Pandemic preparedness: Overcoming Challenges in a 2000 Liter Scale Up for Adenovirus-based Vaccine Manufacturing

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1. Motivation

The COVID-19 pandemic demonstrated that the mass production of vaccines is crucial for our healthcare system[1]. In order to provide large quantities in a short time span, high capacity technology is inevitable[2,3]. IDT Biologika has established a pandemic preparedness concept that demonstrates our readiness for viral vector vaccine mass production. In this manner, we have developed an exemplary process for Adenovirus (AdV) vector manufacturing and successfully scaled it up into a 2000 L stirred tank reactor (STR). Here we describe our work flow and the challenges that we overcame during the process development.

2. Work flow

The development of the process can be summarized in four stages. **(Fig.1)**.

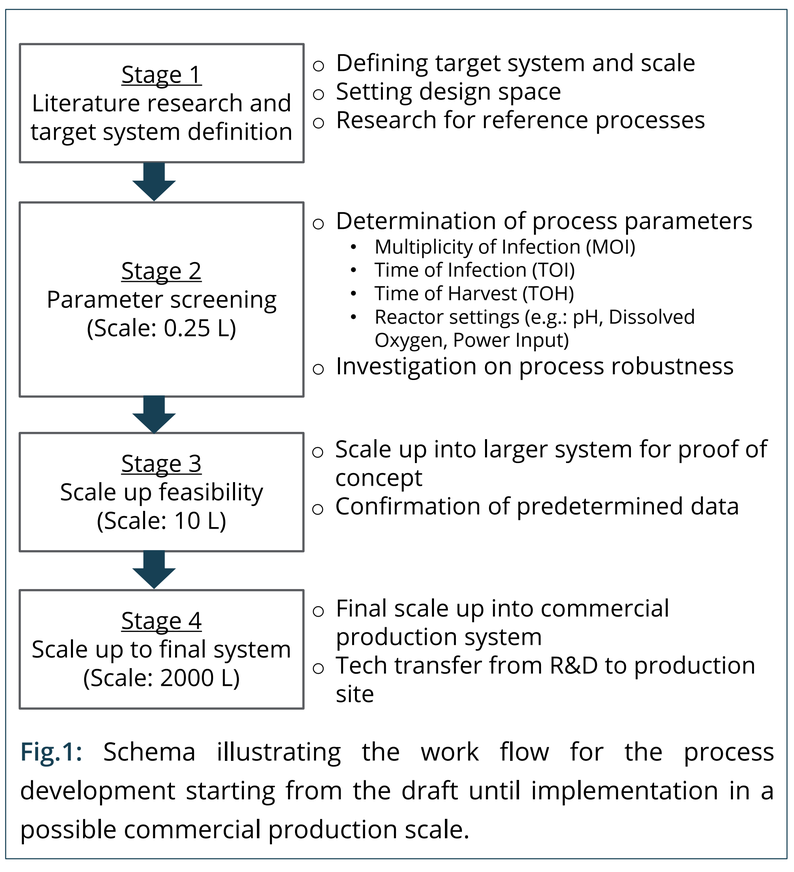

Fig.1: Schema illustrating the work flow for the process development starting from the draft until implementation in a possible commercial production scale.

3. Challenges

Besides determining the process parameters for sufficient AdV production in our cultivation system there were numerous challenges to overcome.

Seed train design for max. volume coverage

The goal was to make use of the maximum capacity of a 2000 L STR. The seed train layout and the volumetric dimensioning needed to be matched to each other **(Fig.2)**. Challenges were:

- layout of cell expansion in shaker flasks to a manageable amount
- continue expansion in a reactor cascade to final cell growth volume
- Minimize media removal or addition steps, to maintain stable process parameters throughout the process
- optimal use of the working volume in regard to cell expansion, infection and lysis in one batch volume

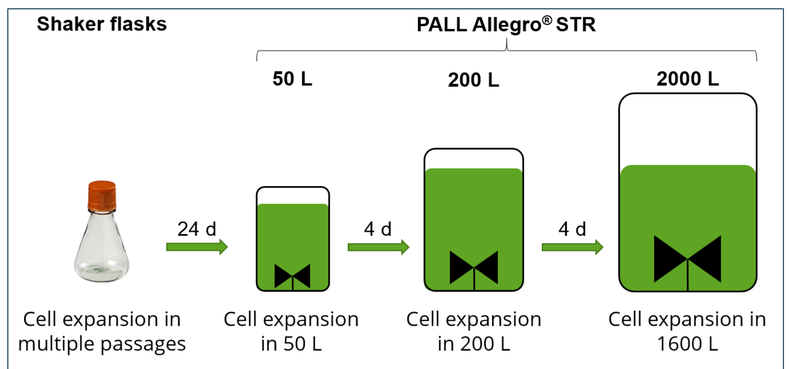

Fig.2: First, HEK-293 suspension cells were cultivated and expanded for multiple passages in shaker flasks. Afterwards, cells were transferred into a 50 L and subsequently into a 200 L STR. The full capacity of these reactors was used. In the last step, cells were transferred to inoculate only 1600 L, leaving room for infection with AdV and buffer for cell lysis. That leads to an increment to the target volume of 2000 L by the end of the process.

