CHARACTERIZATION OF HUMAN ADIPOSE STEM CELLS GROWN IN OPTIMIZED NOVEL SERUM-FREE XENO-FREE MEDIA

Harpreet Sidhu, Ph.D, Dodanim Talavera-Adame, M.D. Ph.D., and Nathan Newman, M.D.*
RINATI SKIN, LLC. Head Office: 9301 Wilshire Blvd., Suite 306, Beverley Hills, CA 90210, USA.
* Corresponding Author

Adipose stem/stromal cells (ASCs) are a type of adult mesenchymal stem cells (MSCs) that can be easily isolated from adipose tissue. ASCs can be cultured *in vitro* over several passages, but conventional culture conditions involve the use of human or animal serum to enhance cell attachment and promote cell survival and proliferation. Since these cells or their conditioned media, which contains many beneficial secretory factors, are increasingly being used in regenerative medicine, it is crucial to eliminate serum supplements to ensure scalability and prevent adverse immune reactions. We have formulated a unique serum-free media (RSGM-100) that supports the growth and expansion of human ASCs *in vitro*. ASCs were isolated from lipoaspirates from human donors and cultured *in vitro* using either media supplemented with 2% human serum or in RSGM-100 media developed in our laboratory. Morphology, viability, proliferation rates, differentiation potentials, and cytokine profiles were evaluated in both groups of cells. Our RSGM-100 supported the growth and proliferation of ASCs up to passage 14 *in vitro* while maintaining their mesenchymal stem cell characteristics and stemness. These cells were successfully cultured in monolayers and three-dimensional scaffolds, and, were able to differentiate into adipocytes, chondrocytes and osteocytes. In addition, they secreted less pro-inflammatory chemokines, unlike ASCs cultured in conventional media supplemented with 2% human serum. These ASCs or their conditioned media, which exhibits biological activity, may thus be safely used for downstream cosmetic or regenerative applications without the limitations, risks, or adverse reactions that may be associated with human serum.

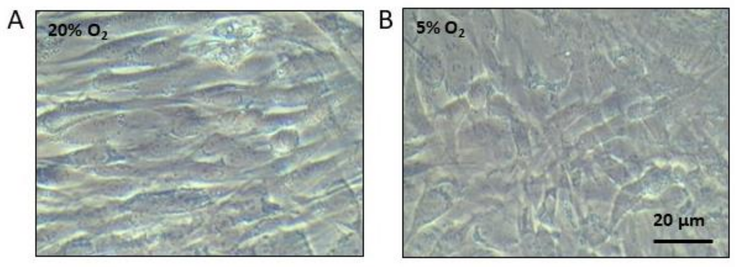

Fig. 1. ASCs cultured at different oxygen concentrations. Representative pictures of ASCs cultured in RSGM-100 under (A) normoxic conditions and (B) hypoxic conditions depicting increased proliferation at lower oxygen conditions. Images taken with a 20X objective.

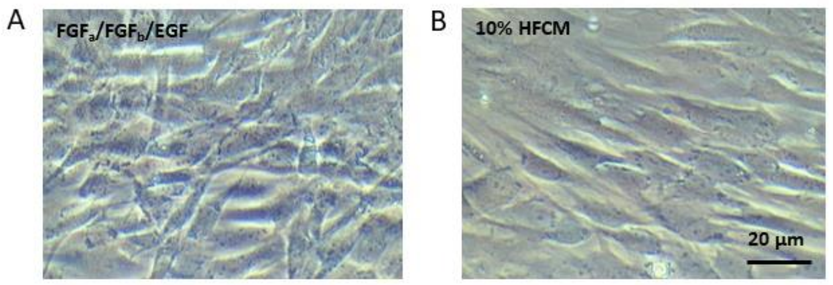

Fig. 2. ASCs cultured with different supplements. (A) ASCs cultured in RSGM-100 supplemented with 2% HS and either FGF_a, FGF_b and EGF, or, (B) 10% human fibroblast conditioned media (HFCM). Over proliferation and a loss of cell to cell inhibition is seen in picture A where ASCs can be seen to grow over other cells in contrast to ASCs cultured with 10% HFCM (B). Images taken with a 20X objective.

