

AggreGuard™ VERO cells case study

Summary of case study

This report highlights the compatibility and optimisation potential of VERO processes when utilising CellRev's proprietary know-how and media supplement AggreGuard™ to prevent microcarrier (MC) aggregation in a batch/fed-batch process. The reported findings highlight the potential to improve VERO processing efficiency in existing batch/fed-batch processes.

General experimental design considerations:

- Cell type and MC type compatibility screens (if data is unknown).
- AggreGuard™ time-dose response screening, considering cell type and media formulation.
- Addition timing of AggreGuard™ as per cell growth rate and usual time to aggregation.
- Specific results (doubling time, cell yield, etc.) upon use of AggreGuard™ will be defined by media formulation and cell type modifications.

AggreGuard™ prevents MC aggregation.

Initial VERO cell-MC compatibility and AggreGuard™ time-dose response screening was completed using 6-well ULA plates. Consequently, VERO cells were grown on 1g of Corning® Untreated MCs in 125mL spinner flasks set at 60rpm in media containing 0.5% FBS. After 3 days in culture, CellRev's media supplement was added and maintained at a constant concentration in spinner flask 1 (**Figure 1A**), whilst no addition was made in spinner flasks 2 (**Figure 1B**). Partial media exchanges (25%) were done every 48h for both spinners, to replenish media (± AggreGuard™). In Spinner 1, cells started growing as multilayers on the MC surface without resulting in aggregation (**Figure 2A**). On the other hand, in Spinner 2, the MCs started aggregating from day 5 and the size of the aggregates increased with the time in culture (**Figure 2A**).

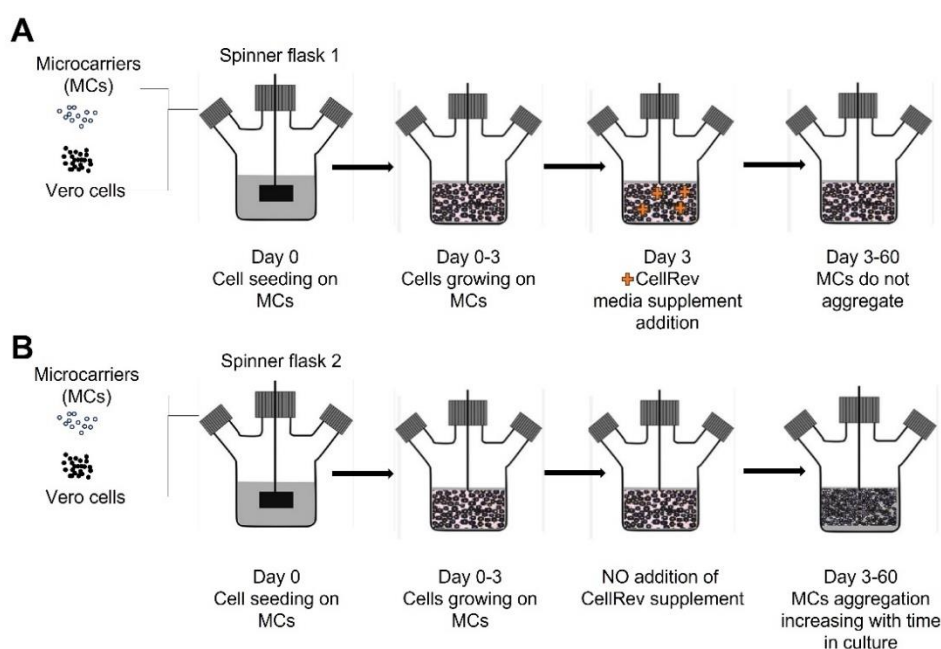


Figure 1. Experimental plan schematic. VERO cells were grown on MCs in spinner flasks with (A) or without (B) CellRev's media supplement for 60 days. The presence of such supplement prevented the aggregation of MCs during the time in culture.

