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Media Optimization and Continuous Production of Extracellular Vesicles from Human Amniotic Fluid Stem Cells during Long Term Culture in a Hollow Fiber Bioreactor.

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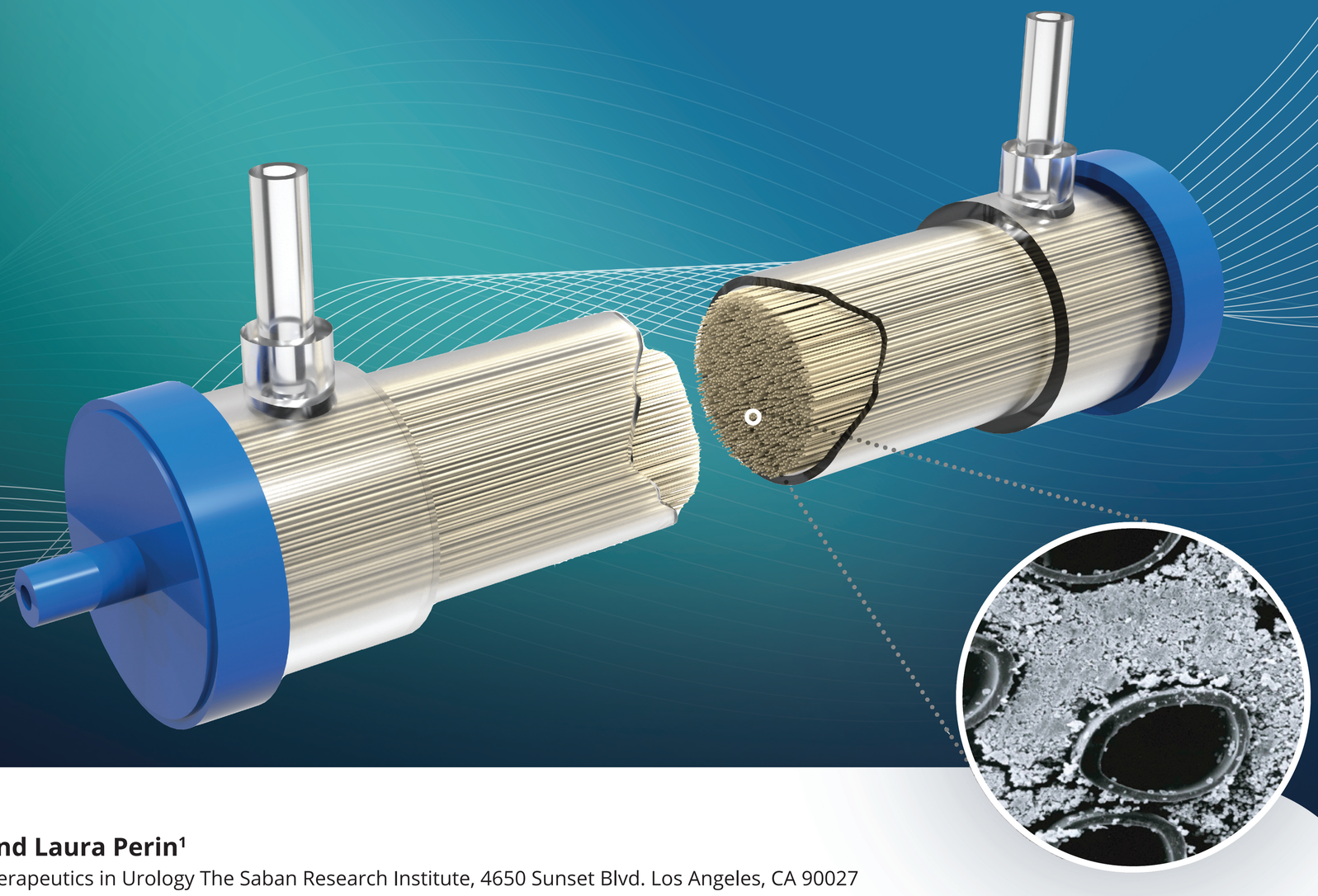

Paolo Neviani[1], Charmi Dedhia[1], John J.S Cadwell[2], and Laura Perin[1]

1) GOFARR Laboratory for Organ Regenerative Research and Cell Therapeutics in Urology The Saban Research Institute, 4650 Sunset Blvd. Los Angeles, CA 90027
2) FiberCell Systems Inc., New Market, Md. 21774

