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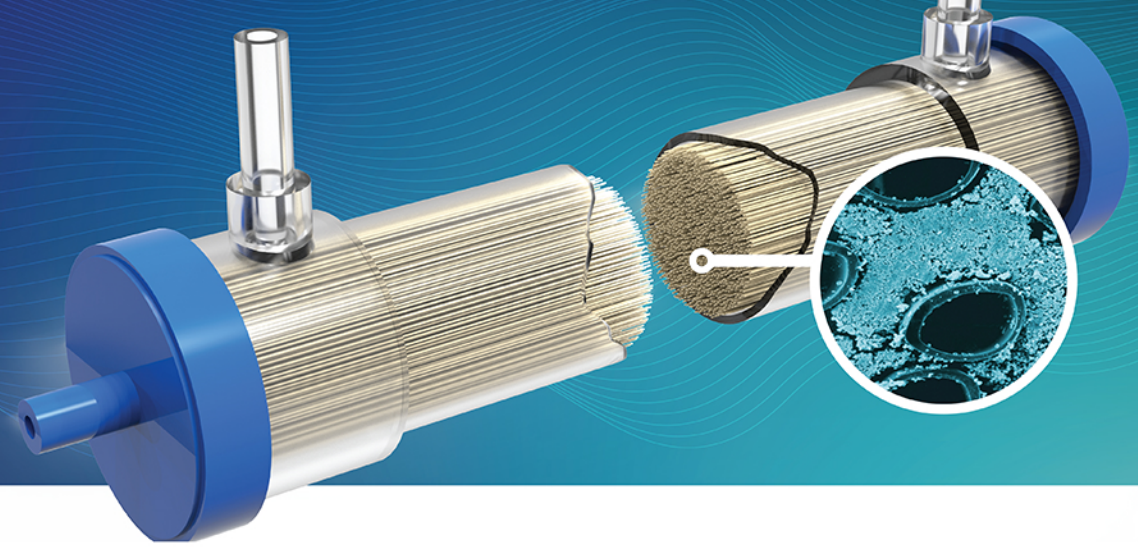

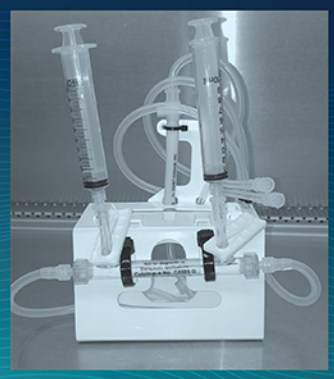

Retroviral Transduction and Production of Palm-GRET Labelled Extracellular Vesicles using Bone Marrow Derived MSC in a Hollow Fiber Bioreactor.

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Introduction

Various methods have been applied to the transfer of genes into mesenchymal stem cells (MSC). The generation of stable MSC transfectants is hampered by the limited number of passages MSC can undergo before they start to differentiate and difficulty in performing at clinical scale. Current data suggests that mesenchymal stem cells, when seeded into a 3-D hollow fiber bioreactor (HFBR) show little proliferation and may be maintained in culture and produce EVs continuously for extended periods of time at high concentrations. Transient transfection under these conditions could result in usefully stable MSC transfectants. To this end a retroviral transfection of bone marrow MSC using an HFBR was performed.

Methods

Bone marrow MSC from ATCC were expanded to $5X10^7$ cells using DMEM/10% FBS and 10 T300 flasks. Retrovirus encoding for green fluorescent protein and Nanoluc luciferase protein was produced in culture. A FiberCell Systems C2025D 20 kD MWCO polysufone cartridge with 450 cm^2 of area and a 2.8 mL volume was seeded as follows. $5X10^7$ cells in a volume of 5 mL was attached to one side-port and $4.5X10^9$ retrovirus in a volume of 4 mL was attached to the opposite side port. Equal volume of cell/virus mixture was flushed into each syringe and then injected into the ECS of the cartridge with the excess volume flowing through the fibers into the medium reservoir, concentrating the cells and the virus together. After 24 hours the ECS was drained, and the medium replaced with basal DMEM (no serum). Harvests were performed at 24-hour intervals for four weeks.

Results

1. Isolated EVs from the first 16 days of harvest collection show strong Nluc signal and approximately 5-12% of all detected particles in those EV samples were GFP positive, indicating release of the GFP-Nanoluc fusion reporter proteins via EVs by MSC transfectants.
2. The particle counts of EVs produced by palmGRET MSC declined from $1.4X10^{10}$ particles/mL in the first harvest to $3.9X10^6$ particles/mL measured in the last harvest (day 27).

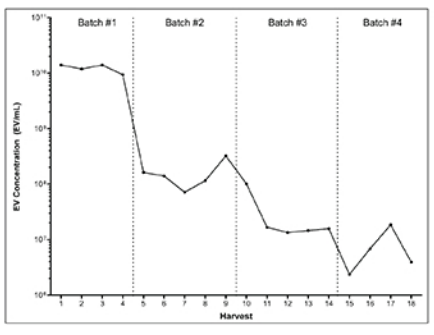
EV production was drastically reduced after 2 weeks of MSC transduction in HFBR.

