NEWLY DEVELOPED XENO-FREE MEDIUM FOR HUMAN MESENCHYMAL STEM CELLS SHOW ROBUST CELL-EXPANSION CAPACITY

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Abstract

Human mesenchymal stem cells (hMSCs) are an attractive candidate for cell therapy due to their multipotential differentiation activities into each cell type or immunomodulatory properties. For therapeutic applications, hMSCs are needed to be expanded to appropriate cell number because primary hMSCs obtained from bone marrow, adipose or cord blood are usually limited and thereby the required cell number can't be obtained. Although many medium for culturing hMSCs are currently proposed, further improvements in their cell-expansion capacity or the maintenance of multipotential differentiation activities have been needed. Furthermore, medium for clinical use should be at least xeno-free formulation because of the potential risks of FBS, such as virus or prion contamination. Thus, we have newly developed xeno-free medium for hMSCs, having especially robust cell-expansion capacity. Both bone-marrow and adipose derived hMSCs expansions using new medium were several-fold higher than those using conventional other medium (commercially available). Moreover, new medium could be used even in the extracellular matrix coating-free condition. After several passages, we confirmed the expression of cell surface markers identified as hMSCs such as CD73, CD90 and CD105 by flow cytometry and colony-forming unit-fibroblast (CFU-F) capacity of the expanded cells. The cells expanded by new medium were shown to be almost 100% of CD73, CD90 and CD105 positive population, but a little decrease of CD105 expression was observed in the cells by other medium. Since CFU-F capacity was almost equal among tested medium, the obtained colony-forming cells in new medium were several-fold larger than those in other medium. We have also confirmed their multipotential differentiation activities into adipocytes, chondrocytes and osteocytes. Overall, it is considered that our newly developed xeno-free medium have desired properties to expand hMSCs for therapeutic application.

Cell growth

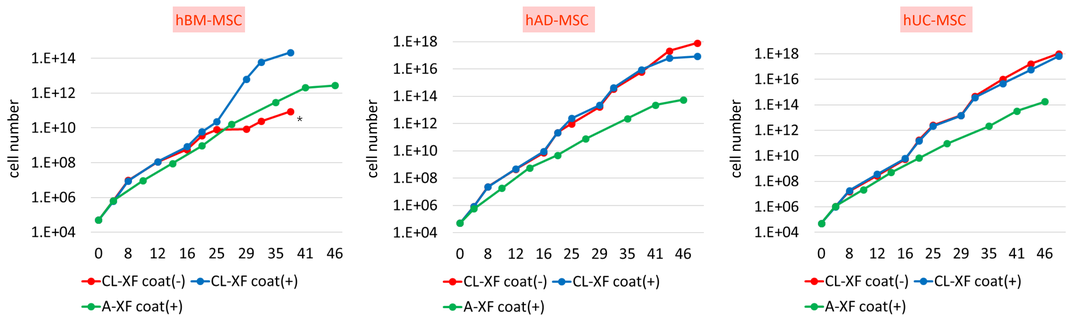
Newly developed xeno-free medium for human MSCs culture

Cellartis® MSC Xeno-Free Culture Medium

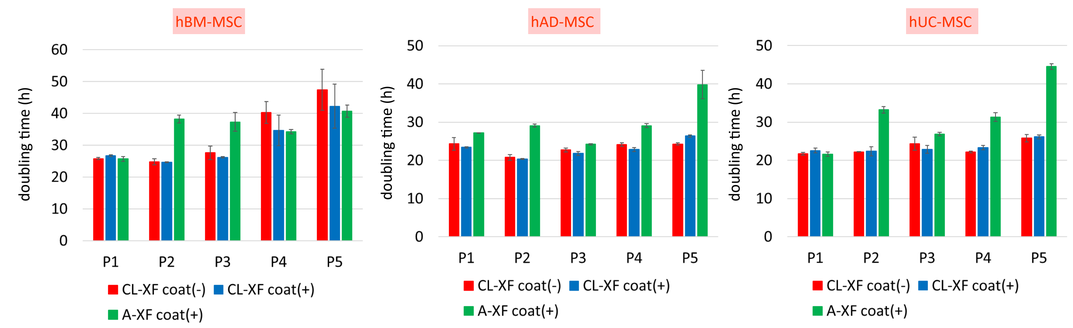
We tested for the ability of MSC culture medium on cell growth using hMSCs derived from bone marrow, fat or cord blood. In general, xeno-free medium for hMSCs are needed to use extracellular matrix coating such as fibronectin for the cell expansion. Thus, we also evaluated the necessity of coating.

