Clinical Scale Production and Wound Healing Activity of Human Adipose Derived Mesenchymal Stem Cell Extracellular Vesicles from a Hollow Fiber Bioreactor

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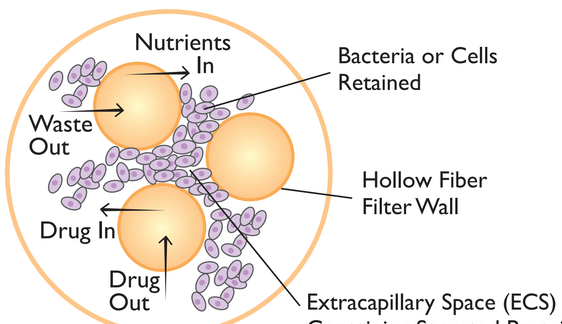
INTRODUCTION

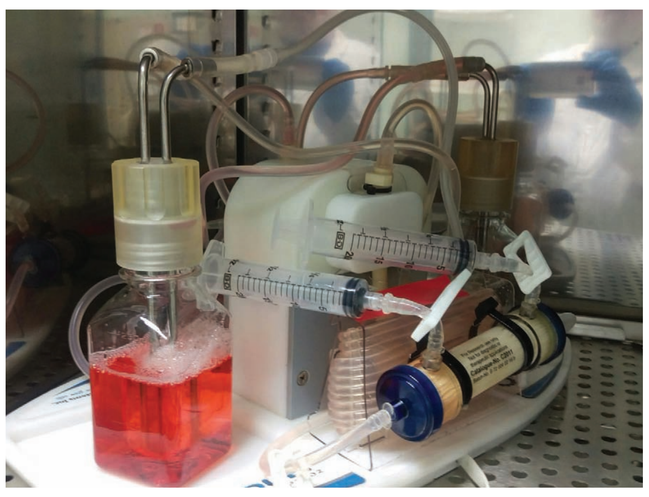
Production of extracellular vesicles (EV) such as exosomes at the scale required for clinical applications remains a challenge. Current methods can utilize large numbers of flasks and may require serum starvation in a batch mode process. EV are secreted in small quantities at low concentrations and a standard preparation can entail a final stage of 200 T225 flasks or more. This method is wasteful, time and space consuming and the cells are growing in a non-physiologic environment. Hollow fiber bioreactors (HFBR) are ideal for producing large quantities of EV at 100x higher concentrations than conventional methods. HFBRs support the culture of large numbers of cells (1-10x10^9 cells) at high densities (1-2x10^8 cells/mL) under more physiologic conditions and do not require serum starvation for EV collection. Cultures can be maintained for several months with continuous collection of EV.



Cross section of a hollow fiber cartridge. Cells are retained in the small volume outside the fiber while media circulates on the inside of the fiber. Small molecules such as drugs can freely cross the fiber along with nutrients and waste products, while bacteria, exosomes, and cells cannot cross the fiber.

METHODS

1x10^9 human adipose derived adult MSC were cultured in a FiberCell Systems HFBR for 8 weeks using DMEM/10% FBS in the circulating medium only. 40 mL of supernatant were harvested weekly from the extracapillary space (ECS) of the cartridge. To isolate EV, the collected serum-free conditioned medium was subjected to two steps of centrifugation 1) 3000 g, 20 mins to remove cell debris 2) 100,000 g to pellet exosomes. Size distribution and concentration of EVs were quantitated by tunable resistive pulse sensing (TRPS; qNano, IZON Sciences) At the end of 8 weeks the cartridge was cut open and cells recovered for phenotypic analysis. This demonstrated no change in cell phenotype over the period of culture. A flow cytometry dye transfer assay was used to demonstrate the ability of EV to deliver cargo to endothelial cells. A scratch closure test and rat skin wound healing assay was used to measure wound healing activity.

