CONSORTIA FACTORS INDUCE DIFFERENTIATION OF ADIPOSE STEM CELLS TO HAIR FOLLICLE STEM CELLS

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Human adipose stem cells (ASCs) can differentiate to lineages of mesenchymal tissues. However, recent evidence suggests that these cells can also differentiate to non-mesenchymal cell lineages.[1] Our objective is to evaluate a set of consortia factors (CFx) derived from human ASCs interactions with specific plant extracts in the *in vitro* differentiation of ASCs to hair follicle stem cells (HFSCs). ASCs were obtained from patient lipoaspirates and isolated from human donors after enzymatic treatment. These cells were expanded and treated with human recombinant differentiation factors and selective plant extracts to produce CFx, The CFx was then used to induce differentiation of ASCs to HFSCs. The CFx was analyzed by cytokine multiplex immunoassay and liquid chromatography mass spectroscopy (LCMS). The expression of cytokeratin 15 was analyzed by immunocytochemistry. The pattern and levels of secretory factors released by ASCs treated with CFx was significantly different compared to ASCs that grew in conventional hair differentiation media (controls). Higher expression of cytokeratin 15 was found in ASCs treated with CFx in comparison to controls. These results demonstrate for the first time that CFx enhance the differentiation of ASCs to HFSCs in vitro and may be benefit in hair regrowth.

METHODS

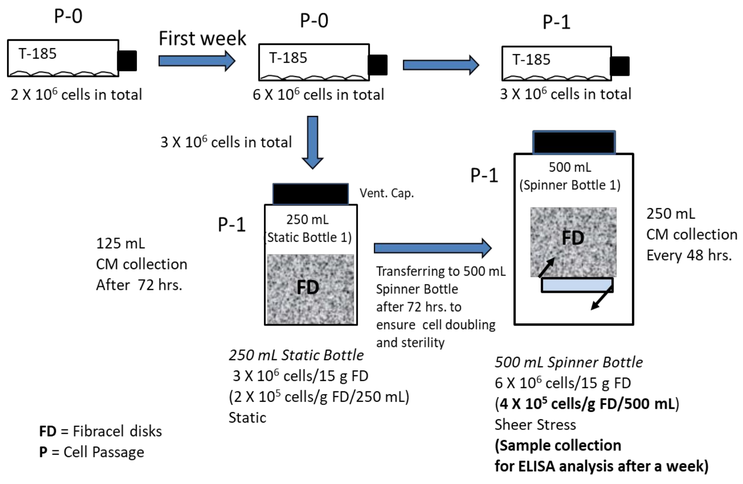

Fig. 1. Consortia Media Bioprocessing. Adipose tissue is harvested from one donor and adipose stem cells (ASCs) are isolated and transferred to tissue culture flasks for expansion (P-0). These cells were then inoculated at passage 1 (P-1) into static bottles that contained Fibracel disks. Once the cells in the static bottles were checked for sterility, they were transferred to spinner bottles. Re-inoculation of ASCs is done in these bottles for CFx scaling up.

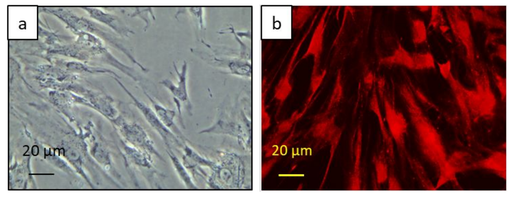

Fig. 2. Adipose Stem Cells (ASCs) growing in culture with serum-free media (SFM) supplemented with conditioned media from human fibroblasts (hFCM). (a) ASCs plated at low density in the media for 48 hrs. (b) Expression of CD10 (red) in subconfulent ASCs.

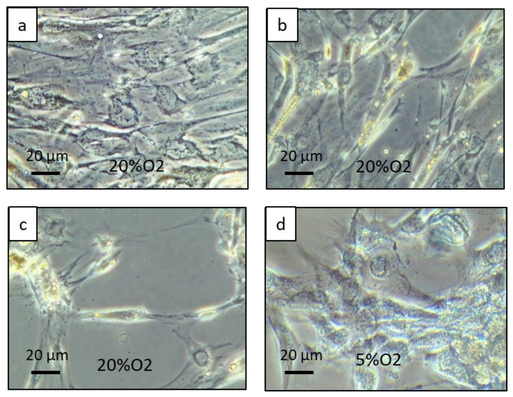

Fig. 3. Adipose stromal cells (ASCs) morphology during hair differentiation. (a) ASCs control plated at high density in regular media. (b) ASCs growing in CFx for six days in 20% O2. (c) ASCs growing in CFx for nine days in 20%O2 or (d) 5% O2.

