Progress report 2022

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[Department Outline]

The Department of Regenerative Medicine and Cell Therapy was established in April 2021 to promote a mass culture of human umbilical cord-derived mesenchymal stem cells (UCMSCs) using polymeric culture substrates and the development of diagnostic imaging of UCMSCs.

Mesenchymal stem cells (MSCs) are somatic stem cells that exist in vivo. MSCs are derived from mesoderm and can be harvested from various tissues such as fat, bone marrow, and umbilical cord.

MSCs have been shown to secrete cytokines and growth factors that have pro-proliferative, anti-inflammatory, and angiogenic effects, and to support tissue repair via paracrine pathways.

MSCs cultures are usually classified into two-dimensional (2D) and three-dimensional (3D) cultures, with 3D cultures reported to be superior to 2D cultures. 3D culture enables the formation of cell aggregates and the growth of cells in a three-dimensional state, a culture environment closer to that of in vivo, and enhances cell function while maintaining the characteristics of mesenchymal stem cells. In this course, we compared and examined the characteristics and functions of 2D, and 3D cultured. UCMSCs using Cellhesion® (Nissan Chemical Co.LTD). MSCs are defined as having CD105, CD73, CD90, etc. as markers, however since the expression of these markers does not determine the quality of the cells, such as whether they are proliferating cells, it is important to have a quality evaluation method for MSCs. MSCs vary widely among tissues of origin, donors, etc., and even young passages. Therefore, it is important to evaluate the quality of MSCs. Using the cell imaging technology Bio-Studio-T (Nikon Corporation), we will evaluate the quality of MSCs during and after three-dimensional mass culture.

[Research Content]

MSCs cultured on Cellhesion® culture substrate show about 7 times more anti-inflammatory activity than conventional 2D cultured cells and are expected to be applied to the treatment of various diseases. In this study, we investigated the effect of 2D and 3D cultured UCMSCs using Cellhesion® as culture substrates. We also compared the characteristics and functions of 2D and 3D cultured UCMSCs using Cellhesion® culture substrate and examined them. In addition, for cell quality evaluation, phase contrast of 2D and 3D cultured UCMSCs image analysis (Bio-Studio-T) of 2D and 3D cultured UCMSCs.

Cellhesion® was diluted at a 1/20 liquid concentration and combined with MSCs in a 10 mm diameter culture dish. This mixture was uniformly agitated to ensure full and even contact between cells and Cellhesion®. Cells and materials were then incubated in a 37°C, 5% CO2 environment within a cell culture incubator to achieve uniform contact. Over time, cells and material aggregated into clusters, with MSCs simultaneously replicating and increasing in number. By the fifth day, the scattered Cellhesion® had reformed into larger clumps.

Following 3D culture, we identified MSCs based on their ability to differentiate into adipocytes, osteoblasts, and chondrocytes. The results demonstrated that this differentiation ability persisted after Cellhesion® 3D culture, which supports the notion that stemness is maintained in stem cells after Cellhesion® culture. Mesenchymal stem cell

marker proteins CD73, CD90, and CD105 tested positive, while CD11b, CD19, CD34, CD45, and HLA-DR were negative. UCMSCs cultured using Cellhesion® 3D were subjected to colony-forming unit (CFU) experiments, which revealed that UCMSCs following 3D culture performed better than those in 2D culture.

We also analyzed the proliferation ability of MSCs in both 2D and 3D cultures. Initially, the 3D culture group exhibited slower growth than the 2D group. However, when UCMSCs were separated from the material by successfully changing the medium and placed in a 2D environment. The 3D culture and 2D culture were re-cultured under the same conditions as their generation of 2D cultured cells. It was found that the proliferation ability of cells cultured in 3D was significantly stronger than that of cells cultured in 2D. The results showed that the UCMSCs cultured in a 3D environment proliferated faster, reached the peak sooner, and the cells proliferated in larger numbers.

The paracrine effect of MSCs cells has a wide range of application prospects. We test the amount of the factors HGF, VEGF, IL-6, and IL-8 secreted from UCMSCs increased with incubation time and peaked 7 days after the start of incubation. We also test the angiogenesis of factors secreted by 2D and 3D cultures, results showed that the 3D culture showed more luminal area, total length, and the number of branching points of angiogenesis.

[Future Outlook]

Since UCMSCs cultured in three-dimensional culture maintained the undifferentiated and migratory capacities of mesenchymal stem cells better than those cultured in two-dimensional culture and showed a large amount and high cell activity that cannot be obtained in two-dimensional culture, three-dimensional cultured mesenchymal stem cells are expected to be applied to regenerative medicine and cell therapy.

[References]

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