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Comparison of Production and Activity of Amniotic Fluid Stem Cell (AFSC) Extracellular Vesicles from 3-D Hollow Fiber Bioreactor and 2-D culture.

Introduction

EV clinical translation is limited by scale-up of EVs production. Hollow fiber bioreactors (HFBR) support culture of large numbers of cells at high densities. Culture conditions may affect EV composition and potency. Here we compare production, potency, identity and therapeutic potential of EVs collected from cells grown in culture dish (2-D) versus the 3-D hollow fiber bioreactor HFBR.

Methods

Human clonal AFSC were derived from patient-consented amniotic fluids. 1 x 10⁶ hAFSC were seeded in 2-D (145 cm²), and 1.6 x 10⁷ hAFSC on a small 20 kD MWCO HFBR (FiberCell-C2025D, 20 kD MWCO, 450 cm²) with fibronectin coating; both cultured in Chang medium with 20% of ES-FBS. Petri dishes starved for 24 hr and then EVs collected, in the HFBR serum was removed from the extra-capillary space on Day 5 while serum remained in the circulating medium. The effect of harvest frequency was tested (1 hrs, 24 hr). 2D-EVs and 3D-EVs were compared by Nanosight, potency assay, identity (by Exoview analysis) and therapeutic effect (*in vivo* in an animal model of kidney disease, Alport Syndrome)

Results

2-D production was 2.8 x 10¹⁰ total EV, volume of 20 mL, while 3-D HFBR was ~2.8 x 10¹⁰ EV/mL (first four 24 hr harvests,) and ~4.4 x 10¹⁰ EV/mL (two days of hourly harvests). Harvest volume from the bioreactor was 2 mL per harvest. A total of 2.2 x 10¹² EVs in 50 mL was collected from the two days of hourly harvests. The HFBR produced as many EVs as 25 petri dishes, every day. 2-D and 3-D EVs had similar size distribution (~130 nm). There was little difference in EV concentration from different HFBR harvest intervals; possibly indicating either significant EV re-uptake or inhibition of EV secretion dependent upon free EV in the supernatant. 3-D EVs trapped VEGF (an *in vitro* established potency assay) as efficiently as 2-D EVs and expressed high level of CD63 relative to 2-D EVs evaluated using the Exoview technology. 3-D and 2-D EVs demonstrated similar amelioration of proteinuria when injected into Alport mice.

Conclusion

In conclusion, 3-D EVs and 2-D EVs had comparable properties including size and potency using an established VEGF potency test as well as disease-modifying activity *in vivo* in a model of chronic kidney injury. EVs collected from HFBR presented a slight modification in tetraspanin compared to the 2-D culture; this could be due to lack of cell passage in hollow fiber, 3-D culture conditions, or the effect of serum proteins on the cells in the hollow fiber bioreactor. Independently from the tetraspanin expression difference, both EVs showed potency *in vitro* and bioactivity *in vivo*. HFBR cell culture conditions for hAFSC still need optimization, however an available 1.2 m² cartridge provides a 30X scale up in a closed, cGMP compatible system. 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. It should be noted that this represents the first time hAFSC have been cultured in a HFBR and many conditions remain to be optimized.

Amniotic stem cell EVs produced in a 3-D hollow fiber bioreactor have enhanced bioactivity when compared to 2-D petri dish EVs, with yields that can support clinical applications in a cGMP compatible system.



