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Address for Correspondence/Yazışma Adresi: Ersin Töret, M.D., Altınbaş University Faculty of Medicine, Medicalpark Bahçelievler Hospital, Department of Pediatric Hematology-Oncology & Bone Marrow Transplantation Unit, İstanbul, Turkey
Phone : +90 505 799 42 34
E-mail : drersintoret@hotmail.com ORCID: orcid.org/0000-0002-6379-8326

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Comparison of Different Culture Conditions for Mesenchymal Stem Cells from Human Umbilical Cord Wharton's Jelly for Stem Cell Therapy

Kök Hücre Tedavisi için İnsan Kordon Kanı Wharton Jel'inden Üretilen Mezenkimal Kök Hücreler için Farklı Kültür Ortamlarının Karşılaştırılması

Yu Bao¹, Shumin Huang², Zhengyan Zhao²

¹Zhejiang University Faculty of Medicine, Children's Hospital, Department of Nephrology, Zhejiang, China

²Zhejiang University Faculty of Medicine, Children's Hospital, Clinic of Division of Child Health Care, Zhejiang, China

To the Editor,

Many recent studies have demonstrated that the umbilical cord is an excellent source of mesenchymal stem cells (MSCs) [1,2,3]. However, in order to use human umbilical cord Wharton's jelly-derived mesenchymal stem cells (hUC-MSCs) in clinical therapy, a suitable culture procedure for good manufacturing practice-compliant production is mandatory. Nutritional deficiency is the major pathophysiological situation in an ischemic microenvironment in the clinic [4]. Thus, the development of serum-free culture systems is needed [5]. Furthermore, hypoxia is common in vivo in mammals [6]. The average oxygen tension falls to 1% in some cases of pathological ischemia, including fracture hematoma, and in cases of myocardial ischemia [7]. Hence, the investigation of biological characteristics of hUC-MSCs exposed to hypoxic and/or serum-free conditions is of great interest.

In our study, we conducted parallel assays by using four cell groups. For the hypoxic controls, cells from group A (n=10) and group B (n=10) were exposed to 5% CO₂ and 94% N₂ in an airtight modular incubator chamber (Billups-Rothenberg Inc., Del Mar, CA, USA). The final oxygen tension was 1%-3% as

measured by an oximeter (Oxybaby M+, Witt Technology, Solza, Italy). For the normoxic controls, cells from group C (n=10) and group D (n=10) were placed in an incubator at 37 °C, 5% CO₂, and 21% O₂. Cells from group A and group C were expanded in a mixture of Dulbecco's modified Eagle's medium and nutrient mixture F-12 (GIBCO, USA) supplemented with 10% fetal bovine serum (GIBCO, USA). Cells from group B and group D were expanded in StemPRO MSC serum-free medium (StemRD, USA). Flow cytometric analysis, differentiation potential, proliferative activities, cell cycle analysis, and apoptosis analysis of these four cell populations were evaluated. We repeated all these experiments 3 times.

Flow cytometry analysis of MSC-specific surface marker expression showed that hUC-MSCs cultured under four experimental conditions for six passages were positive for CD44, CD73, CD90, CD105, CD29, and HLA-ABC (BD Pharmingen, USA) and negative for CD34, CD45, CD14, and HLA-DR (BD Pharmingen, USA); no significant differences were detected between the four cell populations (Figure 1). This finding indicates that culturing cells under hypoxic and/or serum-free conditions did not induce significant variations in the typical MSC marker expression profile. hUC-

MSCs maintained their multilineage differentiation potential in vitro after expansion under various conditions [8]. MSCs from all groups demonstrated a similar osteogenic phenotype, as evidenced by positive staining for alizarin red S (Sigma-Aldrich, USA) and deposits of calcified matrix (Figure 2). In the case of adipogenic differentiation, cells from all groups formed lipid vacuoles detected by oil red O (Sigma-Aldrich,

USA). There were no significant quantitative changes among the groups (Figure 3). Thus, the results presented in this report indicate that hypoxic and/or serum-free conditions do not affect the biological characteristics of hUC-MSCs. Under hypoxic and serum-free conditions, hUC-MSCs have higher proliferation according to their growth curves (BD Pharmingen, USA) and MTT assays (BD Pharmingen, USA) than cells grown under normoxic and serum-containing culture conditions, but without more apoptosis (Figures 4 and 5).