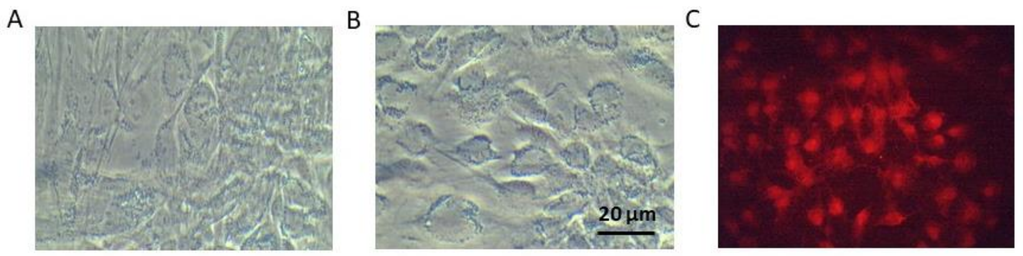

Fig. 3. ASCs cultured on fibronectin coated culture vessels survived and proliferated till passage 14. Representative images of ASCs cultured in media supplemented with 2% HS (A) or RSGM-100 supplemented with 10% HFCM (B). (C) ASCs cultured in RSGM-100 supplemented with 10% HFCM maintained the expression of CD10 through all passages (image depicted here at P10). All images were taken with a 20X objective.

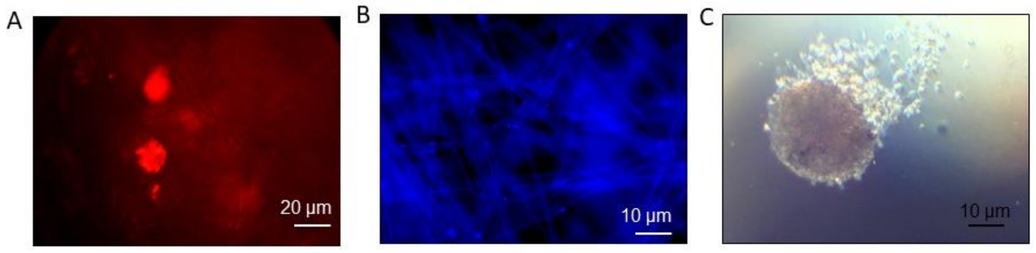

Fig. 4. ASCs in three dimensional scaffolds. (A) ASCs cultured on Fibra-cel® disks prelabeled with quantum dots. (B) Unlabeled ASCs on Fibra-cel® disks stained for DAPI (nuclei in blue). (C) ASCs grown in hanging drop cultures for embryoid body formation.