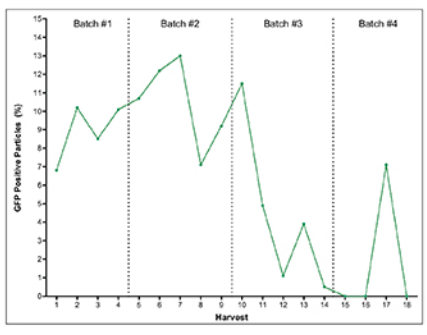
3. It was not really possible to directly determine transduction efficiency under these conditions.

Discussion

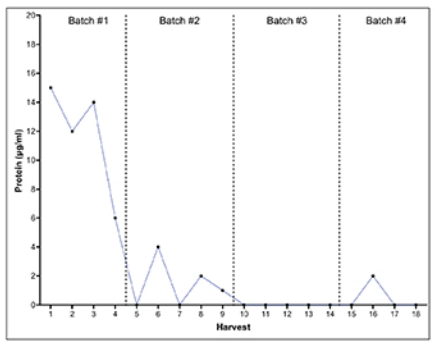
A hollow fiber bioreactor can reduce the volume required to perform transductions by 100X, and utilizes a closed, cGMP compatible format. Overall, these promising preliminary data warrant further optimization and refinement of the transduction protocol, particularly by modifying the viral titer, selection strategy, and length of the experiment. Cell viability assays will also be performed to determine whether EV concentration declined after 14 days as a result of decreased cell viability or if other factors are at play. Increasing scale by a factor of 100x or more is possible using existing hollow fiber systems.

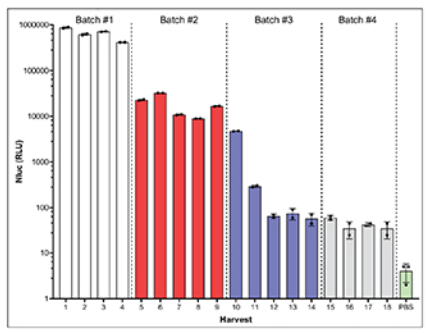
Characterization of MSC EVs





Harvest	EV Concentration (EV/mL)	GFP + (%)	GFP Count
1	1.40E+10	6.8	287
2	1.19E+10	10.2	1028
3	1.40E+10	8.5	966
4	9.37E+09	10.1	730
5	1.62E+08	10.7	275
6	1.40E+08	12.2	278
7	7.12E+07	13	129
8	1.15E+08	7.1	119
9	3.21E+08	9.2	460
10	1.01E+08	11.5	183
11	1.66E+07	4.9	13
12	1.34E+07	1.1	3
13	1.45E+07	3.9	6
14	1.57E+07	0.5	1
15	2.35E+06	0	0
16	6.79E+06	0	0
17	1.84E+07	7.1	2
18	3.93E+06	0	0



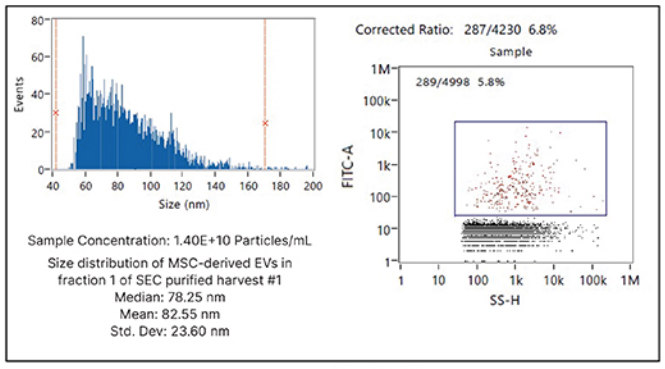


Fraction 1

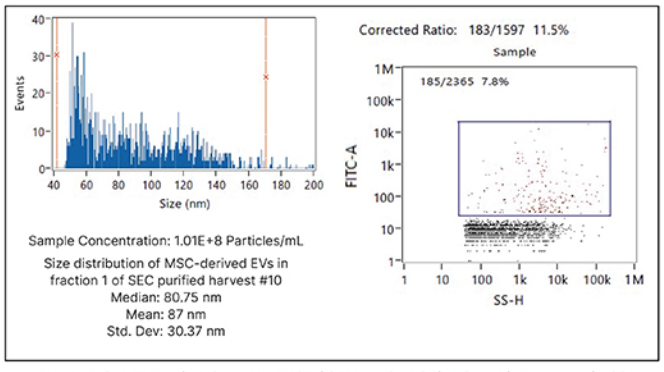
Batch	Nluc Average	Nluc fold-change
1	655,181 ± 190,341	
2	18,258 ± 9,522	36
3	1,038 ± 2, 058	631
4	39 ± 12	16,675

- Nanoluciferase activity measured in MSC EVs significantly decreased over time
- The particle count of EVs produced by palmGRET MSCs declined from 1.4E+10 particles/mL in the first harvest to 3.9E+06 particles/mL measured in the last harvest (day 27).

Harvest #1 (02.08.2022)
EVs (Fraction 1)



Harvest #1 (02.08.2022)
EVs (Fraction 1)



Here we determined the # of particles and the % of GFP+ particles in fraction 1 of harvest #1 of MSCs using NanoFCM