Abstract
The emergence of gene therapy for genetic diseases has rapidly evolved with the approval of several gene therapy products. New research and clinical developments suggest that ex vivo transfection may deliver corrected genes more efficiently and safely than historical direct transfection systems. Cell lines such as HEK293 and H1458 produce high quality and reproducible viral vectors for this purpose. These cell lines, however, are often adherent cultures that require dated and labor-intensive techniques to produce these vectors. In contrast to gene therapy, monoclonal antibody (mAb) production utilizes suspension cells such as CHO (Chinese hamster ovary) to efficiently produce well-characterized biologics.

Suspension cell lines for gene therapy are being evaluated as an alternative to adherent cultures to improve infective titers and produce high quality virions. Additionally, adapting high density cell culture production from mAb technology will likely increase output with the potential of treating more patients with less equipment, personnel, time, material waste, and cost.

Using tangential flow perfusion (alternating and single pass), we have successfully and reliably grown suspension HEK293 cell cultures up to 80 x 10^6 viable cells/mL. This platform sustains high density bioreactor cultures that do not become nutrient depleted during transfection and can continually harvest to ensure high quality viral vector production.

This next generation gene therapy platform has potential to transform gene therapy manufacturing into a cost-effective, low waste, and superior technology.

Background and Experimental Overview
Tangential Flow Filtration (TFF) and Alternating Flow Filtration (ATF) perfusion processes enable high cell density cultures for biologics manufacturing. In both, a collection device maintains cell culture within the bioreactor while spent media is continually harvested, allowing byproducts and waste to be removed while introducing fresh medium to the bioreactor to maintain nutrients. In TFF, culture runs tangential to the surface of the membrane. In ATF, a diaphragm pump is used to continually alter the direction of the culture between the bioreactor and filter unit.

To develop a perfusion platform, ATF and TFF bioreactor experiments were designed to optimize perfusion method with serum-free and suspension adapted clonal HEK293 cells. For all experiments, the cells were cultured in Irvine Biosystems TFF925 membrane bioreactor that utilizes hollow fiber technology. Daily samples were taken from the bioreactor harvest lines to monitor cell count and viability as well as nutrients and metabolites. Perfusion was initiated on day 3 post-incubation for all experiments.

Comparison of TFF and ATF Perfusion

Both methods of perfusion support densities of 80 x 10^6 cells/mL, yet each method has its own advantages and drawbacks. Many factors must be considered when selecting the best platform for viral vector manufacturing.

ATF Perfusion Advantages
- Higher cell density up to 80 x 10^6/mL
- Cell culture harvesting prevents cell washout
- Cell culture viability >90% through day 15
- ATF bioreactors utilized for large scale perfusion systems

TFF Perfusion Disadvantages
- Lower cell density up to 50 x 10^6/mL
- More frequent media exchanges
- Cell culture viability >89% through day 15

Discussion of Results
- Both ATF and TFF capable of reaching 80 x 10^6 cells/mL
- ATF growth: likely due to shear stresses
- viability trends similar in both ATF and TFF
- ATF fibers and TFF VHU both maintain cells within the bioreactor system as expected
- Important to regulate shear, pH, and glucose strategies in HEK293 perfusion
- ATF membrane fouls using current parameters

Comparison of Viability and pH

Both the ATF and TFF processes show high viability and stable pH over the time of perfusion.

Conclusions and Future Work
Techniques for CHO per fusion cultures have been adapted to support high-density perfusion cultures of suspension HEK293 cells for gene therapy purposes. From preliminary studies, both ATF and TFF perfusion systems are capable of reaching similar maximum viable cell densities of 80 x 10^6 cells/mL. These platforms have the potential to change conventional viral vector manufacturing methods to a high-throughput, cost-effective, and low waste technology.

To create a platform for viral manufacturing, future studies are designed to improve cell culture health and to produce infectious viral vectors:
- Transfect cultures to optimize large scale transient transfection, production, and harvest methods
- Optimize perfusion method to continually produce and harvest high-quality viral vectors
- Increase maximum viable cell densities
- Decrease time to reach maximum viable cell densities
- Elongate culture duration and viral production by maintaining cell culture health
- Minimize material usage and production waste