Rapid Expansion of Human Adipose Stem Cells using Corning® HYPER*Flask*® Cell Culture Vessel

Customer Application Note

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N.M. Vieira, V. Brandalise, E. Zucconi, M. Secco, M. Zatz
Human Genome Research Center
Biosciences Institute, University of São Paulo
São Paulo, Brazil

Abstract

Human Adipose Stem Cells (hASCs) represent an abundant and accessible source of multipotent adult cell population (1). Although liposuction fat can be obtained in large quantities, the large number of viable and undifferentiated cells required for cellular therapeutic applications will need efficient *in vitro* expansion methods. For instance, in an experimental therapeutic trial in a mouse model for progressive muscular dystrophy, we obtained positive results when we injected 10^6 cells monthly (2). If we extrapolate for human use, an estimated quantity of stem cells for a person weighing about 60 kg would be about 3 x 10^9 cells per month. We have tested several protocols for *ex vivo* expansion, and using Corning HYPER*Flask* cell culture vessels, we were able to generate a great quantity of mesenchymal stem cells for research, as well as aiming future cellular therapeutic approaches.

Results

hASCs were isolated from adipose tissue obtained from elective liposuction procedures. After isolation, hASCs were characterized by flow cytometry for the expression of 12 cell surface proteins. Myogenic, adipogenic, chondrogenic and osteogenic differentiation was demonstrated by the expression of myogenic markers, lipid vacuoles, mucopolysaccharide-rich extracellular matrix and calcium deposits, respectively (1,2).

hASCs are easy to expand in HYPER*Flask* vessels (Fig. 1) and show a fibroblast-like morphology consistent with that of hASCs cultured in T-175 flasks with vent caps (Fig. 2). Cells were cultured with the standard proliferation media (Dulbecco's modified Eagle's media – high glucose containing 10% FBS) for hASCs, and an initial seeding inoculum of 1.72 x 10^7 per HYPER*Flask* vessel. Cultures were harvested using PBS and TrypLE™ (Invitrogen™). Cells were counted by Guava® EasyCyte™ using the Guava ViaCount reagent.

After expansion using HYPER*Flask* vessels, hASCs maintained their immunophenotype and multilineage differentiation

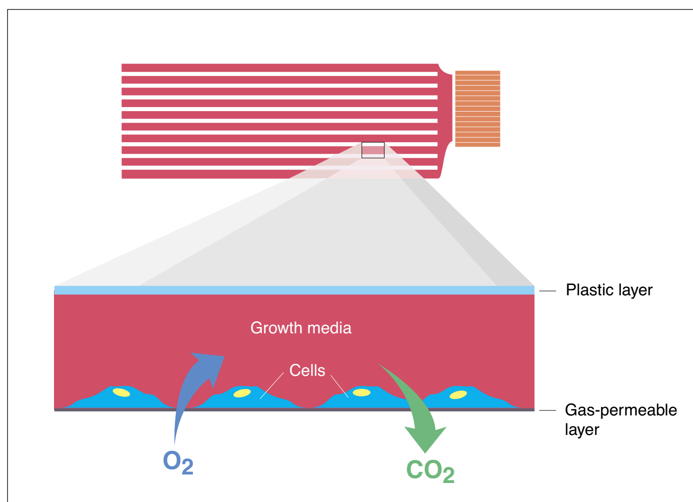

Figure 1. Corning HYPER*Flask* Cell Culture Vessel Principal of Operation. Ten equivalent layers, each containing the same surface area of a gas-permeable material, are joined together to form a multi-layered cell culture vessel.

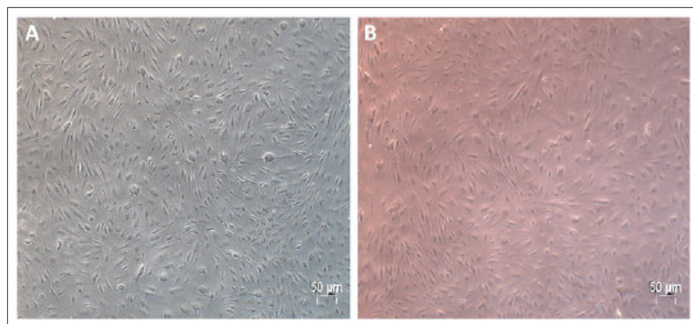

Figure 2. Typical morphology of hASCs in A) T-175 flasks and in B) HYPER*Flask* vessel, 45 minutes after seeding.