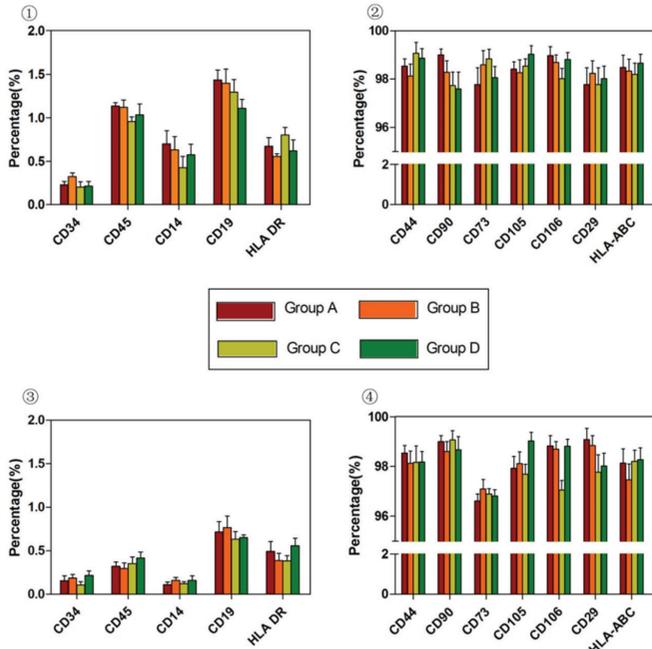


Figure 1. Flow cytometry of hUC-MSC samples [mean percentage \pm SD (%)]. 1 and 2: Passage 3; 3 and 4: Passage 6.

SD: Standard deviation, hUC-MSC: Human umbilical cord Wharton's jelly-derived mesenchymal stem cells.

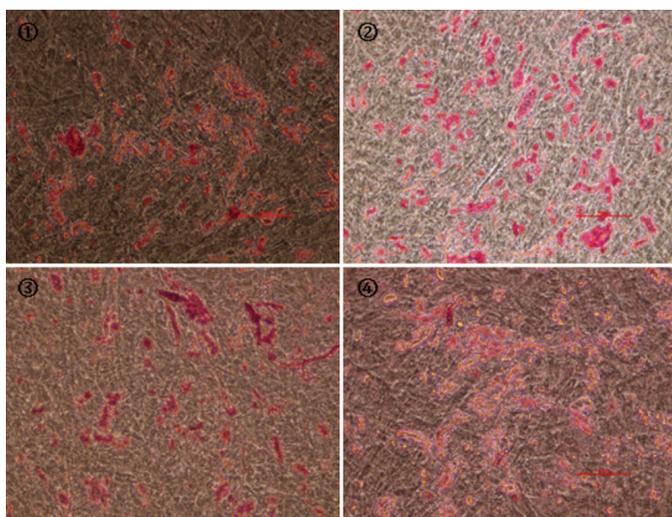


Figure 2. Multilineage differentiation potential of hUC-MSCs. Formation of mineralized matrix was detected by alizarin red S staining. 1: Group A; 2: Group B; 3: Group C; 4: Group D (original magnification: 200 \times , bar: 50 μ m).

hUC-MSC: Human umbilical cord Wharton's jelly-derived mesenchymal stem cells.

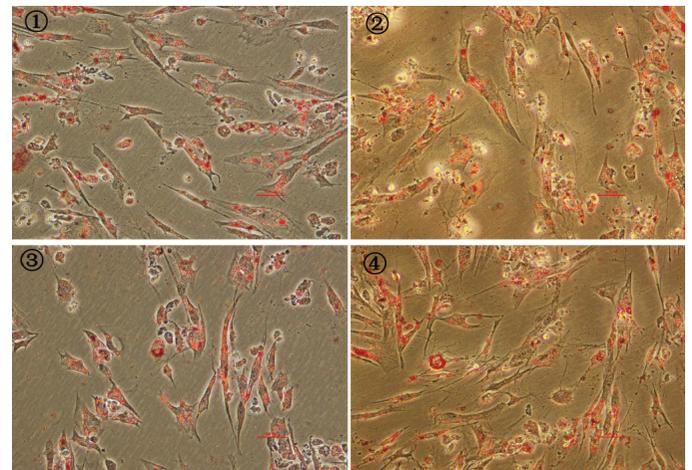


Figure 3. Multilineage differentiation potential of hUC-MSCs. Adipogenesis was confirmed by neutral oil droplet formation stained with oil red O. 1: Group A; 2: Group B; 3: Group C; 4: Group D (original magnification: 100 \times , bar: 50 μ m).

hUC-MSC: Human umbilical cord Wharton's jelly-derived mesenchymal stem cells.

