Rapid HEK Platform Scale Up to 200 L Using k_La Modeling

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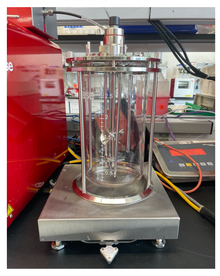
INTRODUCTION

- A HEK293 triple transfection process was successfully scaled up from shake flask to 200 L XDR bioreactor using various scale-up considerations such as k_La based on oxygen uptake rate calculations.
- k_La was measured for both 200L bioreactor and a 2L scale down model and mass transfer rates were estimated with agitation and sparging.
- Since a particular k_La can be achieved across a range of agitation and sparge rates, we used the 2L scale down model to assess the impact of mechanical and bubble shear across a range of agitation and sparge rates.
- These data suggested the HEK293 cell line was tolerant to a wide range of agitation and sparge rates – a higher agitation rate was chosen to reduce sparging and its impact on the transfection step was assessed.
- 200 L bioreactor scale-up parameters selected based on successful small scale bioreactor results.
- Results from 200L scale is comparable to our 2L scale-down model.

2 L Bioreactor

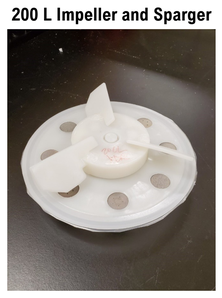


Scale Up

200 L Bioreactor

2 L Impeller and Sparger

200 L Impeller and Sparger

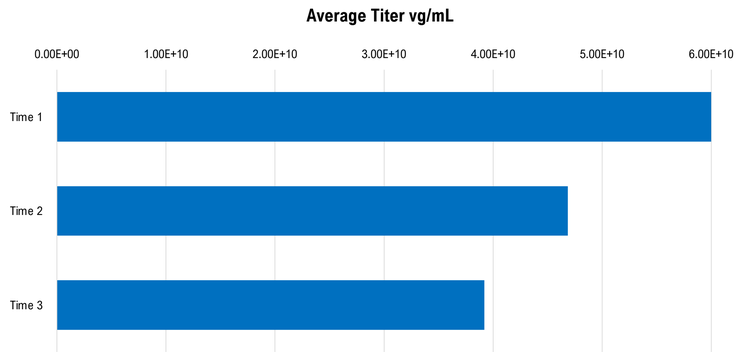


Scale Up Considerations

Parameter	Rationale	Small Scale Bioreactor	Large Scale Bioreactor
Working Volume (Post transfection)	N/A	2.1 L	210 L
Overlay setpoint		0.04 L/min	1.2 L/min
Agitation	Agitation setpoint chosen based on matching mass transfer coefficient between small scale and large scale	0.6 N/m^2-0.8 N/m^2	0.5 N/m^2-0.7 N/m^2
Transfection Mixing Mode		Manual mix by inverting 3x	Rocking in Wave reactor
Complexation Mixture Transfer Mode		Peristaltic pump, pumping for 5 minutes	Peristaltic Pump, pumping for 5 minutes