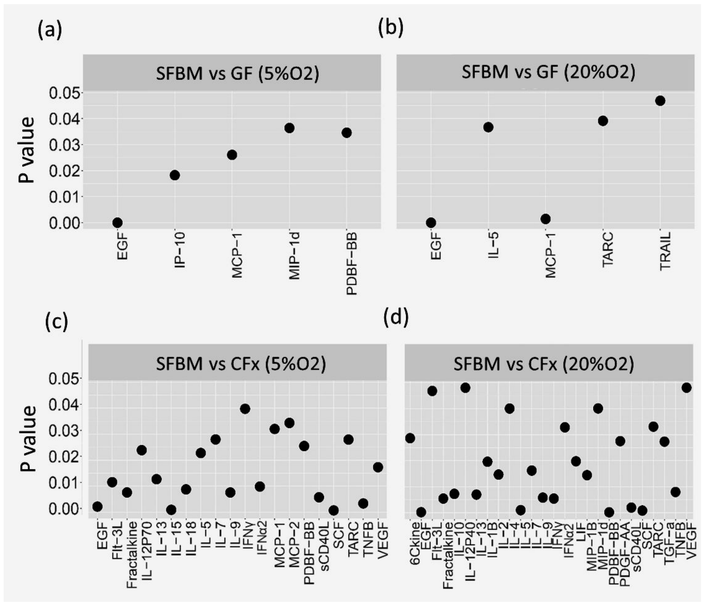

Fig. 4. Cytokine multiplex immunoassay results. Pairwise t-tests between different treatments to induce ASC differentiation to HFSCs. (a) SFBM (Serum Free Base Media) vs GF (differentiation media with recombinant growth factors) at 5% O2, (b) SFBM vs GF at 20% O2, (c) SFBM vs CFx (Consortia Factors) at 5% O2 and (d) SFBM vs CFx at 20% O2. The plots show the factors that are significantly different ($p<0.05$) between the treatments.

RESULTS

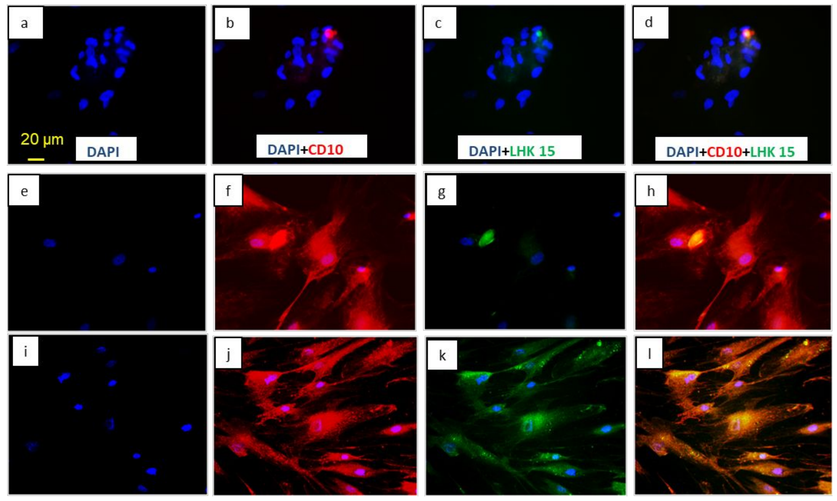

Fig. 5. Expression of HFSC markers in ASCs treated with CFx. (a – d) ASCs used as negative control for staining to (a) DAPI (blue), (b) CD10(red), (c) Cytokeratin 15 (LHK 15 - green), and (d) merged images. (e – h) ASCs growing in regular media and stained for (e) DAPI, (f) CD10, (g) Cytokeratin 15, and (d) merged images. (i – l) ASCs were treated with CFx and stained for (i) DAPI, (j) CD10, (k) Cytokeratin 15, and (l) merged images.