The presence of MCs aggregates is a common challenge in the field. As aggregate size increases, the cells in the inner core of the aggregate become necrotic affecting not only the final yield, but also the viability of the neighbouring cells. This was highlighted as well in our study, reporting the considerable presence of dead cells in the aggregates (**Figure 2A, 2C**). The addition of AggreGuard™ maintained high cell viability (**Figure 2A**) via preventing MCs aggregation (95% vs 75% without supplement; **Figure 2C**). The overall cell yield was also improved. At the time of harvest (day 60) there was an 80.5x fold increase in cell number from day 0 when using the media supplement. This was only a 62.5x fold increase without the supplement (**Figure 2B**). The cells harvested from the MCs at day 60 were able to reattach and proliferate in standard t-flasks, maintaining their initial doubling time (\pm 21h).

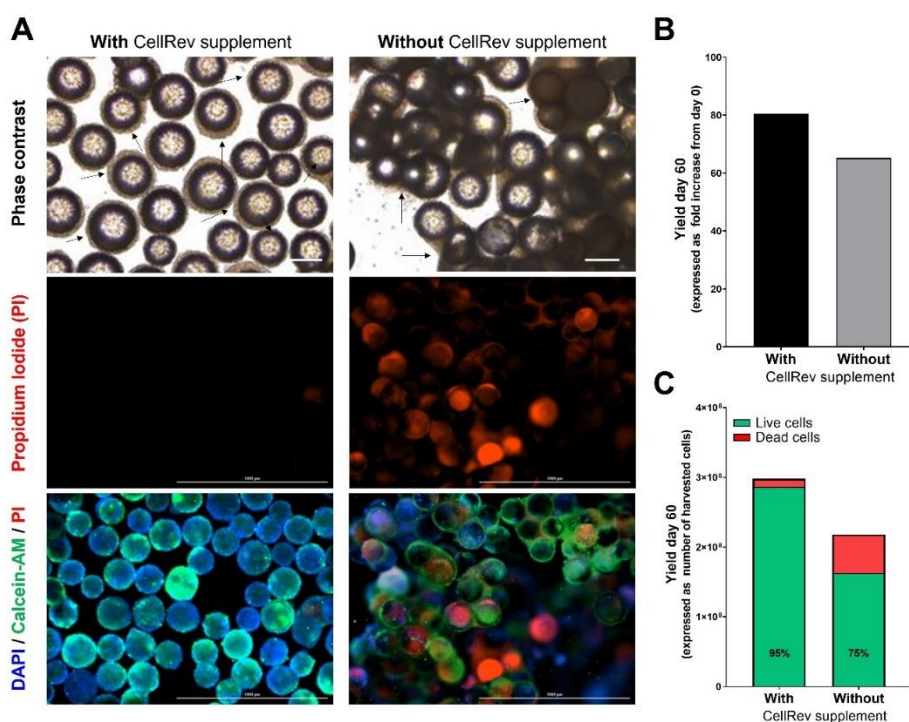


Figure 2. AggreGuard™ increases final cell yield. A) Images taken at day 42 showing the presence of dead cells in the MC aggregates (without supplement), whilst no MC aggregates or dead cells are visible with the addition of CellRev's supplement. Cells were harvested from the MCs at day 60 from both spinners with both yield (B) and viability (C) higher when the media supplement was present. Scale bars in panel A correspond to 200 μ m and 1000 μ m for phase contrast and fluorescent images, respectively.

Conclusion

Through this study, CellRev has highlighted the opportunity to improve overall cell yield & viability by preventing MCs aggregation, facilitating uniform supply of nutrients and oxygen to MC adherent cells. This demonstrates high potential to improve existing VERO cell processes, particularly increased cell surface area for vaccine related processes. AggreGuard™ can be integrated into existing biopharmaceutical manufacturing, after initial optimization, to facilitate increased process productivity with high potential for process intensification.

Please email enquiry@cellrev.co.uk to discuss your processing needs.

CellRev

Scalable adherent cell processing for research and manufacturing.

The company's patented manufacturing platform is an industry-first, facilitating faster, cheaper, and more sustainable production of cellular products. CellRev's platform offers seamless translation from research to market with superior automation, control, and stability versus existing technologies.