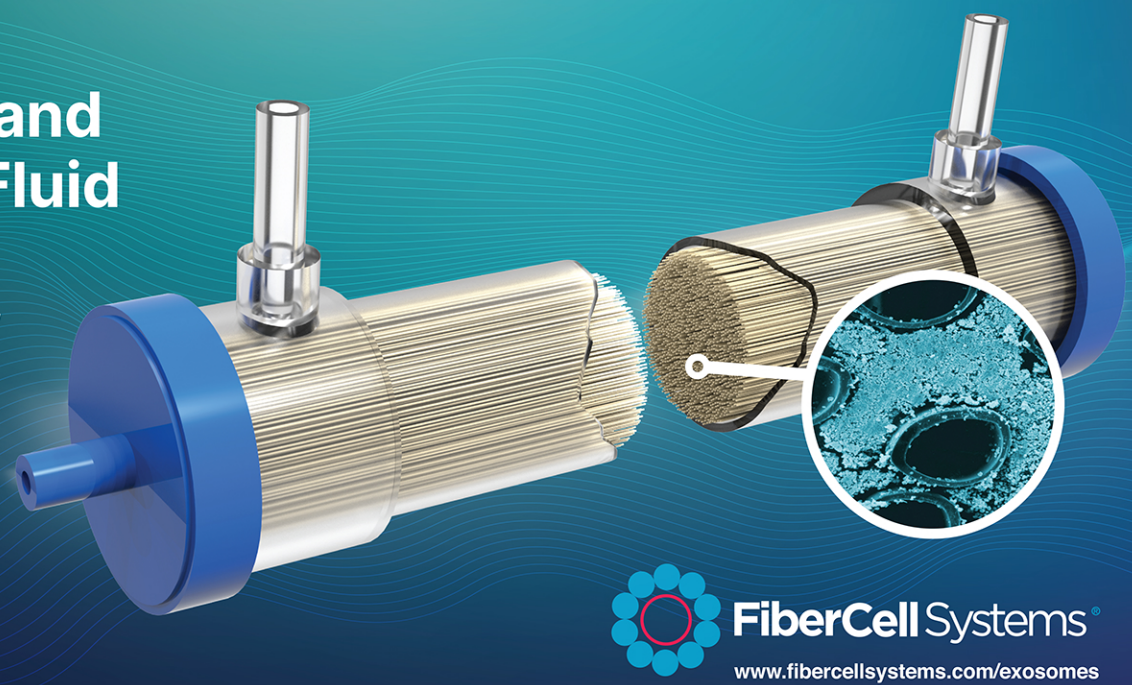
Scalability of Production and Bio-Activity of Amniotic Fluid Stem Cell Extracellular Vesicles from 3-D Hollow Fiber Bioreactor and 2-D Culture.



C Dedhia (1), P Neviani (1), JJS Cadwell (2), S Dostalik (2), S Sedrakyan (1), L Perin (1)
1) USC-Children's Hospital Los Angeles, USA
2) FiberCell Systems, Frederick, Md. USA

Introduction

EV clinical translation is constrained by limitations in scale-up of EVs production. Hollow fiber bioreactors (HFBR) support the culture of large numbers of cells, at high densities, producing significant numbers of EVs at high concentration. The high cell densities present in a HFBR can facilitate the use of xeno-free/chemically defined mediums, such as CDM-HD. Here we compare production, potency, identity and therapeutic potential of EVs collected from cells grown in culture dishes (2-D) vs. a HFBR (3-D).

Methods

Human clonal Amniotic Fluid Stem Cells, hAFSC, were derived from consented donor's amniotic fluid. 1×10^6 hAFSC were seeded in 2-D petri dishes (145 cm^2), and $9.2X10^8$ hAFSC were seeded into a 20 kD MWCO HFBR (FiberCell Systems C2011, 20 kD, 4,000 cm^2) with fibronectin coating; both cultured in Chang's medium with 20% FBS. At confluence in the petri dish the medium was replaced with basal medium, starved for 48 hr and EVs collected. After three days the medium in the ECS of the HFBR was replaced with Chang's medium alone, without 20% FBS, complete Chang's with 20% FBS remained in the central reservoir. The ECS was flushed with basal Chang's over the next 3 days and then harvesting of EVs every day was initiate. After two weeks of production serum in the reservoir was reduced stepwise to 5% and 5% CDM-HD introduced. After one more week serum was completely removed and replaced with 10% CDM-HD. The final weeks of EV production were produced using chemically defined medium, CDM-HD alone. Glucose consumption was monitored on a daily basis. 2-D EVs and 3-D EVs were compared by Nanosight, potency assay and by WBand therapeutic effect (*in vivo* injections in an animal model of chronic kidney disease, Alport Syndrome).