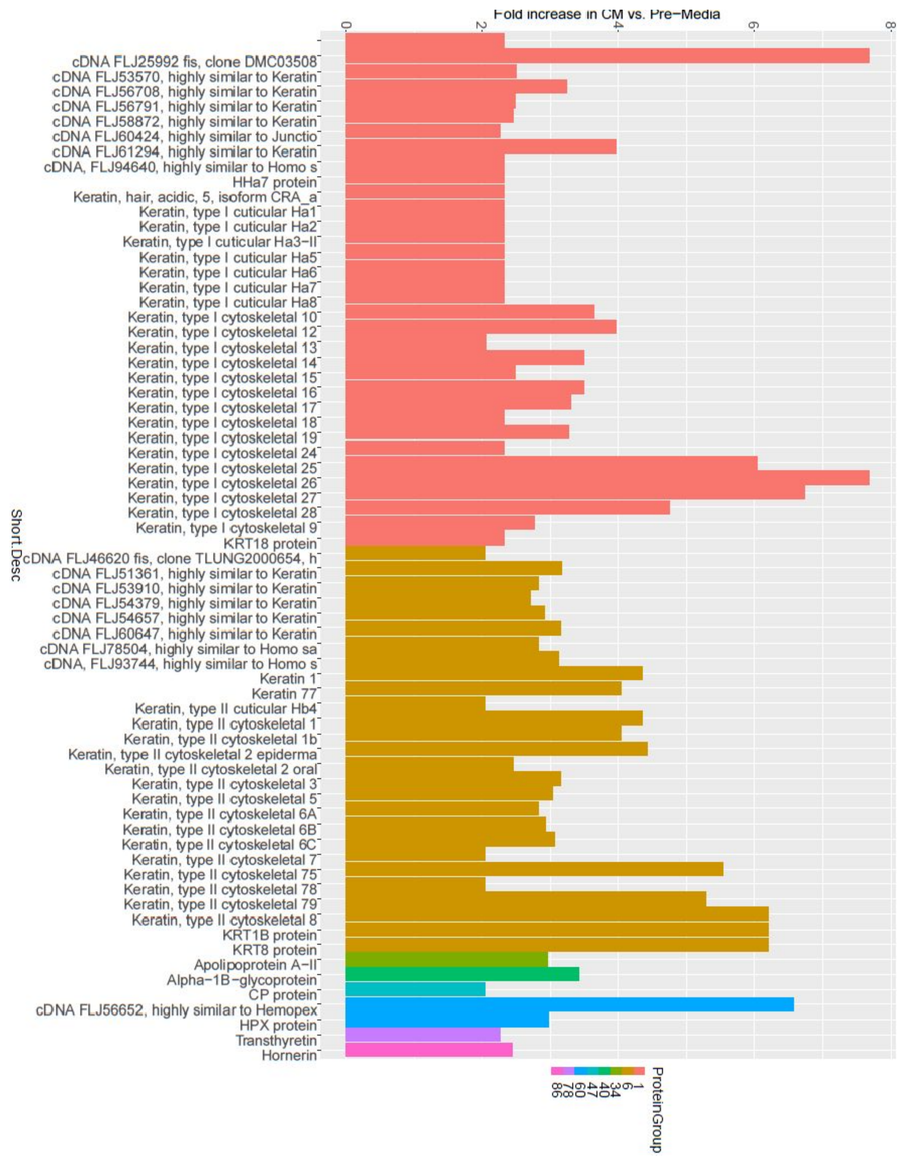

Fig. 6. LCMS analysis of consortia media

CONCLUSIONS

We characterized the consortia media with factors (CFx) and found upregulation of about 24 factors in comparison to those secreted by induction of growth factors. Together with the increase in LHK15 expression, these results suggest that CFx enhance the secretion of more factors associated with ASC differentiation to HFSCs.

[1] Talavera-Adame D, Newman D, Newman N. Conventional and novel stem cell based therapies for androgenic alopecia. Stem Cells Cloning. 2017 Aug 31;10:11-19. doi: 10.2147/SCCAA.S138150. eCollection 2017.