potential. hASCs did not express endothelial markers (CD31- PECAM1), nor hematopoietic markers (CD34, CD45 and CD117-c-kit). The majority of hASCs expressed high levels of CD13, CD44, adhesion markers (CD29-integrin β1, CD90-Thy-1), and mesenchymal stem cell marker CD73 (SH3). Expression of some markers, such as CD105 (SH2), was variable among the donors. hASCs were negative for HLA-class II (HLA-DR) but positive for HLA-class I (HLA-ABC) (Fig. 3).

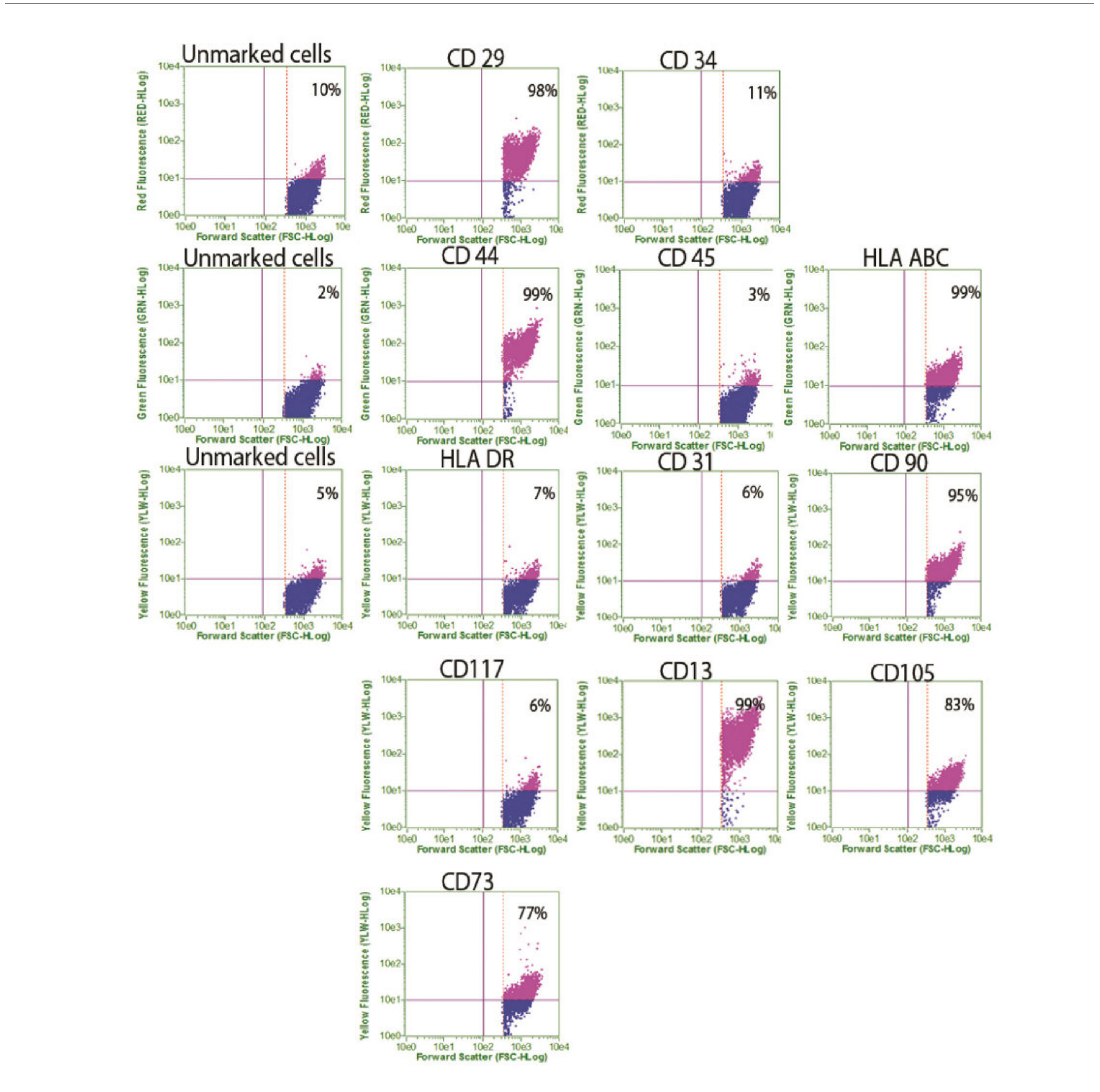

Figure 3. Immunophenotyping of hASCs cells after expansion in HYPER*Flask*® vessels. Values represent the mean percentage of all assessed cells positively stained by the indicated antigens and analyzed by flow cytometry. Graphics show forward scatter versus fluorescence intensity.