Introduction

The clinical translation of extracellular vesicles (EVs) faces significant challenges, particularly in the scale-up of EV production. Hollow fiber bioreactors (HFBRs) provide an attractive solution by supporting high-density cell cultures and enabling large-scale EV production. Specific cell types, such as mesenchymal stem cells and human amniotic fluid stem cells (HAFSC), do not proliferate in 3-D perfusion hollow fiber bioreactors. This characteristic permits long term culture, 30 days on up to several months, without differentiation, and continuous production of secreted EVs. In standard 2-D flask culture HAFSC require specific stem cell selected fetal bovine serum in concentrations of 20% which is impractical for cGMP manufacturing. Previous work has demonstrated high concentration and enhanced bioactivity of EVs produced in a hollow fiber bioreactor for treating kidney disease in Alport Syndrome Mice (Perin et al., ISEV 2022). EV production in 3-D perfusion hollow fiber bioreactors is optimized by manipulating culture media and harvest conditions over time. This study compares the production efficiency, potency, and molecular profiles of EVs harvested from HAFSCs cultured in various mediums including a serum-free medium under controlled bioreactor conditions.

Methods

Clonal HAFSCs were derived from amniotic fluid of consented donors and seeded at a density of 5 × 10^8 cells into a medium HFBR (FiberCell C2011) with fibronectin coating. Cells were initially cultured in Chang's medium containing 20% ES-FBS, various FBS and human platelet lysate (HPL) formulations and finally in Rooster Nourish serum free medium. FBS or HPL were placed in the circulating medium only of the bioreactor preventing contamination with endogenous EVs. Glucose consumption was monitored daily as an indicator of cell metabolism. EVs were harvested, and their concentration and size were measured by Nanoparticle Tracking Analysis (NTA). Potency assays were conducted via Western Blotting (WB) and identity analysis was performed using Exoview technology to profile EV surface markers.

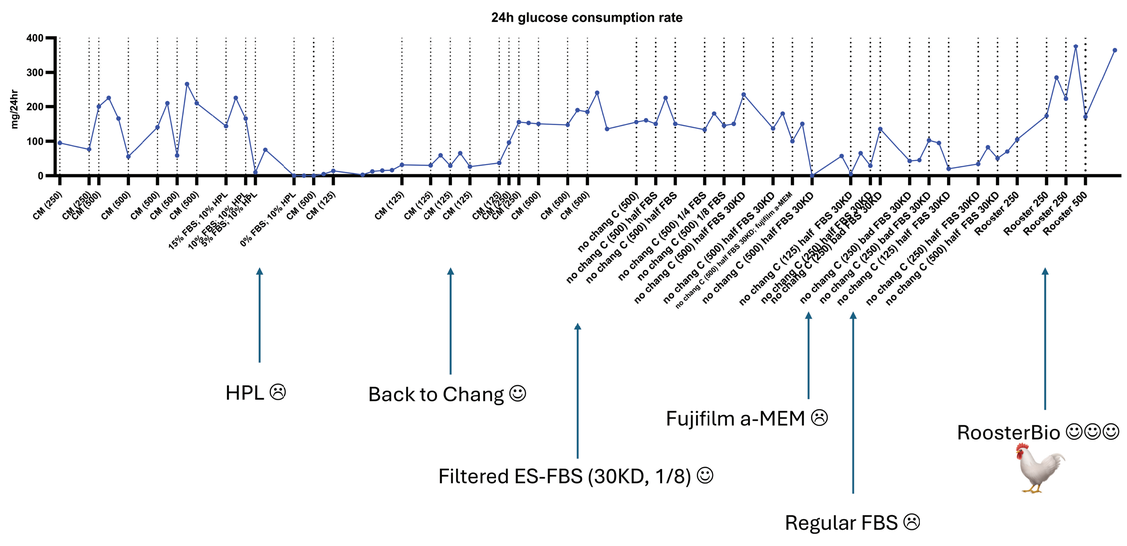

Figure 1 Effect of different mediums on glucose consumption rate of C2011 cartridge seeded with 5X108 HAFSC over 112 days of culture.

Mediums:
1. Chang's, 20% ES-FBS
2. Chang's, 15% ES-FBS, 10% HPL
3. Chang's, 10% ES-FBS, 10% HPL
4. Chang's, 5% FBS, 10% HPL
5. Chang's, 0% FBS, 10% HPL
6. Chang's, 20% ES-FBS
7. Chang's, no Chang C, 20% ES-FBS
8. Chang's, no Chang C, 10% ES-FBS
9. Chang's, no Chang C, 5% ES-FBS
10. Chang's, no Chang C, 2.5% ES-FBS
11. Chang's, no Chang C, 10% 30kd Filtered ES-FBS
12. Chang's, no Chang C, 10% 30kd Filtered ES-FBS, Fujifilm a-MEM
13. 50% Chang's, 50% Alpha MEM, 30kd Filtered FBS (non-ES)
14. Rooster Nourish

