Benchmarking the Scale-X Bioreactor System for Production of Influenza Virus

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INTRODUCTION

Influenza viral strains represent a consistent seasonal threat to global health. Surveillance and diagnostic testing are conducted worldwide by multiple organizations, necessitating a continuous supply of materials and resources. To maintain antigenicity, current strains of influenza A and B are cultured in tissue rather than eggs. Consequently, there is a demand for large-scale production methods for tissue culture-grown influenza. Currently, multiple stationary T-flasks are employed in tissue culture-related productions, which require extensive handling and multiple production lots to achieve large volumes.

Bioreactors have become indispensable in the industry for large-scale cell production. They can control multiple parameters and monitor various variables throughout the growth phases of cells and viruses, ensuring a higher quality product.

This poster presents the results of benchmarking the Hydro Scale-X Bioreactor System for producing qualified influenza viral material. The quality of the bioreactor-produced material was compared to that of a similar production grown in stationary vessels, using a large volume stationary vessel as a comparative benchmark. Both methods' products were evaluated against the original source material used in the growths.

METHODS

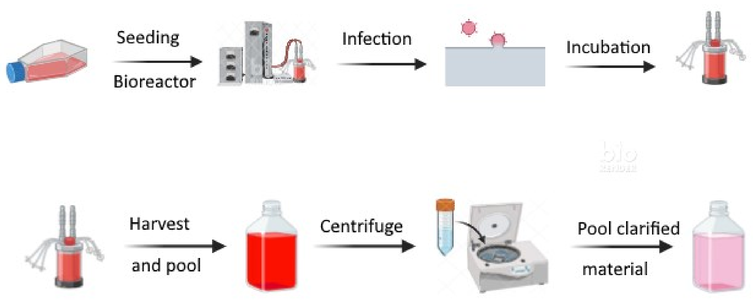

Figure 1. Large scale influenza viral production and characterization. MDCK cells (ATCC® FR-926) are expanded in stationary flasks initially prior to being seeded into a Hydro Scale-X bioreactor. After 24 hours, the cells are infected based on prior infection data. The virus then incubates for 72 hours so as to harvest at the optimum timeframe. The viral material is harvested after 72 hours post-infection, pooled and clarified via centrifugation. The centrifuged material is then pooled and stored. This results in ~5L of tissue culture grown influenza virus that undergoes quality control testing to assess titer and purity.

METHODS

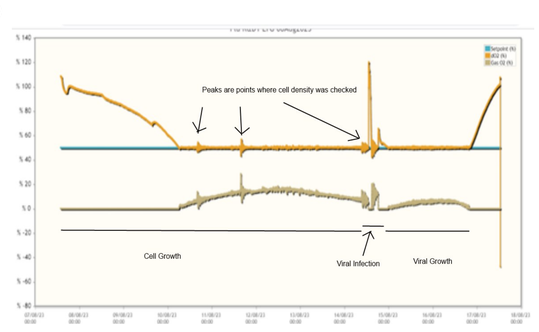

Figure 2. Viral growth process. a) The Hydro Scale-X bioreactor allow monitoring of multiple variables. Distilled oxygen is used to track the expansion of cells and cell death due to viral infection. **b)** MDCK cells confluent prior to infection. **c)** MDCK cells 72 hours after infection, showing signs of CPE (cytopathic effects).

RESULTS

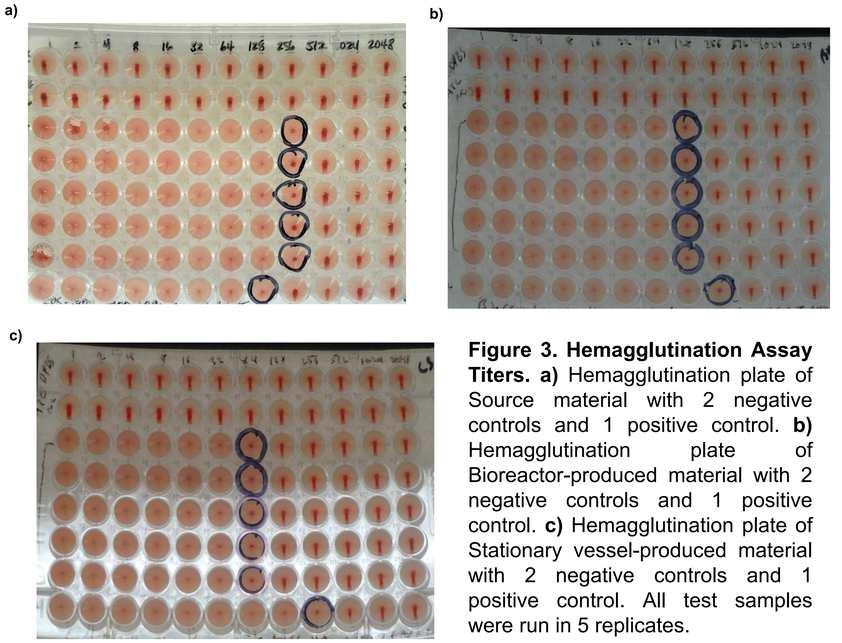

Figure 3. Hemagglutination Assay Titers. a) Hemagglutination plate of Source material with 2 negative controls and 1 positive control. **b)** Hemagglutination plate of Bioreactor-produced material with 2 negative controls and 1 positive control. **c)** Hemagglutination plate of Stationary vessel-produced material with 2 negative controls and 1 positive control. All test samples were run in 5 replicates.

RESULTS

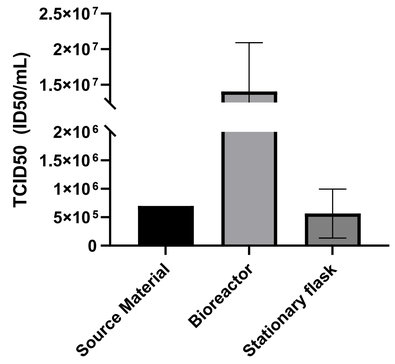

Figure 4. TCID50 Assay. Material harvested from the bioreactor was compared to a proportional volume of material grown from a stationary vessel. These two productions were compared against the original source virus used to grow them. An ID50 titer was calculated using an ELISA based assay. Assays were run in duplicate. Data was analyzed by GraphPad Prism.

CONCLUSION

Viral material produced in the bioreactor was assessed for its hemagglutination titer and $TCID_{50}$ titer. The material was compared against a production grown in parallel in stationary vessels.

Hemagglutination assay: The hemagglutination assays resulted in a titer of 128 for bioreactor-produced material and 64 for stationary vessel produced material. This is lower than the initial titer of 256 seen in the source material, but the drop in titer is an observed phenomena associated with large scale productions. The increased titer of the bioreactor shows that the mechanics unique to it allow for a higher concentration.

$TCID_{50}$ assay: The $TCID_{50}$ assays resulted in increased titers of the stationary vessel and bioreactor when compared to the source material. This is expected in viruses that have been passaged. The material produced in the bioreactor had a log higher titer compared to the stationary vessel material.

This work highlights the potential of utilizing a Scale-X bioreactor in the production of influenza virus strains to produce higher titer viral materials. Future work will include reproducing similar results with different Influenza virus subtypes as well as performing additional analysis (i.e., NGS, FFA titer).

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