Large Scale Production of Extracellular Vesicles in a Hollow Fiber Bioreactor System

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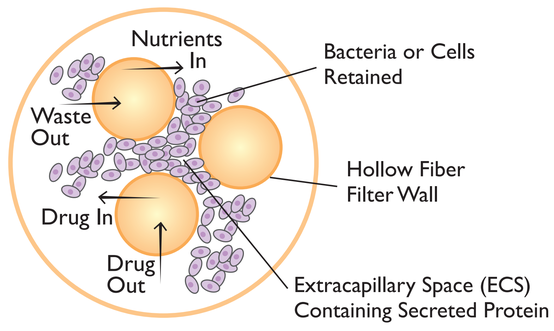
INTRODUCTION

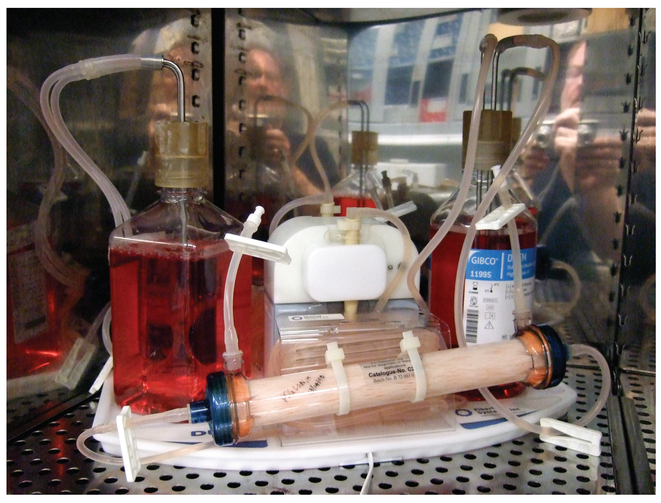
Production of extracellular vesicles (EV) such as exosomes at the scale required for clinical applications remains a challenge. Current methods can utilize large numbers of flasks and serum starvation in a batch mode process. Hollow fiber bioreactors (HFBR) are ideal for producing large quantities of EV at 100X higher concentrations than conventional protocols. HFBRs support the culture of large numbers of cells at high densities, 1-2X10^8 cells/mL. Cells are bound to a porous support with a 20kDa molecular weight cut off (MWCO) so cell passaging is not required and EV cannot cross the fiber in either direction.

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

5X10^8 adipose derived adult MSC were cultured in a FiberCell HFBR for 8 weeks in DMEM+10% FBS in the circulating medium only. 40 mL of conditioned medium from the extra-capillary space was harvested weekly. An HEK293 culture expressing heterodimeric interleukin-15 was similarly maintained in hollow fiber culture for over 4 months, with 3 harvests of 20 mL per week.



RESULTS

Comparison of Exosomes From Human Adipose Derived MSC Produced in T225 flasks vs. FiberCell Systems C2011 20 mL 20 kd MWCO PS Cartridge

Flasks were split 1:5 in DMEM/10% FBS until a total of 130 T225 flasks were obtained. DMEM/10% FBS was replaced with DMEM alone and exosomes collected after 2 days. 5X10^8 adipose derived adult MSC were cultured in a FiberCell HFBR for 8 weeks in DMEM+10% FBS in the circulating medium only. Cells did not expand (monitored by glucose uptake rate) nor did they differentiate (by multiple immunocytochemistry assays) over this time. Contaminating exosomes in serum cannot cross the fiber allowing it's use in culture. Total exosomes collected from two HFBRs under these conditions equaled that from 3600 T225 flasks in a total volume of 360 mL.