Adipogenic, chondrogenic, and osteogenic differentiation was demonstrated by the expression of lipid vacuoles, mucopolysaccharide-rich extracellular matrix and calcium deposits, respectively (Fig. 4). These results confirmed the mesenchymal nature of the expanded cells, as well as their multipotent potential.

Conclusion and Prospective Research

The results from the present study demonstrate a strategy to expand viable and undifferentiated hASCs. *Ex vivo* expansion has been attempted in several ways such as static cultures combining growth factors, or mimicking the natural microenvironment using co-culture systems. We used the standard proliferation media for hASCs and an initial seeding inoculum of 1.72×10^7 per Corning® HYPER*Flask*® vessel. After 7 days, we obtained 1.72×10^8 cells per flask. These cells may be injected or inoculated into a larger-scale vessel, such as Corning CellSTACK® chambers or a bioreactor, to be further expanded. With this approach, we were able to generate a great quantity of mesenchymal stem cells for research, as well as aiming future cellular therapeutic approaches.

References

1. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Eng. 2001; 7:211-228.
2. Vieira NM, Bueno Jr CR, Brandalise V, et al. SJL dystrophic mice express a significant amount of human muscle proteins following systemic delivery of human adipose-derived stromal cells without immunosuppression. Stem Cells. 2008; 26:2391-2398.

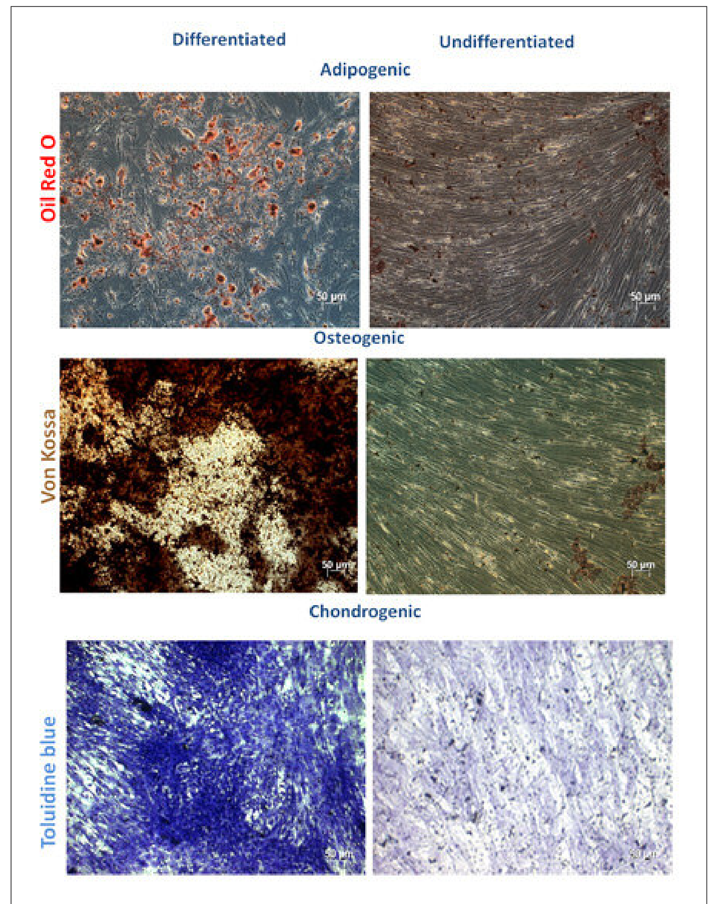

Figure 4. Differentiation potential of hASCs after expansion in HYPER*Flask* vessels.

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Corning Incorporated
Life Sciences

Tower 2, 4th Floor
900 Chelmsford St.
Lowell, MA 01851
t 800.492.1110
t 978.442.2200
f 978.442.2476

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