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IMPACT OF OXYGEN LEVELS ON UMBILICAL CORD DERIVED MESENCHYMAL STROMAL CELL EXPANSION

Background/Aim. Umbilical cord-derived mesenchymal stromal cells (UC-MSCs) are a promising source for regenerative medicine due to their differentiation potential and rich secretome. One of the key factors affecting the *in vitro* cellular micro-environment is oxygen concentration. Since MSCs naturally reside in tissues with reduced oxygen levels, determination of optimal oxygenation conditions is important for standardizing expansion protocols and improving their further therapeutic application. **Methods.** UC-MSCs at passage 3 were cultured under 5% and 10% oxygen conditions, while all other culture parameters remained unchanged. Cell viability was assessed using the trypan blue exclusion assay. Colony-forming unit (CFU) assay, adipogenic and osteogenic differentiation assays confirmed by cytochemical staining (Oil Red O and Alizarin Red S correspondingly), and flow cytometry analysis were performed for cell characterization. **Results.** UC-MSCs cultured under both oxygenation conditions retained the typical morphology of MSCs. The highest cell yield was observed under 5% O₂ conditions, reaching $11.50 \times 10^6 \pm 0.04 \times 10^6$ cells, compared with $8.00 \times 10^6 \pm 0.03 \times 10^6$ cells under 10% O₂ conditions. Cell viability was also higher at 5% O₂, reaching approximately 95%, whereas under 10% O₂ conditions it was approximately 92%. Colony-forming activity was 48% at 5% O₂ and 21% at 10% O₂. Immunophenotypic analysis confirmed that the cells cultured under both oxygenation conditions retained a typical MSC phenotype. Intracellular lipid accumulation was significantly greater at 5% O₂ than at 10% O₂, as indicated by more intensive Oil Red O staining. However, osteogenic differentiation was pronounced at both 10% and 5% O₂, as evidenced by equal mineralized matrix deposition detected with Alizarin Red S. **Conclusion.** Overall, cultivation under 5% O₂ provided more favorable conditions for maintaining MSC proliferation, viability, clonogenic potential, and balanced differentiation capacity compared with 10% O₂ conditions. These findings indicate that cultivation under 5% O₂ may represent a promising approach for the standardized large-scale expansion of UC-MSCs intended for further biomedical and industrial applications.

Keywords: mesenchymal stromal cells, umbilical cord, oxygenation, immunophenotype, proliferation, clonogenic potential, differentiation.

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