Scale up into different reactor dimensions

As a proof of concept the small – scale model has been scaled up into a larger mid – scale system. Among others comparable cell growth was an indication of successful scale up **(Fig.3)**. Challenges were:

- sufficient mixing and oxygen supply
- avoid increase of shear stress

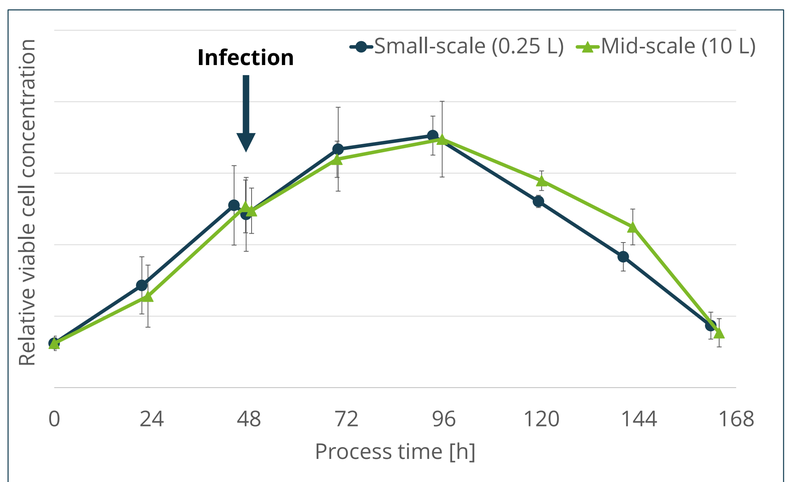

Fig.3: Growth curves of a HEK-293 suspension cell line in the small (blue line) and mid – scale (green line) systems proving scalability due to high curve comparability. Each curve includes the average viable cell concentrations of n = 4 STRs

Optimization: MOI reduction

As part of the pandemic preparedness agreement, 46 batches are to be provided each year, which results in an enormous amount of infective material. Therefore we have screened for lower MOI in our small scale system and transferred the optimum to the Allegro® 2000 STR. As a consequence the cell growth profile was altered **(Fig.4)** but comparable titers were achieved **(Fig.5)**.

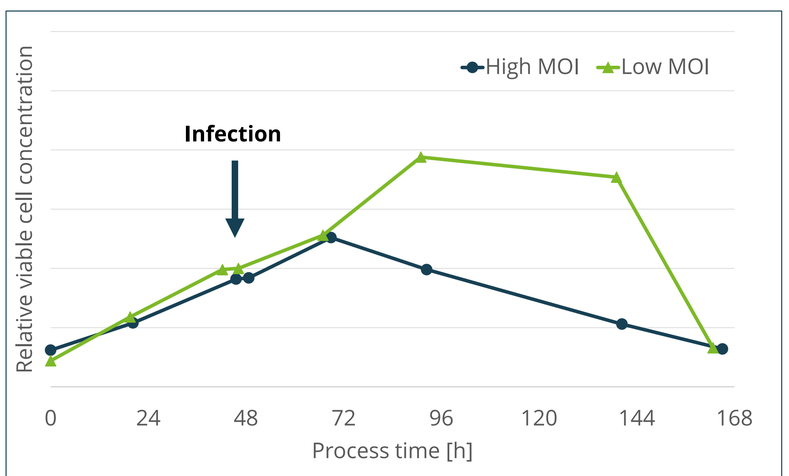

Fig.4: With low MOI (green) cell growth continued after infection for a longer time period in comparison to the use of high MOI (blue). Both processes were performed in n = 1 STR.

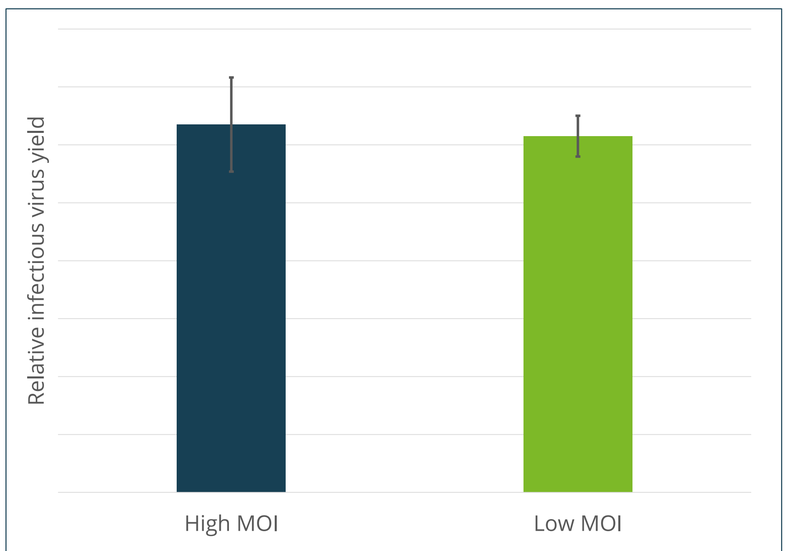

Fig.5: The infectious virus yield produced with low MOI (green) is comparable to the one with high MOI (blue). Both processes were performed in n = 1 STR.

Conclusion

Overall we overcame challenges developing a process for the production of AdV-based vector vaccines in a HEK-293 suspension cell line. As a result we have established an exemplary process and demonstrated the scalability and practical feasibility in our full scale GMP – Unit. The process design mimics a possible mass production scenario and therefore matches our concept of the pandemic preparedness, that has been approved by the German government. We demonstrated our capabilities in large scale vaccine manufacturing and furthermore generated key-knowledge of our suspension platform process for upcoming large scale manufacturing.

4. Outlook

In order to fulfill our pandemic preparedness responsibility, we will maintain our status in order to quickly response to any cases of potentially emerging pandemic viruses. For this purpose, we will practice our handling multiple times annually in order to show robust working procedures, that can be applied to any process at that scale. We also strive to continuously optimize our process platforms in regard to performance and efficiency.

Inquiries are welcome

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References

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3. Vaccine, 24 July 2017, The complexity and cost of vaccine manufacturing – An overview