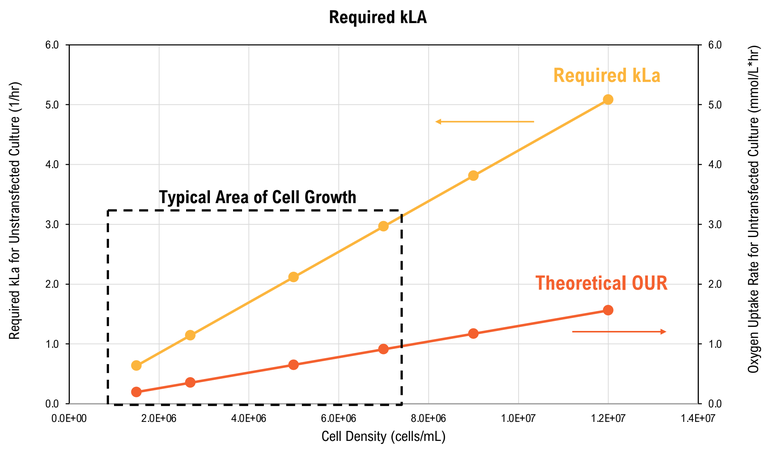
Complexation Mixture Time

Time 1 Found to be Optimal Time



Oxygen Uptake Rate and Corresponding k_La Requirements

Untransfected Culture

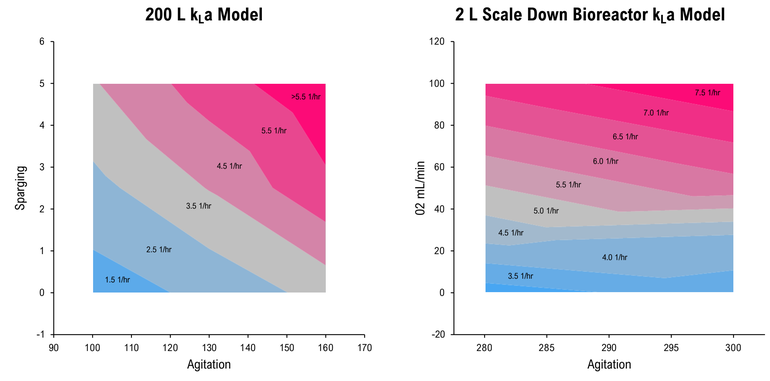


Oxygen Demand Assessment

- Oxygen demand was estimated using a specific uptake rate of 1.3e-10 qO2 mmole/cell.hr from Gálvez et al 2012.
- Goal of scale up was to meet oxygen demand while managing impact of mechanical shear and bubble shear on the cells and especially the transfection step.
- Specifically, selection of the appropriate agitation rate, sparge rate and sparger type.

RESULTS

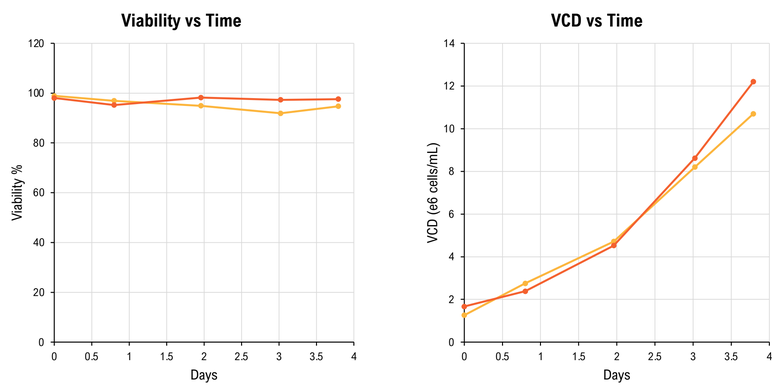
Figure 1. Measurement of Mass Transfer Coefficient (k_La)



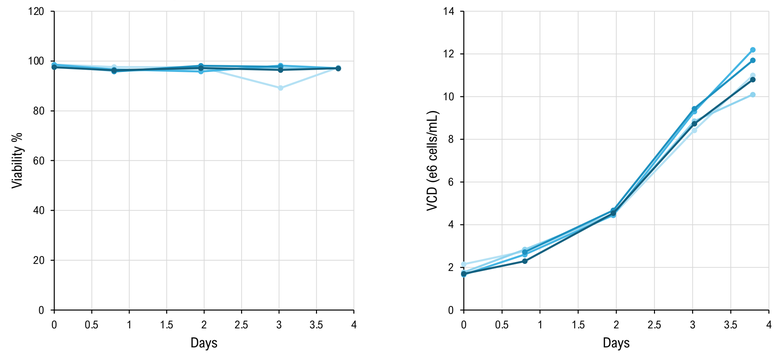
- It was observed that both scales can readily achieve the required KLA, thus oxygen demand.

Figure 2. Mechanical and Bubble Shear Stress Tolerance

Sparger	Shear Rate (N/m^2)	Air (sLPM)	Oxygen (sLPM)
Macro	0.6	On Demand (max 0.1)	On Demand (max 0.15)
Macro	1	On Demand (max 0.1)	On Demand (max 0.15)



Sparger	Shear Rate (N/m^2)	Air (sLPM)	Oxygen (sLPM)
Micro	0.6	On Demand (0.1)	On Demand (max 0.15)
Micro	0.6	Constant (0.01)	On Demand (max 0.15)
Micro	0.8	Constant (0.01)	On Demand (max 0.15)
Micro	1	Constant (0.01)	On Demand (max 0.15)
Micro	1.2	Constant (0.01)	On Demand (max 0.15)

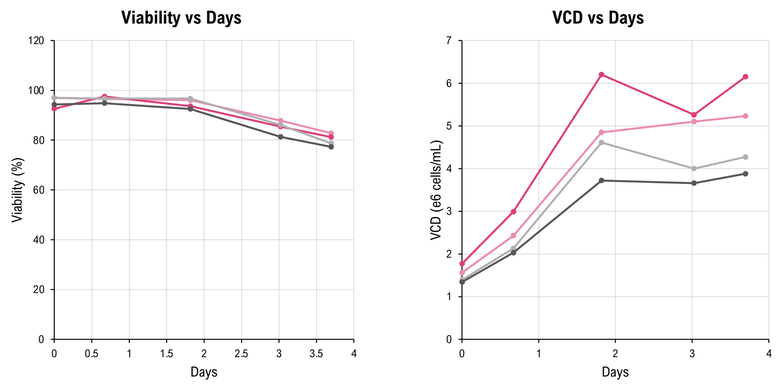


- In order to understand the mechanical and bubble shear stress tolerance of the cell line, a range of agitation rates was tested along with a few gas sparging schemes for the parameters frequently used for transfected culture.

