A NOVEL XENO-FREE PROLIFERATION CONTROL CULTURE SYSTEM FOR HUMAN ADIPOSE DERIVED STEM CELLS

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Abstract

Human mesenchymal stem cells (hMSCs) have shown great promise as a cell source not only for cell therapy but also for exosome therapy. However, when cells are cultured or transported alive, it is vital to keep the cells on a routinely passage in order to avoid overcrowding which can cause poor cell growth, senescence even death. We have developed the Xyltech cell proliferation control system for human pluripotent stem cells. In this study, we developed the novel Xeno-Free proliferation control medium (Xyltech, MSC-01) on the basis of “Xyltech technology”, and validated its applicability on human adipose-derived stem cells (hADSCs). Xeno -free growth medium (Xyltech Growth, MSC) was used as a control.

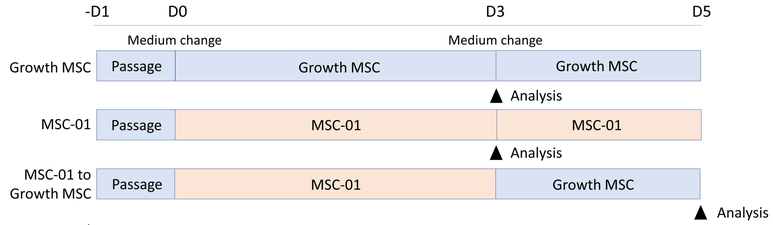
Materials & Methods

Cells & Culture Medium

Primary Cells: Normal human adipose-derived stem cells (hADSCs, Lonza)
Growth medium: Xyltech™ Growth MSC (**Growth MSC**),
Proliferation control medium: Xyltech™ MSC-01 Xeno-Free (**MSC-01**)

Proliferation Control Culture

About in the conditions of Growth MSC, MSC-01 and MSC-01 to Growth MSC (Proliferation control culture) are shown in the scheme 1.



Scheme 1 | The hADSCs were cultured in Growth MSC. The day after passaging, the culture media were changed to MSC-01 for 3 days to control proliferation. Subsequently, the cells were switched back to Growth MSC for 2 days to promote re-proliferation (MSC-01 to Growth MSC). In both the Growth MSC and MSC-01 conditions, culture were initiated the day after seeding and maintained for 3 days.

Differentiation

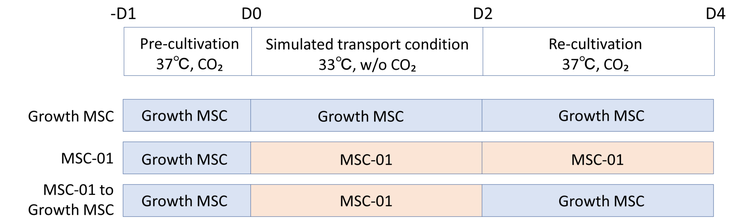
The following kits were used to differentiate hADSCs into adipocytes, osteoblasts, and chondrocytes; Mesenchymal Stem Cell Adipogenic Differentiation Medium 2 (Promo Cell), Mesenchymal Stem Cell Osteogenic Differentiation Medium (Promo Cell) and Stem Pro™ Chondrogenesis Differentiation Kit.

Flow Cytometry

The following antibodies were used in the flow cytometric analysis: CD90 (BD#51-9007657), CD73 (BD#51-9007649), CD105 (BD#51-9007648) as positive markers and CD34 (BD#560941), CD45 (BD#560975) as negative markers.

Simulated live Cell Transport

The pseudo-live cell transport conditions are shown in the scheme.



Scheme 2 | The hADSCs were seeded into flasks (iP-TEC, sanplatec) at a density of 8,000 cells/cm². The next day, the vessels were filled to capacity with MSC-01 and placed under transport conditions (33° C, non-CO_2, static) for 2 days. Subsequently, the medium was exchanged with Growth MSC and cultured again at 37° C under CO_2 conditions. As a control, the medium used for transport was either Growth MSC or MSC-01, and the same medium was used for re-cultivation as during transport.

Result 1： Comparison of Xyltech™ Growth MSC XF with other Xeno-Free culture medium

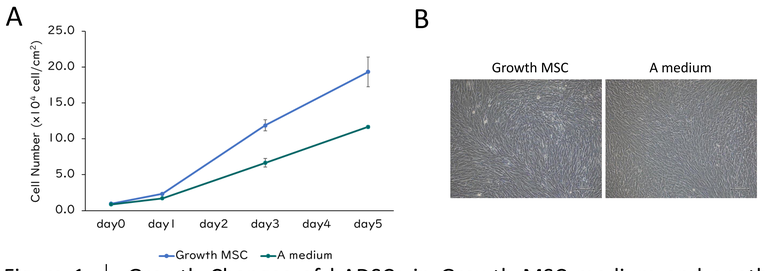


Figure 1 | Growth Changes of hADSCs in Growth MSC medium and another commercial medium. hADSCs cultured in Growth MSC medium showed higher growth compared to A medium (A). hADSCs reached 100% confluency on day 3 in both Growth MSC medium and A medium (B).