	Mesenchymal Stem Cell : MSC	Cellartis® MSC Xeno-Free Culture Medium : CL-XF		company A xeno-free medium: A-XF	
1	hBM-MSC (bone marrow)	coating (-)	coating (+) (fibronectin)	coating (-)	coating (+) (fibronectin)
2	hAD-MSC (fat)				
3	hUC-MSC (cord blood)				

Growth curve



Doubling time



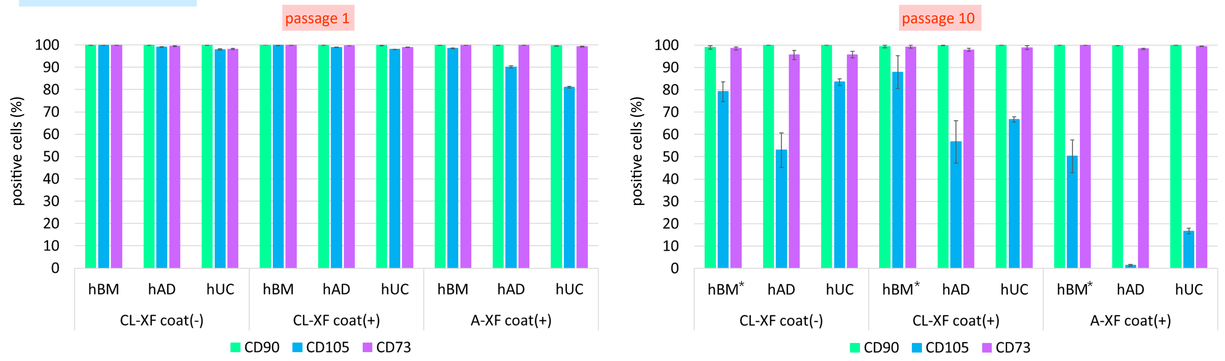
Cellartis® MSC Xeno-Free Culture Medium (CL-XF) showed superior proliferation capacity for hMSCs derived from various tissues even in coating-free condition. On the other hand, A-XF could not maintain the culture of each MSC in the coating-free condition. Further, each MSC cultured by CL-XF showed stable doubling time in both with and without coating (typically 20-30 hours in CL-XF, but 30 hours in A-XF).

(*hBM-MSCs by CL-XF coat(-) condition in this experiment might be affected by the passage timing. Another experiment showed similar growth curve between with and without coating. It should be confirmed the reproducibility.)

Characteristic of hMSC after expansion

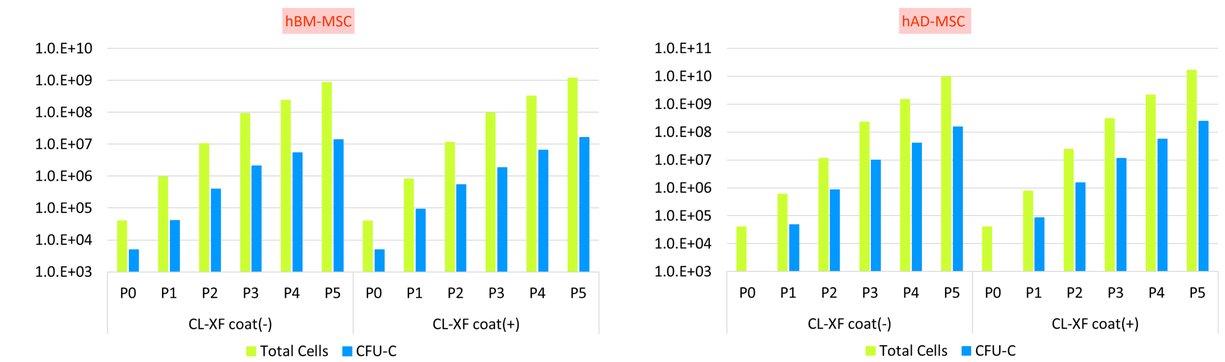
Human mesenchymal stem cells (hMSCs) cultured by Cellartis® MSC Xeno-Free Culture Medium (CL-XF) were evaluated their characters such as MSC surface marker, colony-formulation and differentiation into three types of cells (adipocyte, chondrocyte or osteoblast).

