

**Practical methods for handling human periodontal ligament stem cells in serum-free and serum-containing culture conditions under hypoxia: implications for regenerative medicine**

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**ABSTRACT** Stem cell-based therapies depend on the reliable expansion of patient-derived mesenchymal stem cells (MSCs) in vitro. The supplementation of cell culture media with serum is associated with several risks; accordingly, serum-free media are commercially available for cell culture. Furthermore, hypoxia is known to accelerate the expansion of MSCs. The present study aimed to characterize the properties of periodontal ligament-derived MSCs (PDLSCs) cultivated in serum-free and serum-containing media, under hypoxic and normoxic conditions. Cell growth, gene and protein expression, cytodifferentiation potential, genomic stability, cytotoxic response, and in vivo hard tissue generation of PDLSCs were examined. Our findings indicated that cultivation in serum-free medium does not affect the MSC phenotype or chromosomal stability of PDLSCs. PDLSCs expanded in serum-free medium exhibited more active growth than in fetal bovine serum-containing medium. We found that hypoxia does not alter the cell growth of PDLSCs under serum-free conditions, but inhibits their osteogenic and adipogenic cytodifferentiation while enabling maintenance of their multidifferentiation potential regardless of the presence of serum. PDLSCs expanded in serum-free medium were found to retain common MSC characteristics, including the capacity for hard tissue formation in vivo. However, PDLSCs cultured in serum-free culture conditions were more susceptible to damage following exposure to extrinsic cytotoxic stimuli than those cultured in medium supplemented with serum, suggesting that serum-free culture conditions do not exert protective effects against cytotoxicity on PDLSC cultures. The present work provides a comparative evaluation of cell culture in serum-free and serum-containing media, under hypoxic and normoxic conditions, for applications in regenerative medicine.

**Keywords** Periodontal ligament • Mesenchymal stem cell • Serum-free culture • Hypoxia • Cytotoxic susceptibility