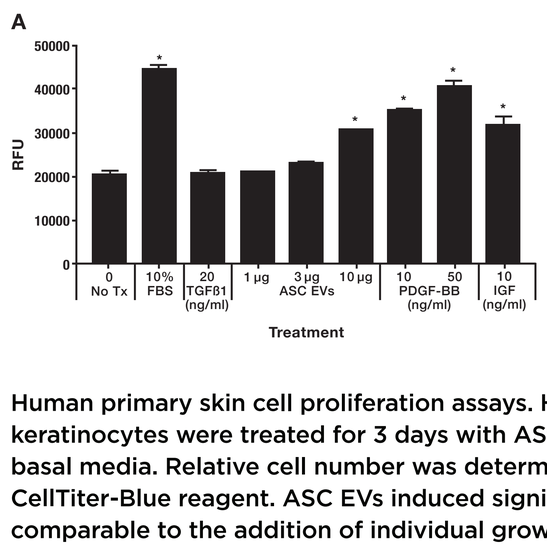


RESULTS

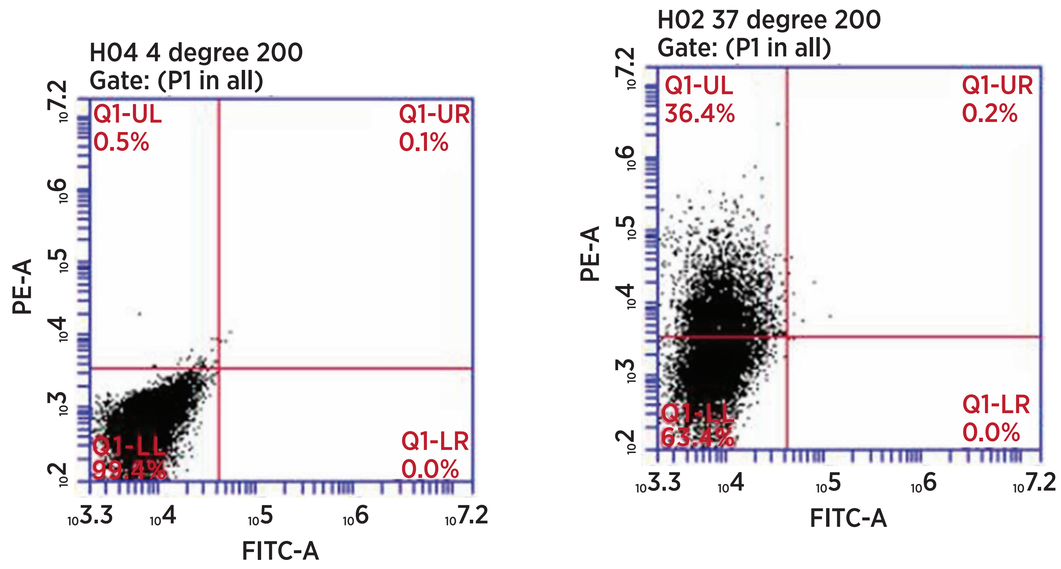
Cell culture production of EVs in flasks vs. HFBR production.

	Volume	Total Number Evs
130 T225 flasks	4000 mL	1.6x10^{10}
C2011 HFBR	120 mL	86.0x10^{10}

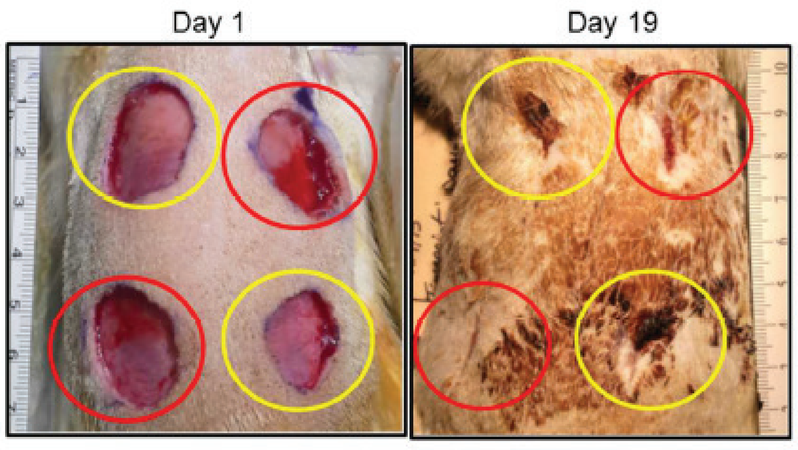
Further characterization of the EV is required but the HFBR produced an equivalent number of EV as nearly 7,000 T225 flasks in a much smaller volume.



