

addition, expression levels of telomerase reverse transcriptase (TERT), histone deacetylase 1 (HDAC1), DNA (cytosine-5)-methyltransferase 1 (DNMT1), dyskerin pseudouridine synthase 1 (DKC1), B-cell lymphoma 2 (BCL2), vascular endothelial growth factor (VEGF), Ki-67 and proliferating cell nuclear antigen (PCNA) were analyzed using quantitative real-time polymerase chain reaction (qPCR).

**Results:** The viability and S phase (the phase of DNA synthesis) of eAD-MSCs were increased significantly after cells were treated with vegetable-based resources (VR #9) under serum-free condition. Also, expression levels of anti-senescence and cell survival-related genes such as TERT, HDAC1, DNMT1, BCL2, VEGF, Ki-67, and PCNA were significantly increased after cells were treated with vegetable-based resources (VR #9) under serum-free condition.

**Conclusions:** Our findings revealed that the vegetable-based resources could promote proliferation of eAD-MSCs under serum-free condition. In addition, results of this study suggest that induction of stem cell proliferation by vegetable-based resources is likely to be related to its expression of anti-senescence and cell survival-related genes such as TERT, HDAC1, DNMT1, BCL2, VEGF, Ki-67, and PCNA under serum-free condition.

#### References

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### Osteogenic Potential and Proliferating Effect of Three-Dimensional Hydrogel Scaffolds on Equine Mesenchymal Stem Cells

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**Introduction:** Physiological cell environment not only connects cells to each other, but also connects cells to the extracellular matrix (ECM) that provide mechanical support, thus exposing the entire cell surface and activating signaling pathways. All cells in tissues consist of a complex three-dimensional (3D) structure and are connected with neighboring cells with ECM [1]. Two-dimensional (2D) culture system is typically used for cell growth, but the method reduces the characteristics of cells. Hydrogel is a polymeric material that swells in water. It can maintain a distinct 3D network structure by cross linking. Its soft and fibrous nature together with high water content and the possibility for diffusion of small components make this class of materials an ideal mimic of ECM. Hydrogel is regarded as excellent materials for encapsulating cells.

In this study, to investigate the enhancing properties of 3D culture system in equine adipose tissue-derived mesenchymal stem cells (eAD-MSCs), we performed encapsulating cells and determined changes in gene expression levels of eAD-MSCs.

**Materials and Methods:** eAD-MSCs were seeded at normal plate ( $1 \times 10^5$  cells/well, condition 1), normal plate with hydrogel ( $1 \times 10^5$  cells/well, condition 2), transwell membrane ( $0.4 \times 10^5$  cells/well, condition 3) and transwell membrane with hydrogel ( $0.4 \times 10^5$  cells/well, condition 4) of 6-well culture plate. After culturing for 5 days, cells from these four different conditions were collected and gene expression levels of Ki-67, proliferating cell nuclear antigen (PCNA), OCT4, and SOX2 were determined by quantitative real-time PCR (qPCR) and normalized against the expression level of GAPDH. Also, differentiation into mesodermal cell lineages was performed by using osteogenic differentiation medium. Then, the mRNA expression level of specific differentiation marker such as osteocalcin (OC) was also examined by qPCR.

**Results:** We observed that the expression levels of Ki-67, PCNA, OCT4, and SOX2 were significantly ( $p < 0.05$ ) increased in conditions including hydrogel (condition 2 and 4) compared to those in conditions 1 and 3. In addition, expression levels of Ki-67, PCNA, and SOX2 were significantly ( $p < 0.05$ ) higher in condition 4 compared to those in condition 2. Also, eAD-MSCs under all conditions were well differentiated into osteocytes in osteogenic differentiation medium. Among them, expression level of OC was significantly ( $p < 0.05$ ) increased in conditions including hydrogel (conditions 2 and 4) compared to those in conditions 1 and 3.

**Conclusions:** These results suggest that 3D culture of eAD-MSCs through hydrogel scaffolds method can enhance the expression of proliferating and osteogenic factors. Also, surrounding culture condition is found to be better than plating culture condition including hydrogel. Therefore, these 3D culture systems can be used to enhance the efficiency of tissue engineering in eAD-MSCs compared to the traditional cell culture system.

#### References

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### Transforming growth factor-beta induced epithelial-mesenchymal transition abolished without TGF-beta condition: Cancer stem cell properties and cisplatin resistance in lungcancer cell lines

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