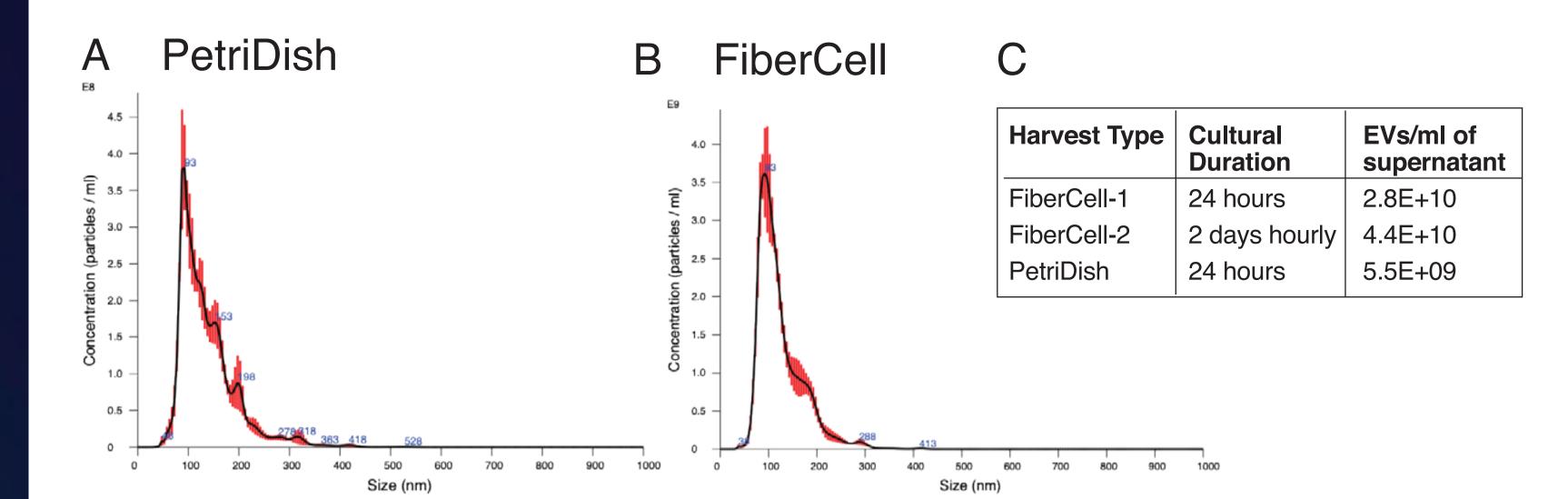


Figure 1: EV characterization by Nanosight. Graphs representing Nanosight analysis of human AFSC-derived EVs grown in PetriDish in vitro cultures (A), and FiberCell Systems 3-D hollow fiber bioreactor (B) Little difference in EV concentration during independent harvest intervals in the bioreactor, and per volume of cell culture supernatant harvested, EVs produced in the bioreactor was 10 times higher in average than EVs produced in PetriDish (C)

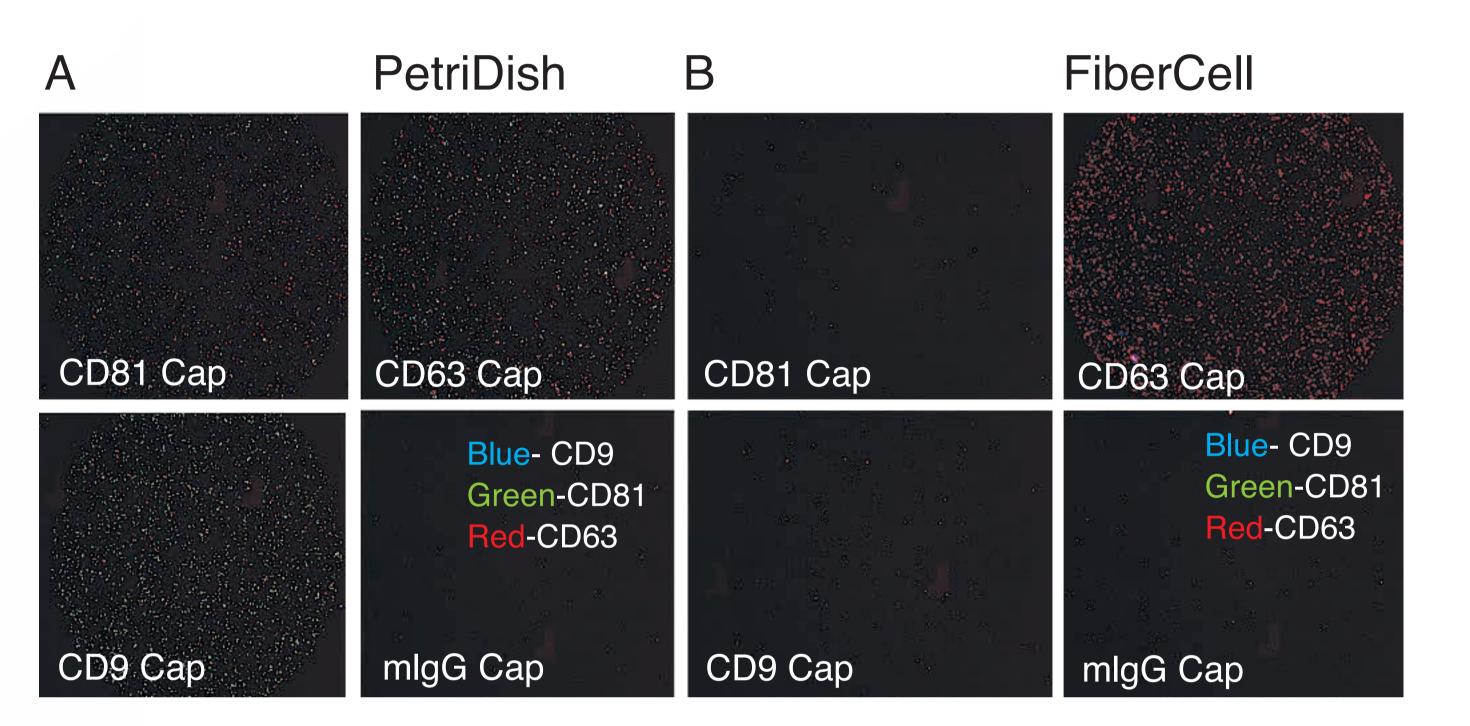


Figure 2: EV tetraspanin expression evaluation by ExoView. Imaging of EVs tetraspanins relative to EVs from PetriDish (A) and relative to FiberCell Systems bioreactor derived EVs (B). EVs were evaluated using 3 color overlay of anti-CD9-AF488, anti-CD81-AF555, and EVs from PetriDish are positive for all 3 tetraspanins while from FiberCell Systems are positive mainly for CD63.