Cell surface marker



hMSCs expanded by Cellartis® MSC Xeno-Free Culture Medium were shown to be almost 100% of both CD73 and CD90 positive population. On the other hand, CD105 expression was decreased by the cultivation, even though still much higher CD105 expression was observed in CL-XF in both coating condition than in A-XF. (*The dataset of hBM at passage 10 was actually at passage 8.)

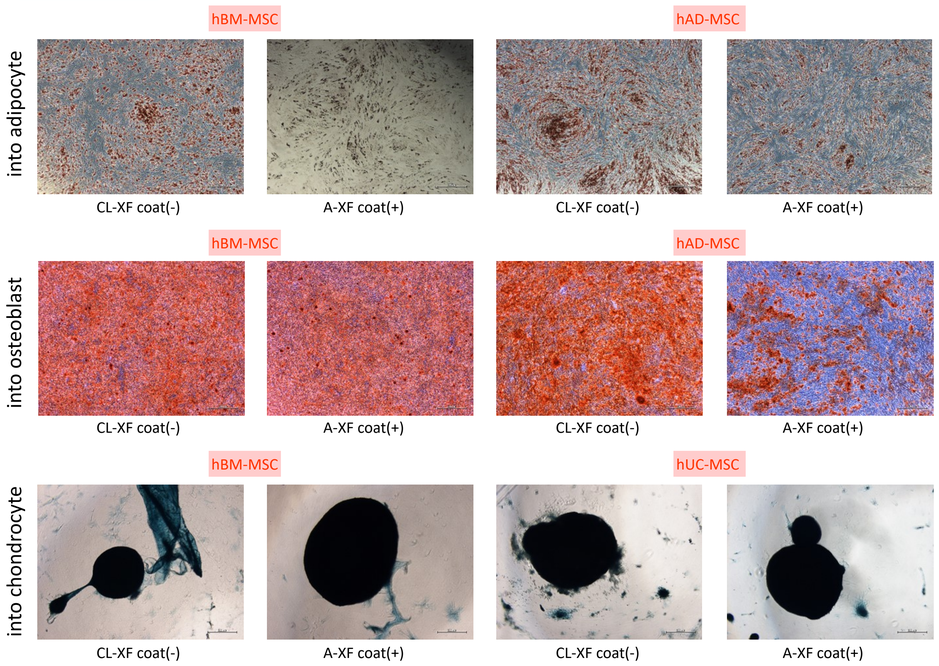
Colony-formulating cells



CFU-C assay was conducted to evaluate the self-renewal ability of MSCs. Each expanded hMSCs were seeded at hundreds of cells per well (6 well plate). After 2 weeks of culture, appeared colonies were stained by crystal violet and then measured.

Colony-formulating cells were maintained after 5 passages in Cellartis® MSC Xeno-Free Culture Medium in both coating condition.

Differentiation



MSCs cultured by each medium were differentiated into adipocyte, osteoblast or chondrocyte by using each differentiation kit (PromoCell). After differentiation, each cell was stained by Oil Red O (adipocyte), Alizarin Red S (osteoblast) or Alcian Blue (chondrocyte).

Although there was a difference on differentiation into each cell type among three cell origins, it was confirmed that MSCs cultured by Cellartis® MSC Xeno-Free Culture Medium (CL-XF) had multipotency. Especially, hAD-MSCs cultured by CL-XF showed superior differentiation ability into adipocyte or osteoblast compared to cells by A-XF.

Summary

Human MSCs cultured by Cellartis® MSC Xeno-Free Culture Medium …

- ✓ showing stable proliferation without any extracellular coating matrix
- ✓ expressing almost 100% of MSC markers (CD73 and CD90) and keeping colony formulation ability
- ✓ differentiating into adipocyte, osteoblast and chondrocyte