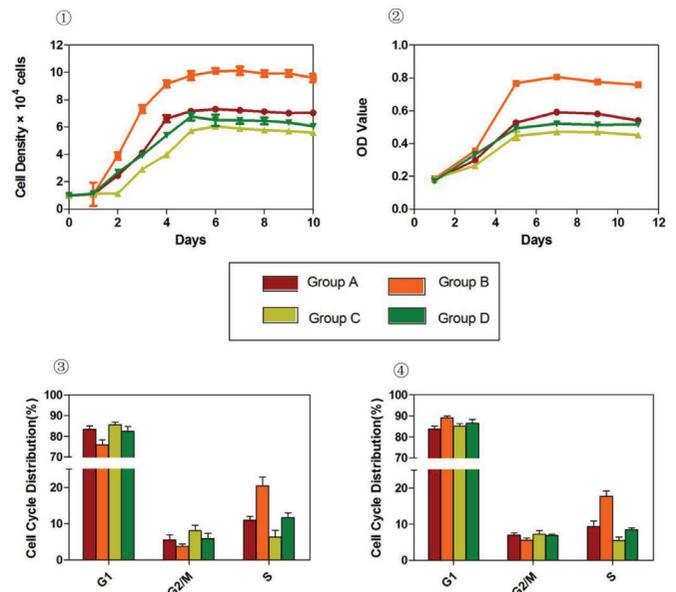


Figure 4. Expansion ability of the four groups. 1: Growth curve at passage 3. 2: MTT assay at passage 3. 3: Cell cycle distribution at passage 3 [mean percentage \pm SD (%)]. 4: Cell cycle distribution at passage 6 [mean percentage \pm SD (%)].

SD: Standard deviation.

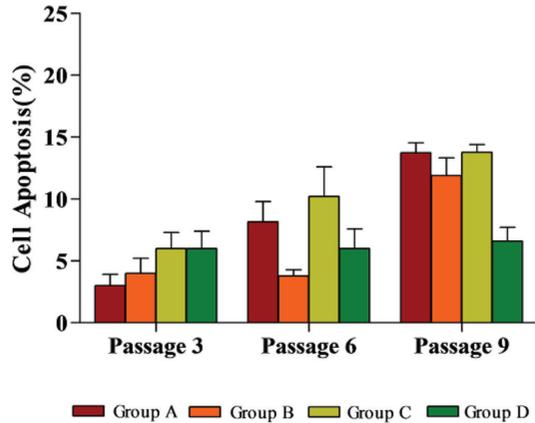


Figure 5. Proportion of apoptotic hUC-MSCs cultured under different conditions (mean \pm SD).

SD: Standard deviation, hUC-MSC: Human umbilical cord Wharton's jelly-derived mesenchymal stem cells.

Taken together, our data indicate that hypoxic and serum-free culture conditions do not influence the major properties of hUC-MSCs. Under hypoxic and serum-free conditions, hUC-MSCs showed higher proliferation, while their apoptosis rate did not increase. This finding is consistent with that of previous reports, which demonstrated enhanced proliferation of bone marrow-derived MSCs (BM-MSCs) under hypoxic or serum-free conditions [9,10]. Therefore, the availability of optimized in vitro conditions, including hypoxia and serum-free media, for hUC-MSC manipulations may have a substantial scientific and clinical impact.

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Ethics Committee Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Medical Ethics Committee of the Children's Hospital School of Medicine, Zhejiang University (reference number 1712048) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

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Address for Correspondence/Yazışma Adresi: Zhengyan Zhao, Ph. M.D., Zhejiang University Faculty of Medicine, Children's Hospital, Clinic of Division of Child Health Care, Zhejiang, China
Phone : +8613805722351
E-mail : zhaoyz@zju.edu.cn ORCID: orcid.org/0000-0002-5741-2431

Authorship Contributions

Data collection or Processing: Y.B., S.H., Z.Z.; Analyses or Interpretation: Y.B., S.H., Z.Z.; Literature Search: Y.B., S.H., Z.Z.; Writing: Y.B., S.H., Z.Z.

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