Results

The bioreactor was maintained for 112 days of continuous culture and harvests performed on a daily basis. Glucose consumption served as an indicator of cell metabolic responses to the various mediums. Ultrafiltered FBS-ES produced similar results to non-filtered FBS-ES while HPL did not support the cells in culture. Cells recovered after HPL removal and FBS-ES reintroduction. Rooster Nourish medium showed the most pronounced increase in glucose consumption and EV production even after 110 days in culture. EVs harvested from FBS-containing media were significantly more abundant than those from serum free medium, although EVs from the latter showed a significantly higher expression of miR-93-5p, a key microRNA associated with therapeutic potency. NTA analysis revealed comparable EV size profiles between the various conditions Potency assays indicated that EVs from both conditions exhibited similar biological activity, though EVs from serum-free medium showed enhanced molecular signatures relevant for therapeutic applications. Harvesting kinetics showed the optimum time interval EV harvesting was 24 hours.

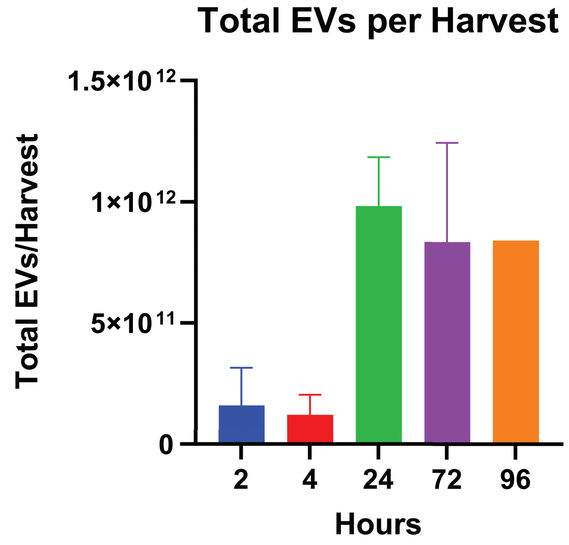

Figure 2 Effect of harvest interval on EV collection. Optimum harvest interval is 24 hours.

Discussion

The effects of various mediums on exosome production and activity during long term (112 days) HFBR culture are presented. FBS-ES has distinct stimulatory effects on HAFSC as opposed to standard FBS, and whatever agent induces this effect can cross the 30kD MWCO filter. HPL has been used in various culture systems as a replacement for FBS, in this case it caused the cells to go into stasis, and remarkably they recovered once FBS was re-introduced. The best results were after 108 days of continuous culture when Rooster Nourish was introduced. After 108 days EVs demonstrated good biological activity and expression of miR-93-5p with this medium. Culture was terminated only because no more Rooster Nourish was available. Despite variable EV production it is estimated that a human dose of EVs (1X10^{13}) could be collected every 5-10 days in a volume of 50-100 mls. The design of the system is cGMP compliant and a 5X scale-up to the C2018 module is readily available. A hollow fiber bioreactor is a viable method to produce clinically relevant extracellular vesicles for regenerative medicine using a cGMP compliant serum free medium.

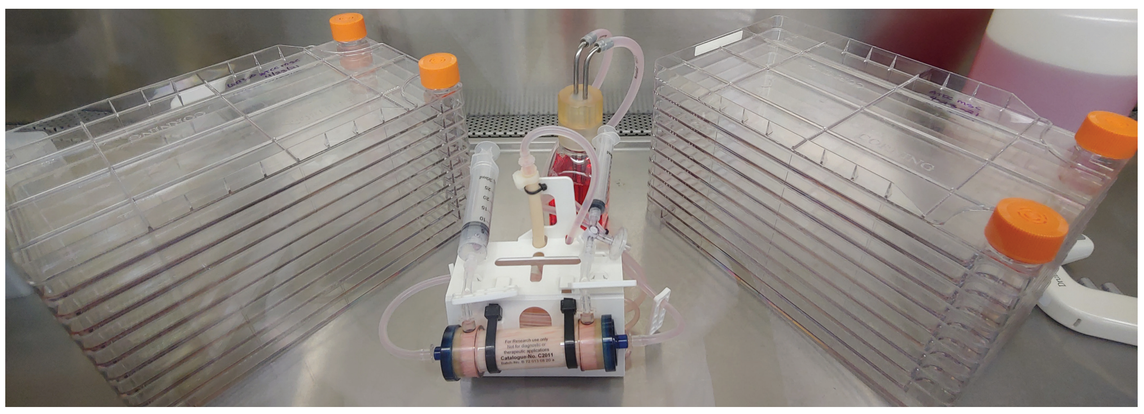

Figure 3 Two Corning ten stacks (1.32 m2) of HAFSC seeded into a C2011 20 kD 4,000 cm2 cartridge.