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# STUDIES ON CULTURE AND OSTEOGENIC INDUCTION OF HUMAN MESENCHYMAL STEM CELLS IN A CO2-INDEPENDENT CONDITION

**Abstract**

Human mesenchymal stem cells (hMSCs) are one of the important factors that regulate bone anabolism. Osteoporosis resulting from microgravity (MG) during space flight may possibly be due to a decrease in osteogenesis mediated by hMSCs. This speculation should be verified through culture and osteogenic induction of hMSCs in a microgravity environment during space flight. Control of CO2 is a key component in current experimental protocols for growth, survival, and proliferation of in vitro cultured cells. However, carrying CO2 tanks on a space flight and devoting space/mass allowances for classical CO2 control protocols makes experimentation on culture and osteogenesis difficult during most missions. Therefore, an experimental culture and osteogenic medium was developed through modifying the components of buffer salts in conventional culture medium. This experimental medium was used to culture and induce hMSCs in a CO2-independent condition. The results showed that culture and induction of hMSCs with conventional culture medium and conventional osteogenic medium in the CO2-independent condition resulted in an increase of pH in medium. The proliferation of hMSCs was also inhibited. hMSCs cultured with experimental culture medium in the CO2-independent condition showed a proliferation potential that was the same as those cultured with conventional culture medium in the CO2-dependent condition. The experimental osteogenic medium could promote hMSCs to differentiate into osteoblast-like cells in the CO2-independent condition. Cells induced by this induction system showed high ALP activity. The expression levels of osteogenic genes in cells induced with experimental osteogenic medium in the CO2-independent condition were not significantly different from those cells induced with conventional osteogenic medium in the CO2-dependent condition. These results suggest that the experimental culture and induction system could be used to culture hMSCs and induce the osteogenesis of hMSCs in the atmospheric conditions common to space flights without additional CO2.