Results

Control: 2-D EVs, Volume: 40mL, $3.07X10^9$ EV/mL, Total EV: $2X10^{11}$

Figure 1: Daily Glucose Consumption - C2011 Hollow Fiber Bioreactor Culturing historical hAFSC

Day 17: 15% FBS
Day 19: 10% FBS
Day 21: 7.5% FBS, 2.5% CDM-HD
Day 22: 5% FBS, 5% CDM-HD
Day 26: 5% FBS, 10% CDM-HD
Day 34: 10% CDM-HD

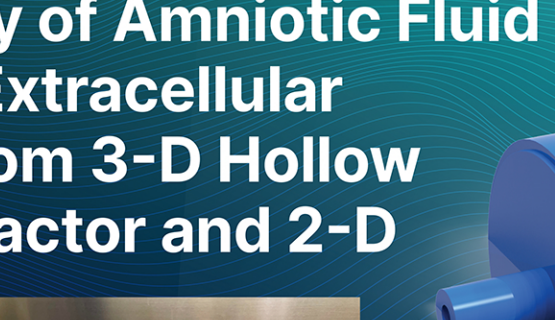


Figure 2: Daily EV Production - C2011 Hollow Fiber Bioreactor Culturing historical hAFSC

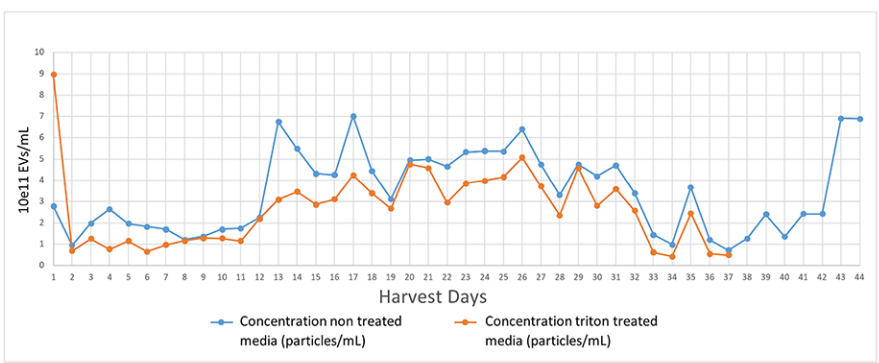
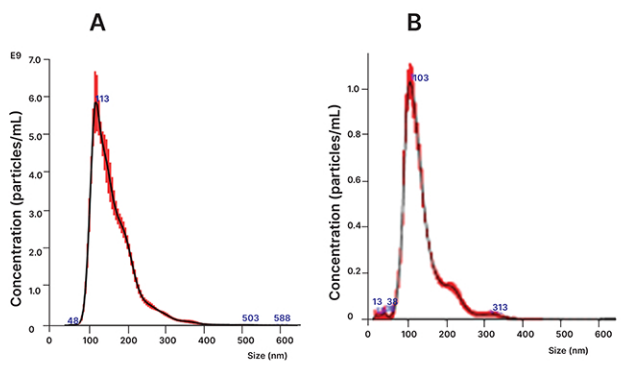


Figure 3 - hAFSC-EV size. Nanosight analysis hEVs derived from 2-D (A) and 3-D (B) average size is 113 nm mode.