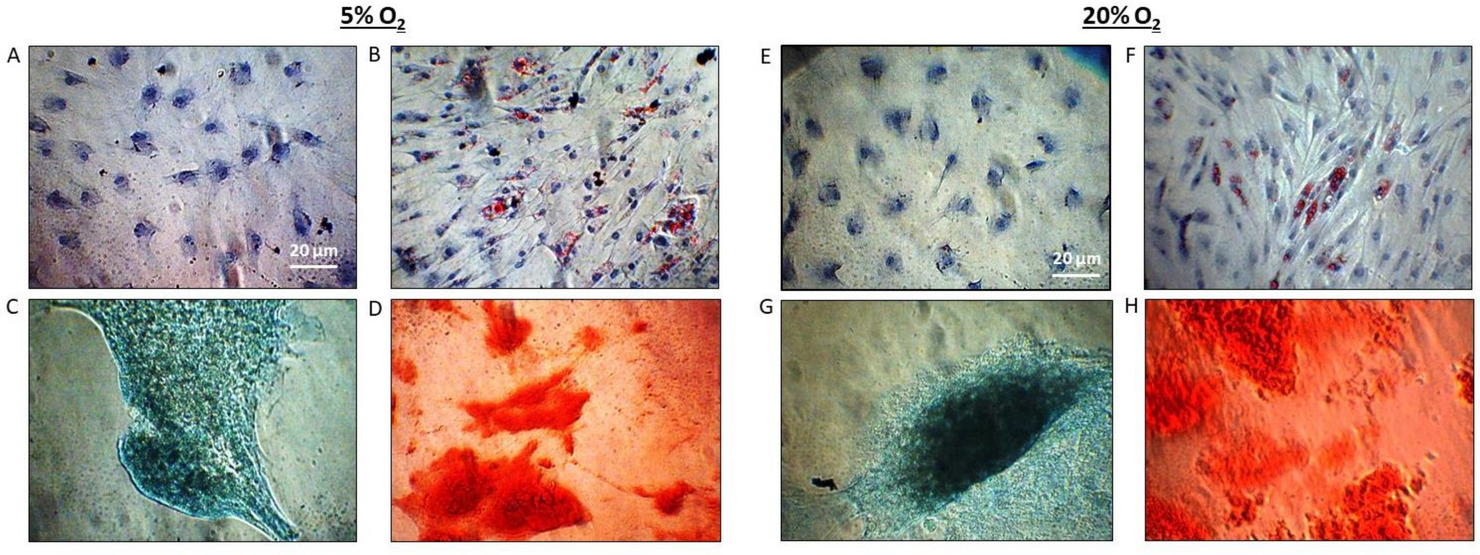

Fig. 5. ASCs cultured in RSGM-100 maintain their stemness and could be directed to differentiate into adipocytes (B, F), chondrocytes (C, G) and osteocytes (D, H) under hypoxic (left panels) or normoxic (right panels) growth conditions. (A, E) Undifferentiated ASCs stained with hematoxylin as a negative control.

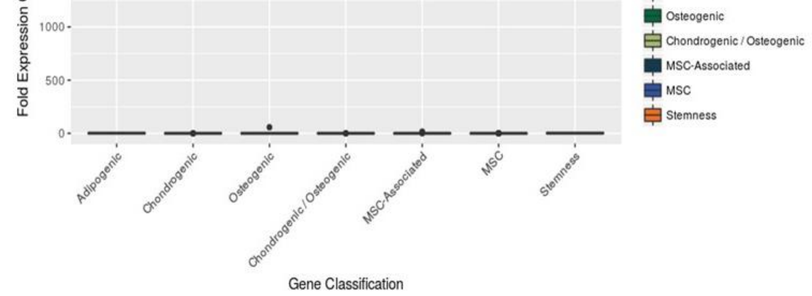

Fig. 6. ASCs cultured in RSGM-100 supplemented with 10% HFCM maintain their stemness and express mesenchymal stem cell markers when assayed using the hMSC qPCR assay (Stem Cell Technologies, Canada).

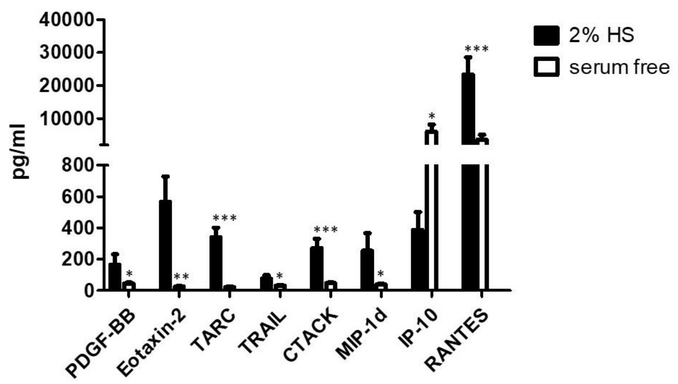

Fig. 7. ASCs cultured in RSGM-100 supplemented with 10%HFCM (white bars) exhibited a significant decrease in the pro-inflammatory cytokines PDGF-BB, eotaxin-2, TARC, TRAIL, CTACK, MIP-1d and RANTES and an increase in the secretion of IP-10 compared to ASCs cultured in media supplemented with 2% HS (black bars). All bar graphs represent mean values and error bars indicate SEM.

CONCLUSIONS

RSGM-100 is suitable for culturing human ASCs up to 10-14 passages, maintains their stemness and they secrete less pro-inflammatory chemokines unlike ASCs grown in traditional serum supplemented media.