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Effects of hypoxia on proliferation and osteogenic differentiation of periodontal ligament stem cells: an *in vitro* and *in vivo* study

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ABSTRACT. Changes in oxygen concentration may influence various innate characteristics of stem cells. The effects of varying oxygen concentration on human periodontal ligament stem cells (HPDLSCs) has not been explored, particularly under hypoxia-related conditions. First, HPDLSCs were cultured from the periodontium of human teeth using the outgrowth method. STRO-1 and CD146 expression of HPDLSCs was investigated by flow cytometry. To detect the multilineage differentiation capacities of HPDLSCs, osteogenic-like and adipogenic-

like states were induced in cells. Next, HPDLSCs (passage 3) were exposed to normal oxygen (21% O_2) or hypoxia (2% O_2) conditions for 7 days and cell proliferation was evaluated. After culture in osteogenic medium for 7 days, osteoblastic differentiation was evaluated by semi-quantitative reverse transcription-polymerase chain reaction analysis to detect 3 osteoblastic markers: core-binding factor a 1/runt-related transcription factor 2, osteocalcin, and osteopontin. In addition, each cell group was incubated with a hydroxyapatite/tricalcium phosphate carrier and transplanted subcutaneously into the back of immunocompromised mice to investigate transplantation differences *in vivo*. HPDLSCs were isolated, cultured, and successfully identified. After exposure of HPDLSCs to hypoxia for 7 days, the proliferation rate was increased and showed higher osteogenic differentiation potential compared to control cells. After 12 weeks of transplantation, hypoxia-treated HPDLSCs differentiated into osteoblast-like cells that formed bone-like structures. These results suggest that oxygen concentrations affect various aspects of HPDLSC physiology and that hypoxia enhances osteogenic differentiation both *in vivo* and *in vitro*. Oxygen concentration may be a critical parameter for HPDLSCs during expansion and differentiation.

Key words: Osteogenic differentiation; Hypoxia; Osteogenesis; Proliferation; Periodontal ligament stem cells