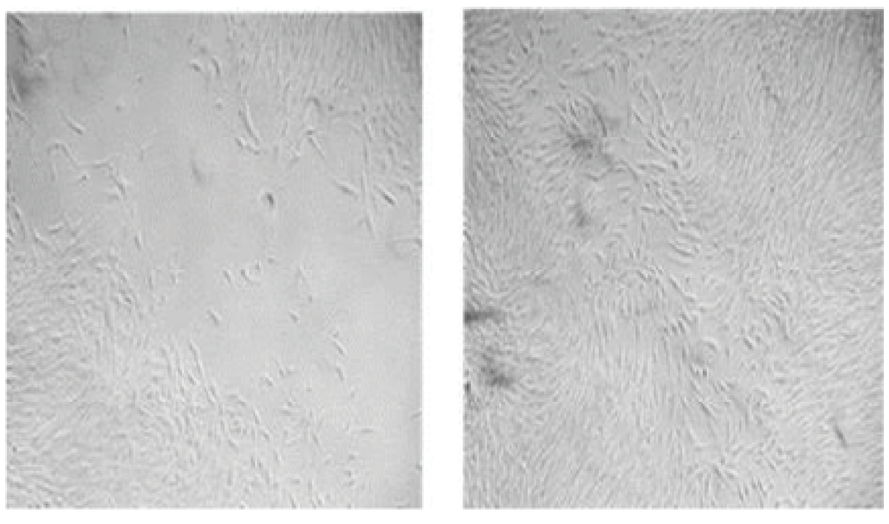
Human primary skin cell proliferation assays. Human primary (A) dermal fibroblasts and (B) keratinocytes were treated for 3 days with ASC EVs or growth factors in their respective basal media. Relative cell number was determined by the fluorescence signal generated by CellTiter-Blue reagent. ASC EVs induced significant ($p<.05$) proliferation in both cell types, comparable to the addition of individual growth factors. No Tx is no treatment.



Incorporation of exosomes into human endothelial cells. Lipophilic dye transferred from exosomes and incorporated into cultured cells has been used to support the function of exosomes to deliver cargo into cells As shown by the shift in the population in the right panel, the cells incorporated dye from the labeled exosomes, supporting the notion that these exosome preparations from the bioreactor are capable of delivering their cargo (i.e., nucleic acid, protein) into the cell.



Wound healing in response to topical application of EVs. Left panel shows fresh 2 cm diameter wounds in the back of the rat. Right panel shows degree of wound healing after 19 days. Yellow circles mark vesicle control treatment. Red circles mark EV treated wounds. All treatments were single application. Pictures are illustrative of 9 different animals.



Scratch closure test. Left panel (negative control) shows the gap in the monolayer 3 days after scraping and culturing in basal media. Right panel (EV-treated culture) shows gap closed after 3 days due to fibroblast migration.

CONCLUSION

HFBRs have demonstrated the potential for manufacturing scale production of EV using cGMP compliant materials and methods. The EV isolated from adult adipose derived MSC cultured in an HFBR show activity that can promote wound healing both in *in vitro* and *in vivo* assays. HFBRs provide a number of significant advantages compared to flask based protocols including higher concentrations, larger capacity, time and space efficiency along with significantly less plastic waste generated. Currently available hollow fiber bioreactors permits the production of gram quantities of EV, with potential use for clinical applications.

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