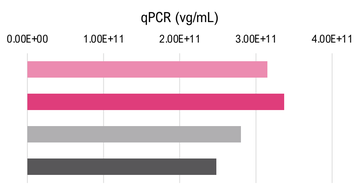
Mechanical/Bubble Stress Tolerance for Untransfected Culture

- Cells can handle high mechanical shear (1.2 N/m^2) and bubble shear imparted by macro as well as the micro spargers at the agitation rates tested.
  - Mechanical shear rates at the higher end of the tested range are expected at large scale due to the geometry and size of the impellers.
  - Significant bubble shear may also be expected at 200L scale since a constant air sparge is employed to ensure sufficient oxygenation and CO_2 removal.
- Since these data suggested the HEK293 cell line was tolerant to a wide range of agitation and sparge rates – a higher agitation rate was chosen, to minimize sparging, as bubble shear has a more significant adverse impact on the culture.
- The next step was to understand the potential impact of agitation and sparge conditions on the transfection procedure and culture performance.

Figure 3. Impact of Agitation/Sparging on Transfection



Sparger	Shear Rate (N/m^2)	Air (sLPM)	Oxygen (sLPM)	Capsid
Macro	0.6	On Demand (max 0.1)	On Demand (max 0.15)	Capsid 1
Micro	0.6	On Demand (max 0.1)	On Demand (max 0.15)	Capsid 1
Macro	0.6	On Demand (max 0.1)	On Demand (max 0.15)	Capsid 2
Micro	0.6	On Demand (max 0.1)	On Demand (max 0.15)	Capsid 2

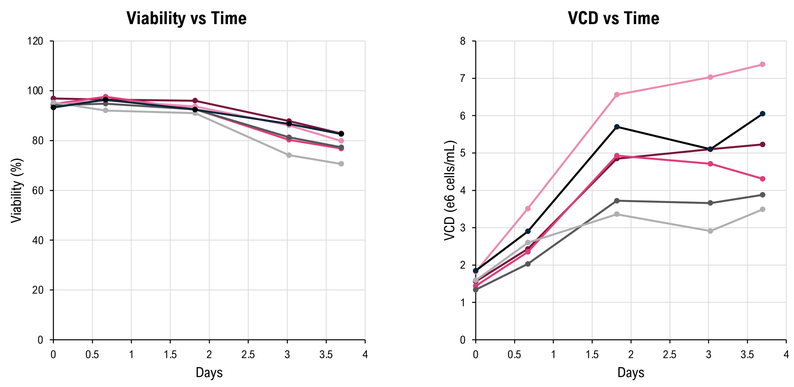


- Results demonstrated that the selected agitation and sparging conditions did not significantly impact productivity regardless of the sparger type.

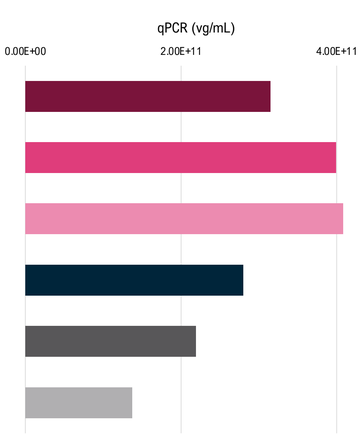
Switch to macrosparger

- Decision to use the macrosparger was made since we had more macrosparger experience in the 200L reactor, as well as to address concerns with foaming observed with microsparger in 2L scale.

