Scale-down of AAV production from commercial scale to a high-throughput mini-bioreactor system

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BACKGROUND

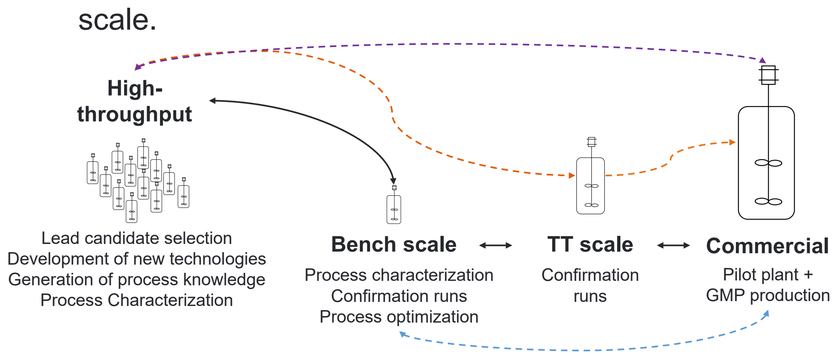
Scale-down models are critical tools for process development, process characterization and generation of process knowledge. By definition, a scale-down model is an incomplete representation of a more complicated, expensive and/or physically larger system that must be used because of the limitations to conduct experimental studies with the at-scale equipment.

Traditionally, scale-down models were developed in bench top bioreactors. However, the increase on demand to accelerate the timeline to develop commercial cell culture processes have opened opportunities for implementing high-throughput technologies.

We introduce the approach to scale down our commercial scale into the mini-bioreactor system ambr250. Mini-bioreactors have been utilized as screening tools during pre-clinical development, but increasing interest is focused on their use as scale-down models of the cGMP manufacturing scale processes during late-phase clinical development.

OBJECTIVES

- Establish a scale down/up platform showing representativeness and comparability across scales.
- ambr250 ↔ Bench scale ↔ TT scale ↔ Commercial scale.



Solid black lines = traditional development path

Dashed lines = proposed development paths to shorten timelines

METHODS

- Similar geometrical space across scales.
- All dimensionless numbers necessary to describe the process in each scale have the same numerical value.

1. SCALE-UP METHODS IN USE

Fundamental methods	Semi-fundamental methods	**Dimensional and regime analysis**	Rules of thumb

1. Obtain knowledge about our manufacturing platform.
2. Identify the potential bottlenecks in our future scale-up (down) strategy.
3. **Define feasible operation conditions for our process.**

2. SCALE-UP BASED ON CONSTANT OPERATING VARIABLES

k_La	Impeller tip speed	Power/ volume	Superficial gas velocity	...?

3. ASSESS COMPARABILITY AMONG SCALES

Cell growth:
- Growth rate (μ_{max})
- Viability

AAV production[1]:
- Productivity
- Empty to full ratio
- Infectivity

[1]Vector genomes – qPCR or ddPCR, capsid proteins - HPLC and infectivity - infectious titer assay.

RESULTS

CELL GROWTH

- Similar growth in ambr250, bench and TT scale.
  - Estimated growth rate in bench and TT scale differ on < 5% compared to ambr250.
  - Viability above 95% for all scales at time < 5 days.

Figure 1. Viable cell density and viability curves of *expreSF+* cells growing at ~30% volume of the STR (~2.8 days) and at full working volume (~3 days).

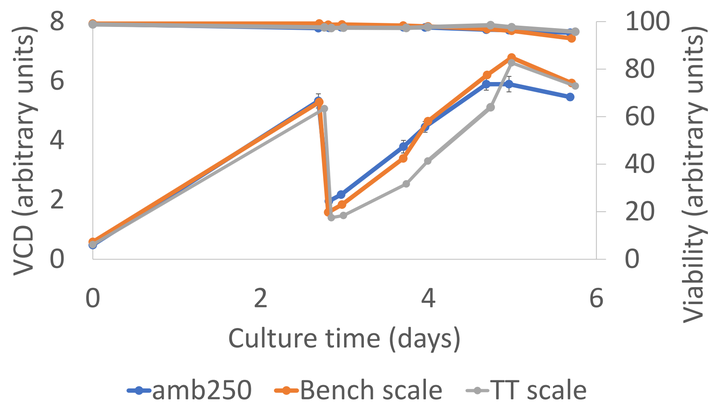


Table 1. Cell growth comparison between bench and TT scale to ambr250 (100%).

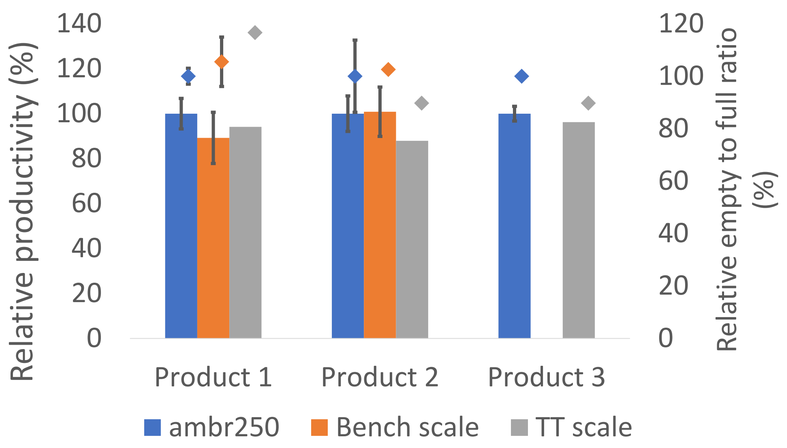
	Relative μ_{max} (%)[2]
ambr250	100
Bench scale	103.9
TT scale	104.3

[2]Individual growth rates for each platform are calculated from the VCD curves at full working volume shown in Figure 1.

