



EX VIVO EXPANSION OF HUMAN AMNIOTIC FLUID STEM CELLS (AFSC)

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Introduction

Human amniotic fluid-derived stem cells (hAFSCs) could be a promising source of cell therapy applications, not only because of their multipotent stem cell characteristics and certain embryonic stem cell properties, but also due to their high capability of proliferation *in vitro*. The aim of this study was to optimize the culture medium for hAFSCs ex vivo expansion, and to characterize expanded cells based on the expression of stem cell markers and differentiation potency.

Methods

The isolated hAFSCs were cultured for over three passages in PRIME-XV™ AFSC Expansion Medium, a complete serum containing medium, and then stained for flow cytometry and immunocytochemistry analysis. The expanded cells were also cultured in varied differentiation conditions for testing specific cell lineages.

Results

We found that human amniotic fluid stem cells could be expanded more than 100 fold over three passages in PRIME-XV™ AFSC Expansion Medium. In addition, hAFSCs were able to sustain the expression markers of stem cells, such as CD29, CD44, CD73, CD90, CD105, and SSEA-4, as well as transcription factors that are critical in regulating pluripotency, such as Oct4-A, SOX2, and Nanog, after extended culturing in the medium. However, these cells do not express CD45 and CD133. Furthermore, we were also able to differentiate the propagated cells into different specific cell lineages, such as adipocytes and osteocytes after incubation with respective induction medium.

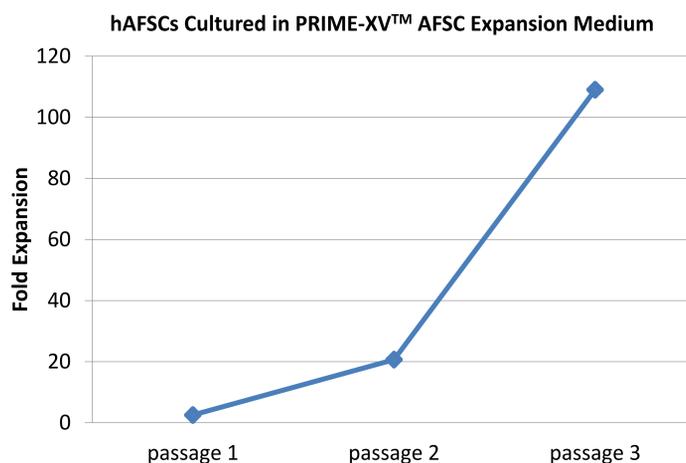


Fig 1. Human amniotic fluid stem cells grown in PRIME-XV™ AFSC Expansion Medium over three passages. Fold expansion was calculated as the ratio of final viable cell count by the initial seeded viable cell count at passage 1.

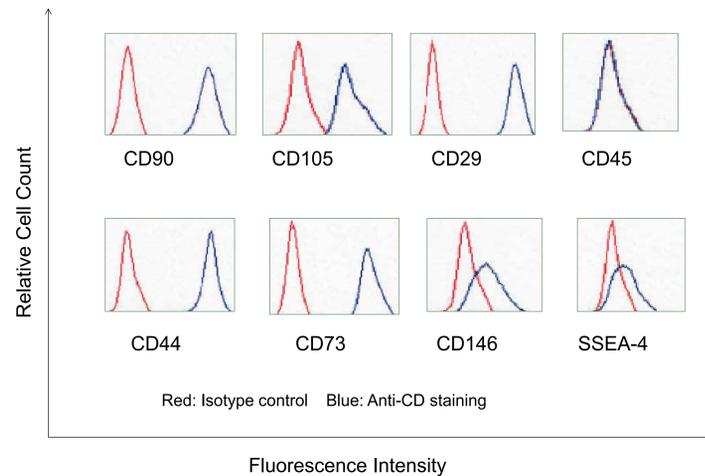


Fig 2. Flow cytometry analysis of human amniotic fluid stem cells after 5 passages of culture in PRIME-XV™ AFSC Expansion Medium. It showed positive staining for typical mesenchymal markers (CD90, CD105, CD29, CD44, CD73, CD146), negative for marker of the hematopoietic lineage (CD45), and positive for stage-specific embryonic antigen (SSEA-4).

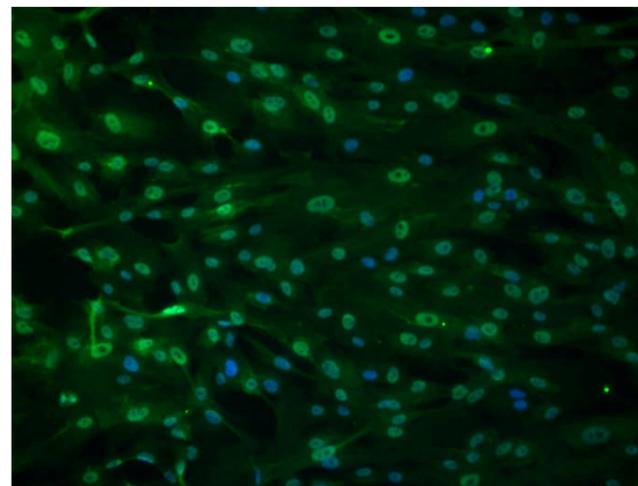


Fig 3. Immunofluorescence analysis of hAFSCs cultured in PRIME-XV™ AFSC Expansion Medium after six passages showed positive OCT-4A staining (in green). Nuclei were counterstained with DAPI (in blue).