Figure 4. Robustness of the Transfection Step



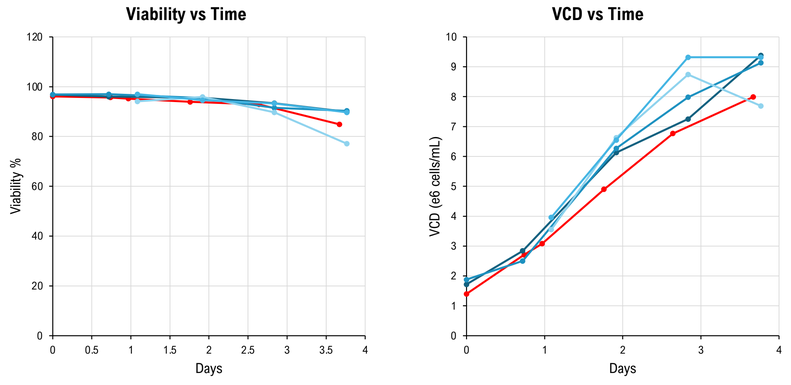
Sparger	Shear Rate (N/m^2)	Air (sLPM)	Oxygen (sLPM)	Capsid	DO % Setpoint
Macro	0.6	On demand (max 0.1)	On demand (max 0.15)	1	40
Macro	0.8	On demand (max 0.1)	On demand (max 0.15)	1	30
Macro	0.8	Constant (0.02)	On demand (max 0.15)	1	30
Macro	0.6	On demand (max 0.1)	On demand (max 0.15)	2	40
Macro	0.8	On demand (max 0.1)	On demand (max 0.15)	2	30
Macro	0.8	Constant (0.02)	On demand (max 0.15)	2	30



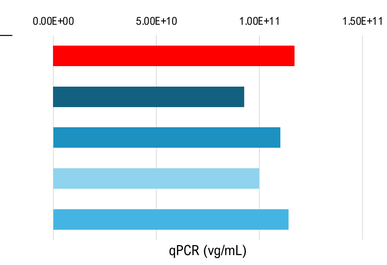
Transfection step robustness

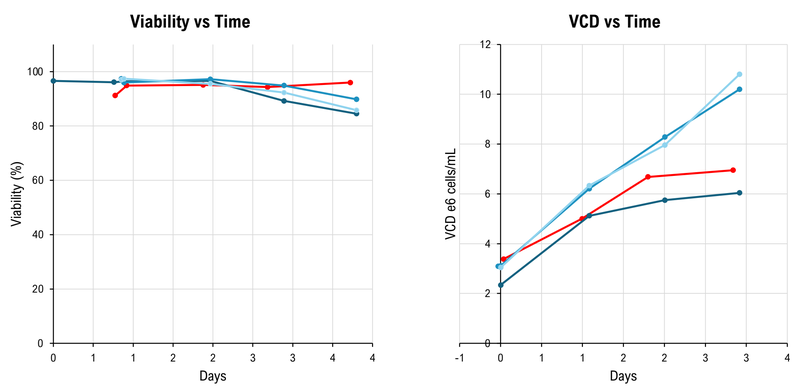
- Constant sparging and higher agitation did not decrease titer significantly at 2 L scale.
- These data allowed us to confirm our preferred conditions for the 200L scale - a higher agitation to minimize sparging.
- Successful scale up of the 200L run was demonstrated by running parallel 2L cultures.

Figure 5. 200 L Bioreactor Performance

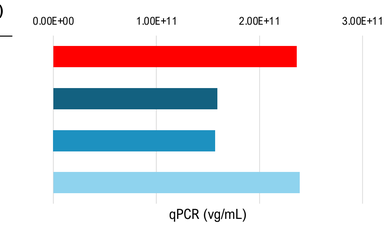


Sparger	Shear Rate (N/m^2)	Air (sLPM)	Oxygen (sLPM)	DO % Setpoint	Scale (L)
Macro	0.7	Constant (0.02)	On demand (max 0.15)	30	200
Macro	0.8	Constant (0.02)	On demand (max 0.15)	30	2
Macro	0.8	Constant (0.02)	On demand (max 0.15)	30	2
Macro	0.8	Constant (0.02)	On demand (max 0.15)	30	2
Macro	0.8	Constant (0.02)	On demand (max 0.15)	30	2





Sparger	Shear Rate (N/m^2)	Air (sLPM)	Oxygen (sLPM)	DO % setpoint	Scale (L)
Macro	0.7	Constant (0.01)	On demand (max 0.15)	30	200
Macro	0.8	Constant (0.01)	On demand (max 0.15)	30	2
Macro	0.8	Constant (0.01)	On demand (max 0.15)	30	2
Macro	0.8	Constant (0.01)	On demand (max 0.15)	30	2



CONCLUSIONS

- To enable scale up a HEK293 triple transfection process, a 2L scale down model was developed and used to understand the impact of agitation and sparging on the culture.
- The model suggested the HEK293 cell line was tolerant to a wide range of agitation and sparge rates – higher agitation rate was chosen, to minimize sparging, as bubble shear has a more adverse impact on the culture.
- Productivity data from 2L satellite cultures for the 200 L bioreactor showed that we successfully scaled up the process.