AAV PRODUCTION

- Similar productivity and empty to full ratio in ambr250, bench and TT scale when running head-to-head (Figure 2).
  - ambr250 vs bench scale: differences <6% and <5% for productivity and empty to full ratio, respectively.
  - ambr250 vs TT scale: differences <8% and <13% for productivity and empty to full ratio, respectively.

Figure 2. Productivity (■) and empty to full ratio (□) comparison between bench and TT scale to ambr250 (100%). Error bars represent std when n≥3. For each product, ambr250 and the other scales were run head-to-head (same raw materials).



- Overall data for Product 1 shows ambr250's representativeness and comparability to bench, TT and commercial scale (Figure 3 and 4).
  - Productivity and infectivity values for all scales fall within the variability of the data.
  - Empty to full ratio differences fall within our success criteria: differences less than assay variability (~30%).

Figure 3. Overall data of Product 1. Productivity (■) and empty to full ratio (□) comparison between bench, TT and commercial scale to ambr250 (100%). Error bars represent std when n≥3.

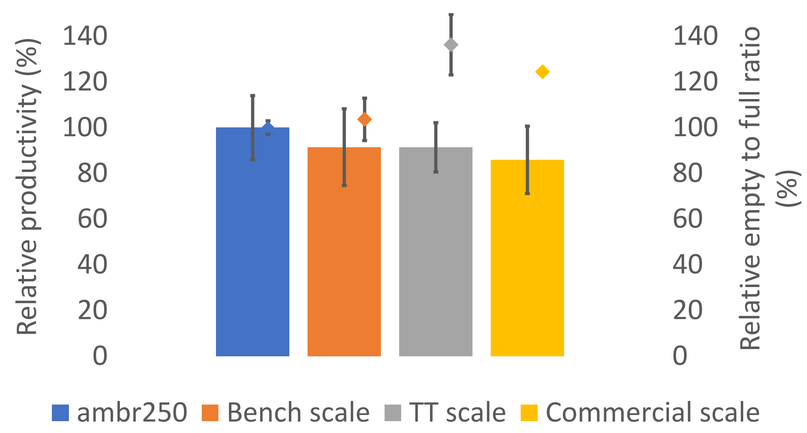
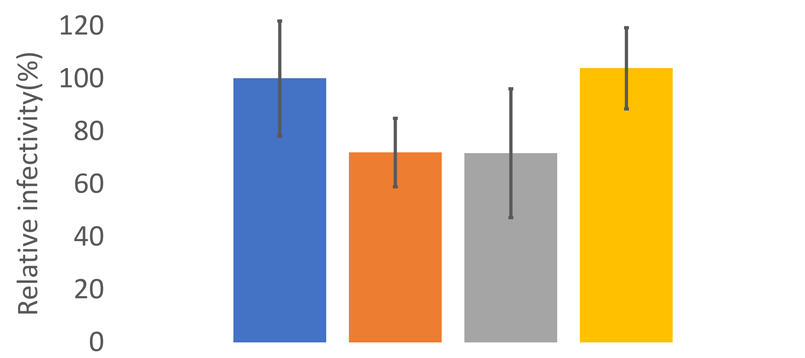


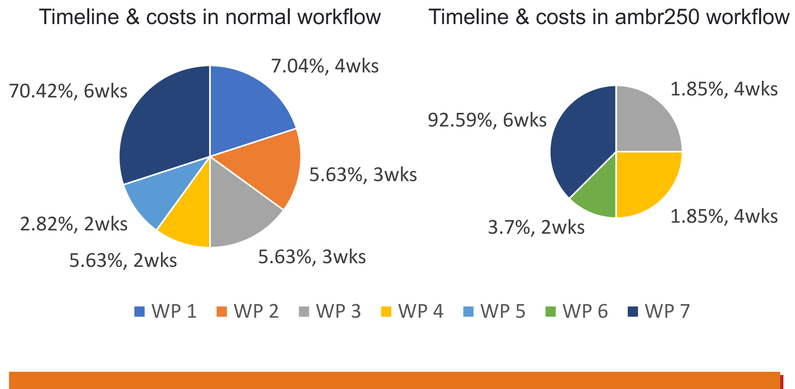
Figure 4. Overall data of Product 1. Infectivity (■) comparison between bench, TT and commercial scale to ambr250 (100%). Error bars represent std when n≥3.



ECONOMIC ASSESSMENT

- ambr250 workflow for each new product:
  - Reduces the timeline by ~1month.
  - Reduces the development cost by ~25%.

Figure 5. Cost and timeline analysis for the development of a new product in our current pipeline and when developing the process directly in ambr250.



CONCLUSIONS

- We have a representative scale down model to speed up process development: ambr250 shows representativeness and comparability to bench, TT and commercial scale.
- Time-to-market will be accelerated by leveraging high-throughput experimentation ensuring translation of results into commercial scale without the need of intermediate experiments.
- Cost of the development of a new product will be reduced by ~25%.
- Future work englobes head-to-head runs ambr250-commercial scale and model validation for process characterization.

REFERENCES

1. Perry's handbook of chemical engineering 9th Edition (2019), ISBN: 978 - 0 - 07 - 183408 – 7.
2. De Wilde D. et al. (2014), Superior Scalability of Single-Use Bioreactors, BioProcess International Volume 12 Supplement 5.

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