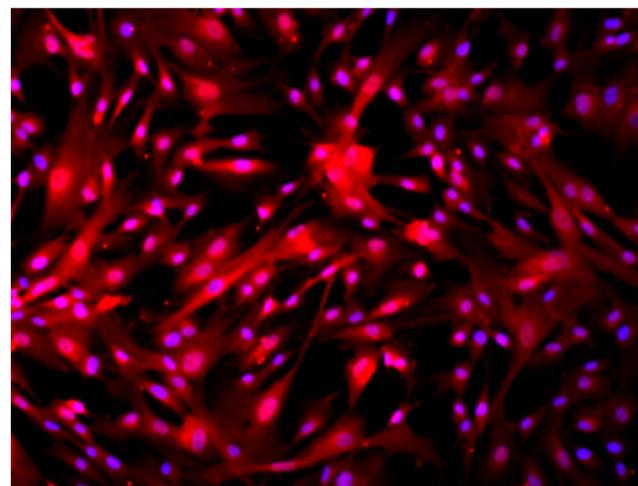


Fig 4. Immunofluorescence analysis of hAFSCs cultured in PRIME-XV™ AFSC Expansion Medium after six passages showed positive Nanog staining (in red). Nuclei were counterstained with DAPI (in blue).

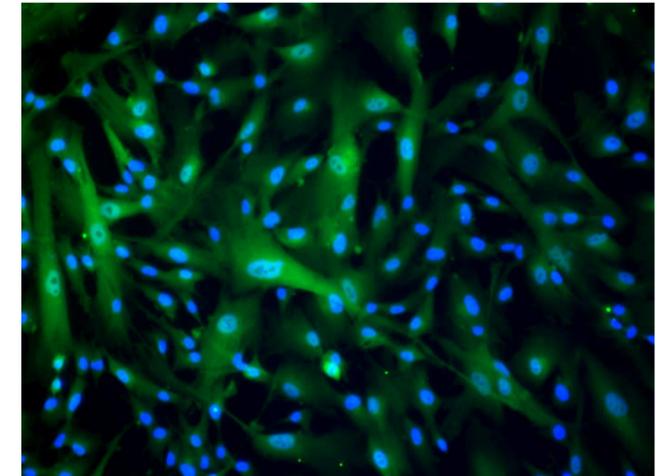


Fig 5. Immunofluorescence analysis of hAFSCs cultured in PRIME-XV™ AFSC Expansion Medium after six passages showed positive SOX2 staining (in green). Nuclei were counterstained with DAPI (in blue).

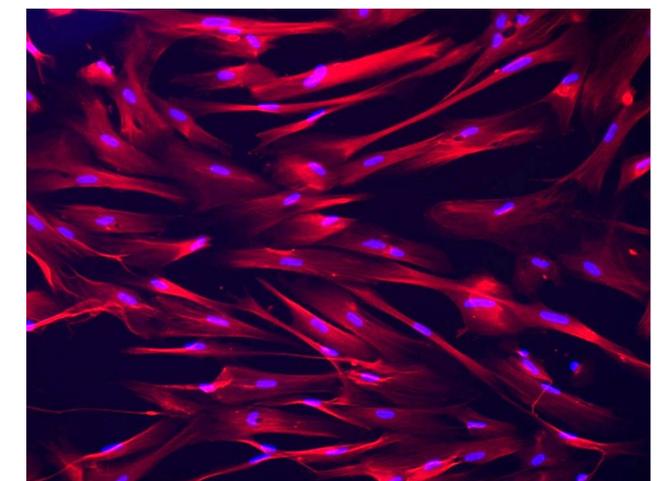


Fig 6. Osteogenic differentiation test of hAFSCs cultured in PRIME-XV™ AFSC Expansion Medium for 6 passages. The cells showed positive osteocalcin staining after induced in the osteogenic differentiation medium for 20 days (anti-Osteocalcin in red, DAPI in blue).

Conclusion

The study showed that PRIME-XV™ AFSC Expansion Medium can effectively expand human amniotic fluid stem cells *ex vivo*, and maintain stem cells expression markers. Based on the platform of this medium, we are continuously developing serum-free and/or xeno-free medium for hAFSC expansion for cell therapy application.

References

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3. Moorefield, E. *et al.* Cloned, CD117 selected human amniotic fluid stem cells are capable of modulating the immune response. *PLoS ONE*, Vol. 6, 10, e26535 (2011).