ADULT ADIPOSE DERIVED MSC

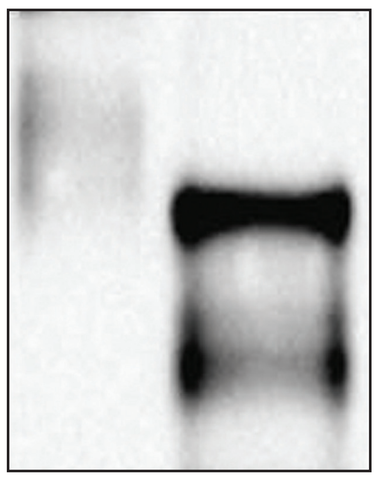
	Collection Volume (mL)	Total Exosome Protein (mg)	Total Exosome Particles (10^{10})
HFBR Cartridge #1 (7 weeks, collection every week)	240	11.82	95.78
Cartridge #2 (4 weeks, 6 collections)	120	14.45	326.9
Flasks 130 T225	4000	0.9	1.6

Total medium consumed: HFB: 7 L per run. Flasks: 24 L

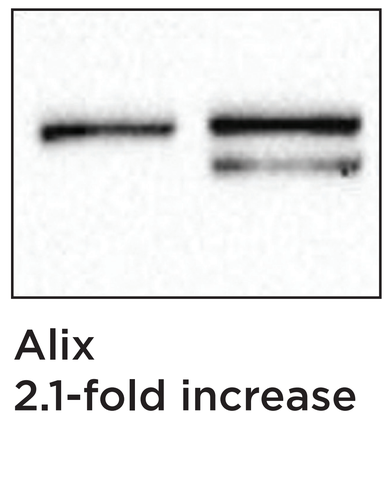
Yield from 293T cells

An HEK293 culture expressing heterodimeric interleukin-15 was similarly maintained in hollow fiber culture for over 4 months, with 3 harvests of 20 mL per week.

The HEK293 bioreactor culture yielded approximately 1X10^{12} EV/mL compared to about 1X10^9 EV/mL in flask culture. CD63 and Alix were greatly enriched from the bioreactor compared to flask culture. Additionally, EV/protein was 10-fold higher in harvests from the bioreactor suggesting higher purity as well. Purified HEK293 cell EV retained their IL-15 bioactivity.

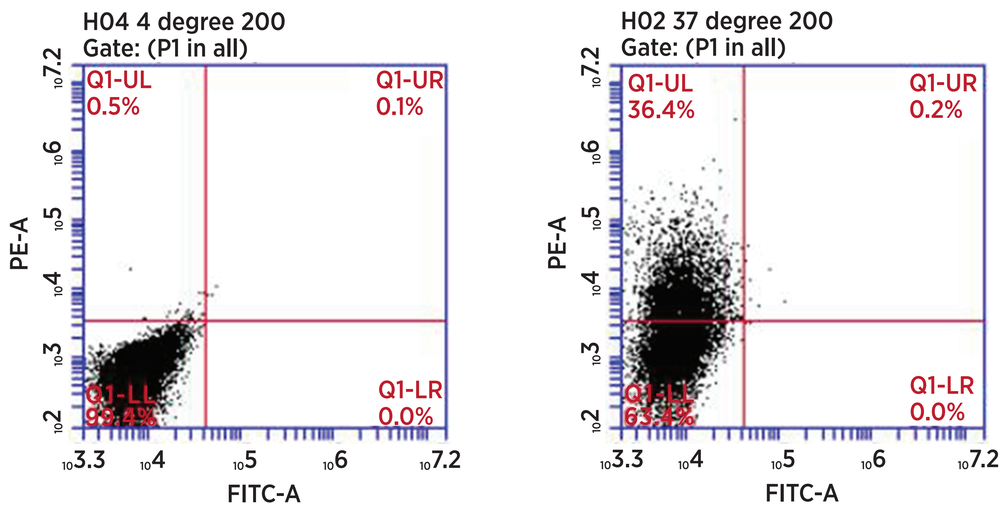


CD63
7.6-fold increase



Alix
2.1-fold increase

Flask Culture **2.7+/- 0.4 ug/mL**
HFBR **55+/-2 ug/mL**



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 DiI 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.

CONCLUSION

HFBRs have demonstrated potential for the manufacturing scale production of EV using cGMP compliant materials and methods. HFBRs provide a number of significant advantages compared to flask based protocols including higher concentrations, large capacity, time and space efficiency, and perhaps EV quality. Current available technology permits the production of gram quantities of EV, with potential use for clinical applications.

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