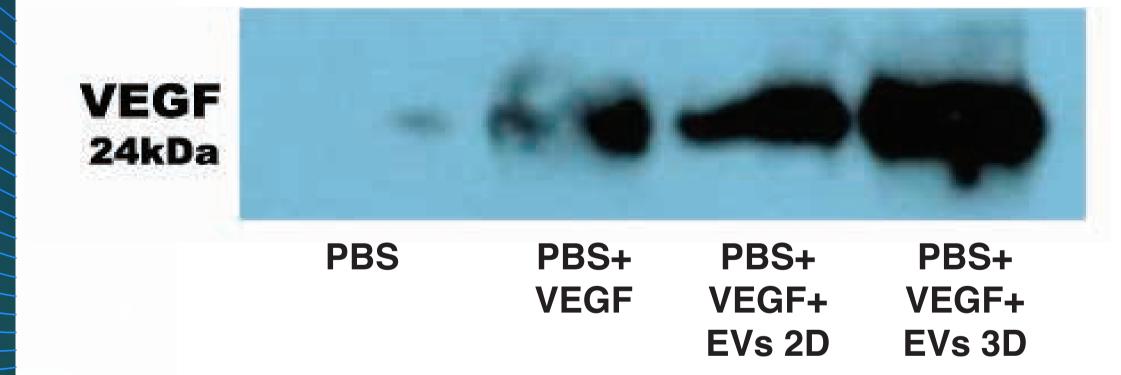


Figure 3: EV potency assay. The ability of EV to bind VEGF was assessed by a co-immunoprecipitation potency assay. Representative immunoblot of VEGF after co-immunoprecipitation with VEGFR1 and treated with 2-D EVs (PetriDish) and 3-D EVs (FiberCell Systems) for 24 hrs showing the ability of EVs to trap VEGF versus non-treated groups.

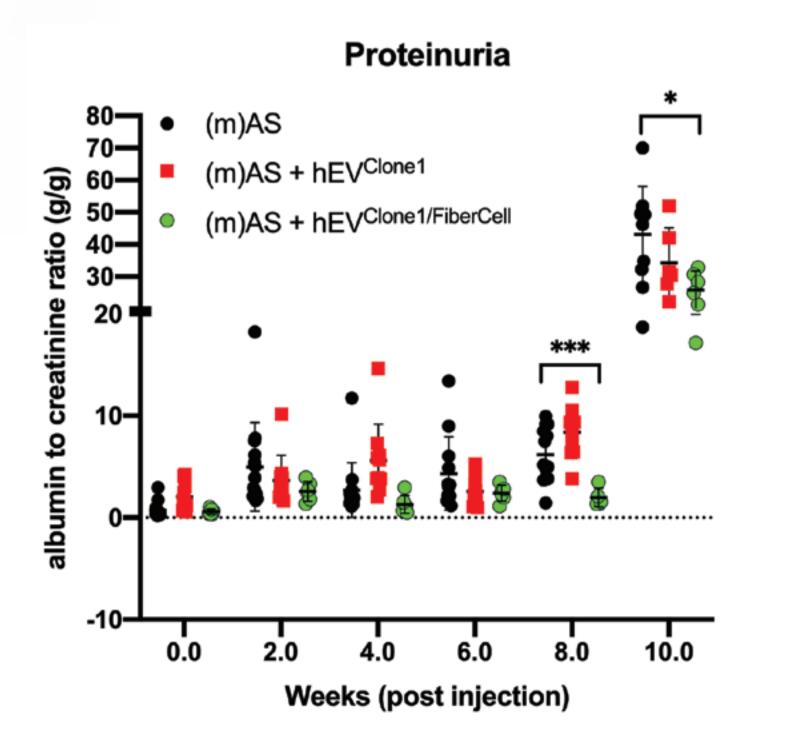


Figure 4: EVs ameliorate proteinuria in CKD mice. Alport mice were injected with AFSC hEVs in FiberCell Systems 3-D (n=6, green) and in Petri Dish 2-D cultures (n=10, red). Control (n=14, black). Injection of 3-D derived EVs ameliorated proteinuria in Alport mice more significantly than the 2-D culture derived EVs.