Result 2： Proliferation control culture of hADSCs using Xyltech™ MSC-01

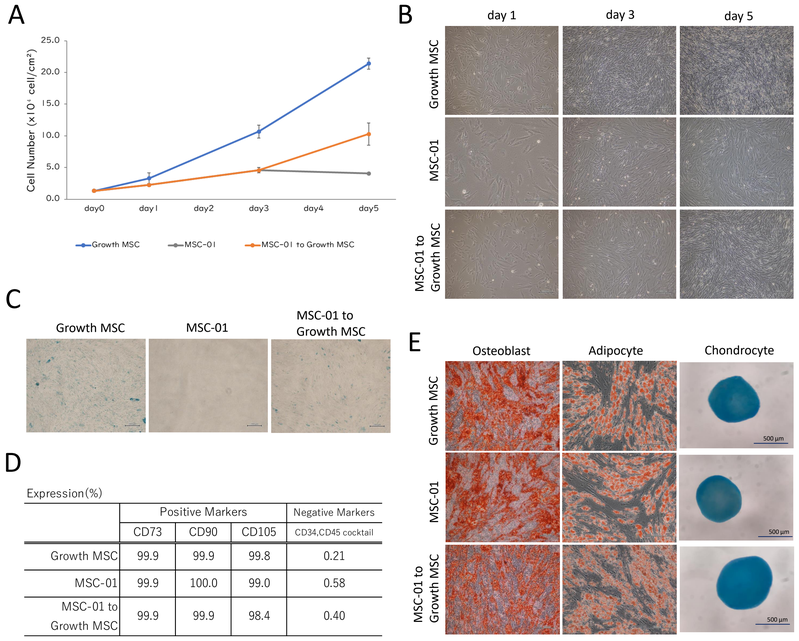
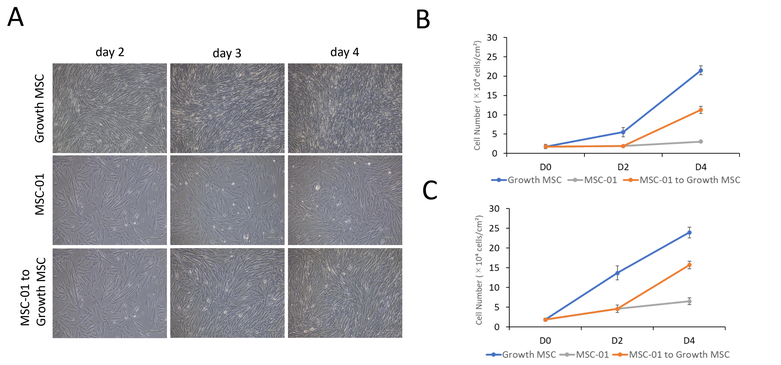


Figure 2 | In the cell count test, hADSCs cultured in MSC-01 exhibited a suppressed proliferation rate compared to Growth MSC. The cells resumed proliferation from days3-5 upon changing the media to Growth MSC (A). The morphology of hADSCs cultured in MSC-01 was similar to that of cells cultured in Growth MSC, and no floating cells were observed during the culture (B). In MSC-01, a few SA-β-GAL positive cells were observed. Conversely, in the replacement from MSC-01 to Growth MSC, there was a slight increase in SA-β-GAL positive cells with increasing cell density. However, the numbers tended to be lower than cells cultured for 3 days in Growth MSC (C). In flow cytometry, the expression rates of both positive and negative marker in MSCs were within the reference values proposed by the International Society for Cellular Therapy (ISCT).(D). Moreover, hADSCs had the ability to differentiate into osteoblast, adipocytes and chondrocytes in all conditions (E).

Result 3： Investigation of Xyltech™ MSC-01 for hADSC simulated live cell transport



D

Expression(%)

	Positive Markers			Negative Markers
	CD73	CD90	CD105	CD34,CD45 cocktail
	99.8	99.8	96.7	0.05

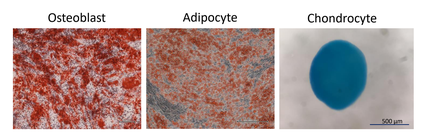


Figure 3 | After simulated transport in MSC-01, no floating cells were observed in hADSCs, and the cell density remained similar to that before transport. Upon resumption of culture, cells tended to increase immediately in MSC-01 to Growth MSC (A). In the cell count test, comparing the simulated transport environment (B) with the normal culture environment (37° C, CO_2) (C), the number of cells in the simulated transport environment showed a suppressed increase during the 2 days of transport compared to the culture environment. Additionally, after culture resumption, the number of cells tended to increase rapidly, especially in cells transported with Growth MSC. In flow cytometry, all markers of the mock-transported cells were within the reference values (D), and the cells also retained their differentiation potential into osteoblasts, adipocytes, and chondrocytes (E).

Conclusions

- MSC-01 was able to maintain hADSCs in a normal culture environment (37° C, CO_2) by suppressing their proliferation, and the cells quickly repopulated when the culture medium was changed to Growth MSC.
- In MSC-01, the cells were barely stained by SA-β-GAL, and the appearance of SA-β-GAL-positive cells after re-proliferation was suppressed compared to growth culture (Growth MSC).
- The proliferation-controlled culture of hADSCs with MSC-01 maintained the characteristics of MSCs.
- MSC-01, under simulated transport conditions, exhibited a suppressed increase in cell number during the transport period, followed by a rapid proliferation after culture was resumed. Additionally, the mock-transported cells maintained MSC characteristics.

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