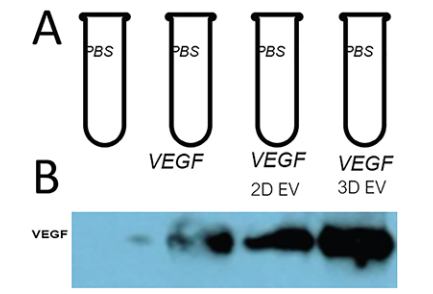


Figure 4: VEGF/VEGFR1 Co-immunoprecipitation. Immunoblot of VEGF (24 KDa, monomer), after co-IP with VEGFR1 in PBS+VEGF (100ng/mL) exposed-EVs from 2-D EVs (A) and 3-D EVs (B). Both EVs trap VEGF in a similar way. The weak band detected in the PBS/VEGF (second band) represents a VEGF carryover due to incomplete removal of VEGF during washing.

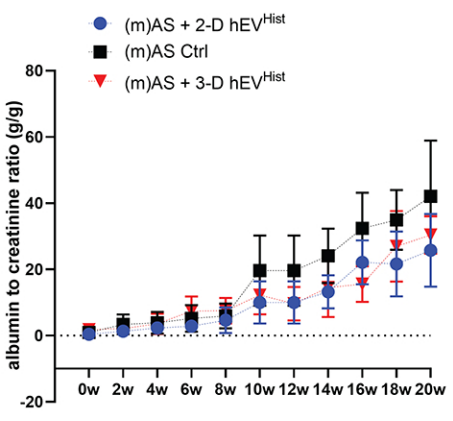
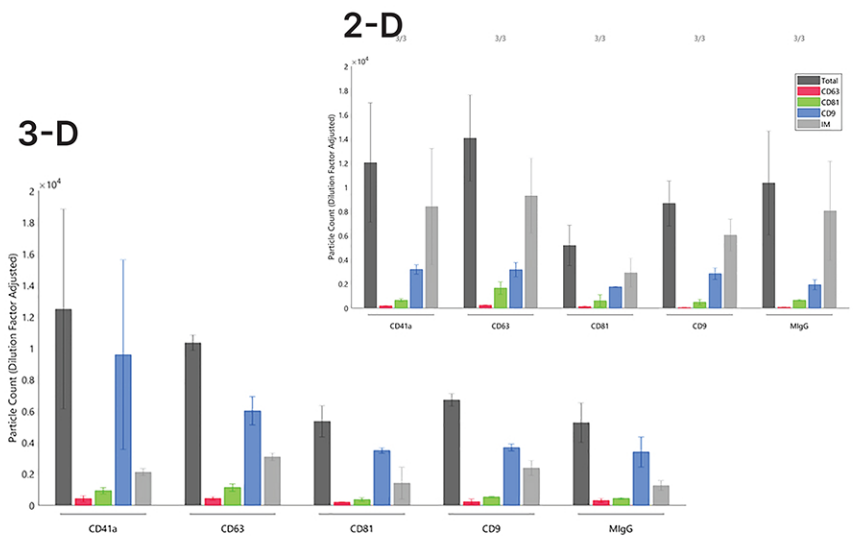


Figure 5: EVs ameliorate renal dysfunction. 2-D EVs and 3-D EVs, reduce proteinuria (measured by ELISA) in treated mice vs untreated mice. AS injected with 3-D: n=6 mice/time point; AS injected with 2-D EVs: n=15 mice/time point; AS non-injected n=16 mice/time point. Mice were 10wks old (week 0) at injection and urine collected every 2wks. WT and non-injected control mice were strain- and age-matched. *p<0.05**; p< 0.01; Mice are still in the study but the protective role of hEVs is evident after injection.

Fig 6: 3-D hEVs present similar tetraspanin profile of 2-D hEVs as evaluated by Exoview



Discussion and Conclusion

3-D EVs had comparable properties and bio-activity relative to 2-D EVs, but the HFBR produced 100x more concentrated EVs per mL. Each daily harvest produced more than $1X10^{13}$ EVs, $1X10^{14}$ would be an estimated human dose. The adaptation of these cells to a chemically defined medium and the demonstrated production range represents a significant step towards enabling therapeutic applications of hAFSC for treating kidney diseases in humans. The C2018 hollow fiber bioreactor module represents an additional 5x scale-up from the data presented here. The HFBR is a closed system that can be cGMP compliant. In conclusion, the HFBR can produce sufficient numbers of EV to support pre-clinical and clinical applications of EVs with at least similar properties to EVs produced by conventional 2-D methods.