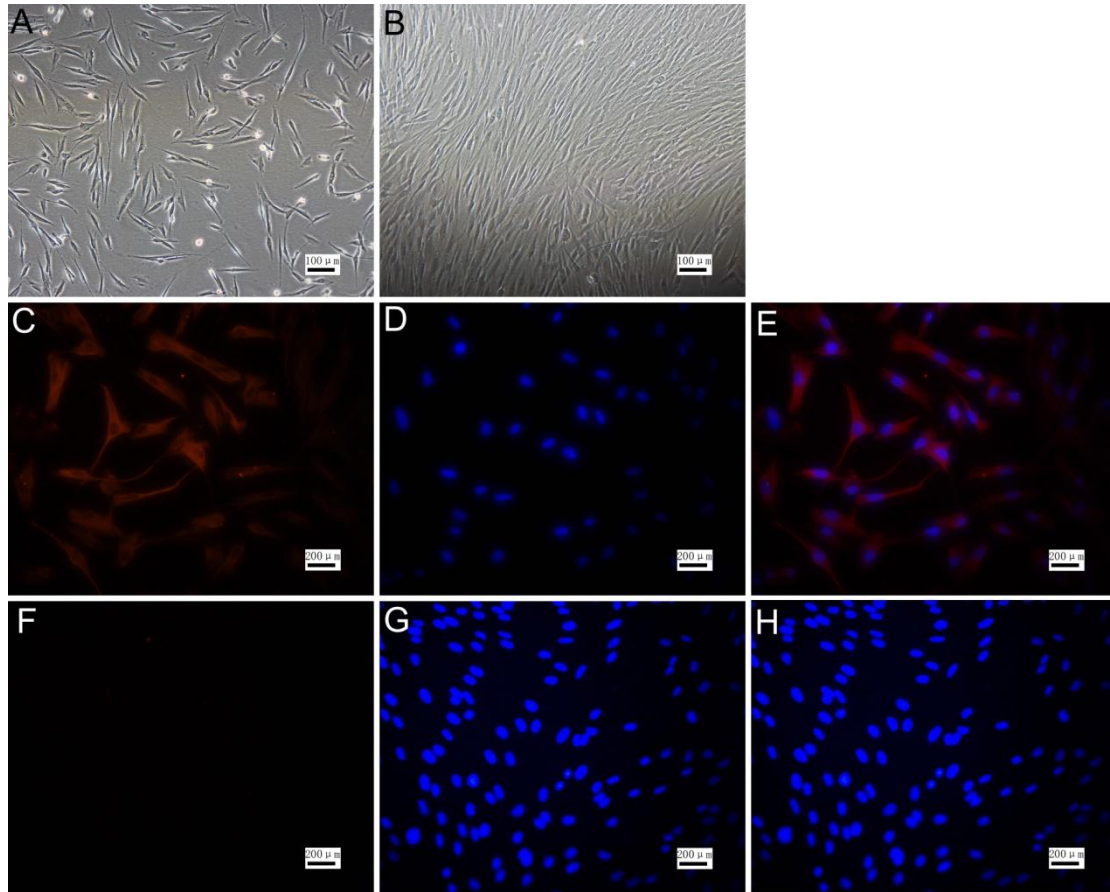


Fig. s2. Phase micrograph of hPDLs and immunofluorescence of hPDLs stained with anti-vimentin and anti-cytokeratin antibodies. Images of hPDLs under inverse microscopy after 24 hours (A) and 72 hours (B) in culture showed cells were fibroblast-like. Immunofluorescence of hPDLs stained with anti-vimentin (C) and anti-cytokeratin antibodies (F). The nuclei were stained using DAPI (D, G). The merge image indicated that hPDLs were vimentin positive (E) and cytokeratin negative (H).

Human periodontal ligament stem cell sorting and preliminary identification

hPDLSCs a group of pluripotent adult stem cells, had been isolated from periodontal tissues. With the ability of self-renew and multi-potent differentiation, hPDLSCs could differentiate into bone, cartilage, nerve, and blood vessels in vitro, and format the cementum-periodontal fiber-alveolar bone structure in vivo[1]. Therefore, hPDLSCs played an important role in periodontal tissue regeneration. However, the amount of the hPDLSCs is very low, which limited its application in periodontal tissue engineering. Flow cytometry revealed that on average 2.6% of PPDL cells were STRO-1⁺/CD146⁺[2]. According to our results, the positive rate of STRO-1 was 4.06% via FCM (Fig.3 A). The hPDLSCs clone formation rate was 38.6% (Fig. s3 B). The hPDLSCs was mainly flat, elongated spindle cells (Fig. s3 C , D). The alizarin red (Fig. s3 E, F) and oil red O (Fig. s3 G, H) staining were positive after induction.

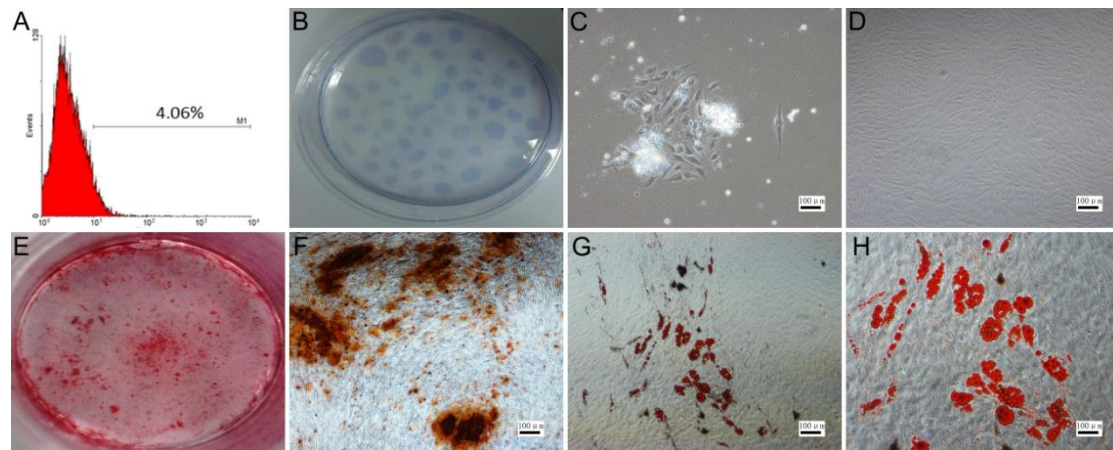


Fig. s3 hPDLSCs sorting and preliminary identification. The positive rate of STRO-1 was 4.06% of the triplicate experiments via FCM (A). The hPDLSCs clone formation rate was 38.6% of the triplicate experiments (B). STRO-1⁺ cells were cultured for 24 hours after sorting, the morphology of the cells was the paving stones (C). After 2-3 weeks culturing, cells reached 90% confluence. The cell shape tends to be consistent, mostly long spindle shape (D). Osteogenic and adipogenic differentiation of hPDLSCs via alizarin red (E, F) and oil red O (G, H) staining were positive after induction.

References

- [1] Seo BM, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet*. 2004 Jul 10-16;364:149-155.
- [2] Jinping Xu, Wei Wang, Yvonne Kapila, et al. Multiple differentiation capacity of STRO-1⁺/CD146⁺ PDL mesenchymal progenitor cells. *Stem Cells and Development*. 2009 Apr;18(3):487-496.