

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. CellRev were able to demonstrate:

- 1) microcarrier (MC) aggregation prevention in a batch process
- 2) the feasibility of a continuous manufacturing process for VERO cells.

The reported findings highlight the potential to improve VERO processing efficiency in both existing batch processes and CellRev's continuous bioprocessing platform.

CellRev's supplement prevents MC aggregation

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). Cells were grown for 60 days in both spinners changing the media every 48h. In Spinner 1, cells have been growing as a multilayer on the surface of the MCs, without resulting in MCs 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).

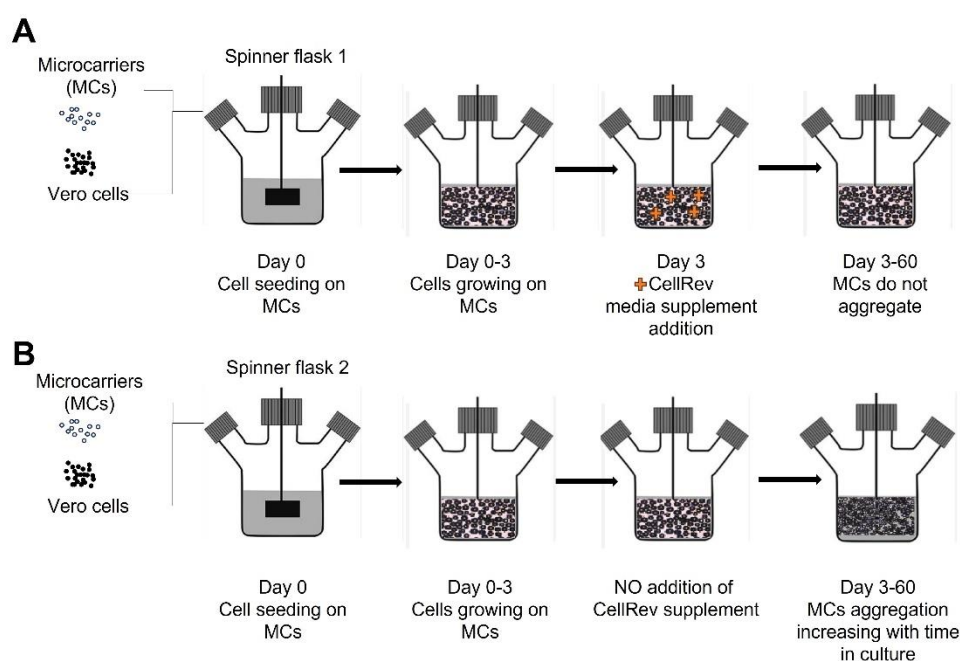


Figure 1. Experimental plan schematic. VERO cells were grown on MCs in spinner flasks with (A) or without (B) CellRev's media supplement for 60 days. The presence of such supplement prevented the aggregation of MCs during the time in culture.

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 CellRev's supplement maintained high cell viability (Figure 2A) via preventing MCs aggregation (95% vs 75% without supplement; Figure 2C). The overall 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 maintaining their initial doubling time on flat surfaces of 21.53h.

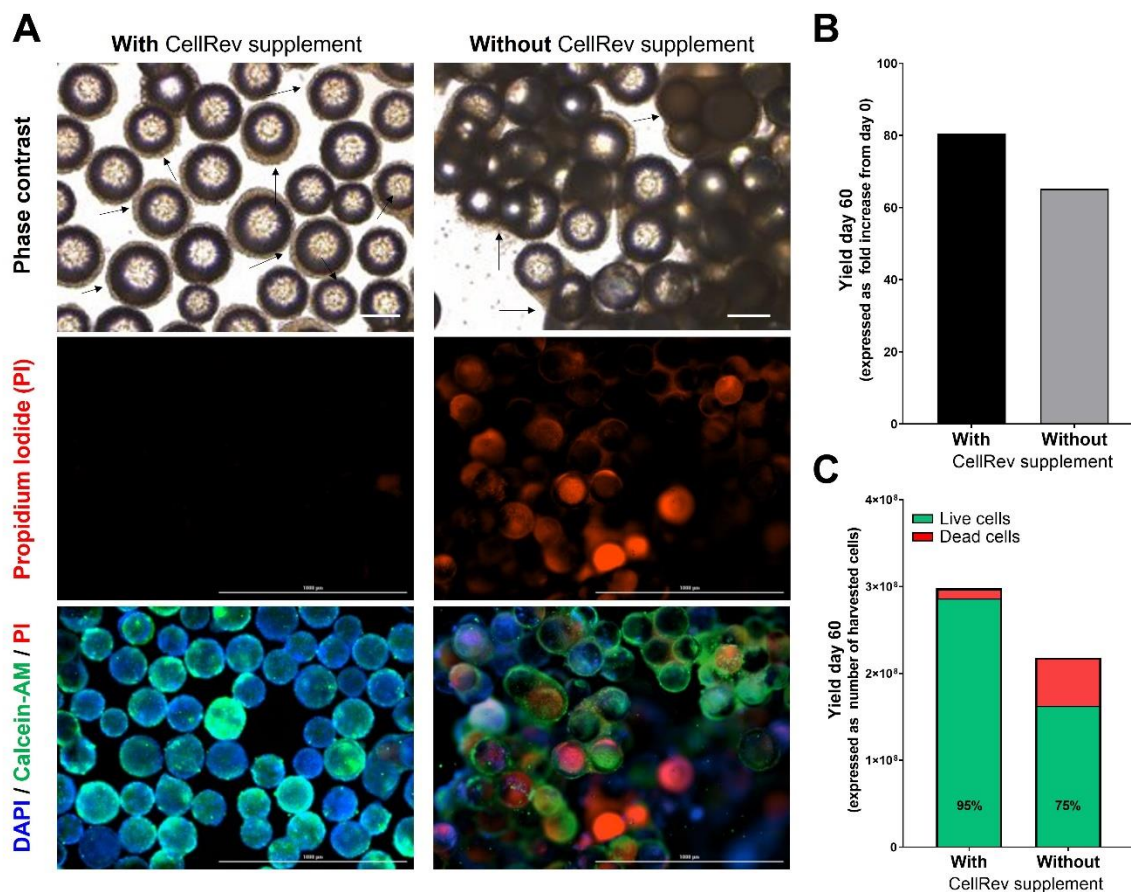


Figure 2. CellRev supplement increases final yield. A) Images taken at day 42 showing the presence of dead cells in the MC aggregates (without supplement), whilst no MC aggregates or dead cells are visible with the addition of CellRev's supplement. Cells were harvested from the MCs at day 60 from both spinners with both yield (B) and viability (C) higher when the media supplement was present.

Scale bars in panel A correspond to 200µm and 1000µm for phase contrast and fluorescent images, respectively.

CellRev's supplement allows for continuous manufacture of VERO cells

A specific concentration of CellRev's supplement allowed for continuous processing of VERO cells at a steady state. The detachment rate of the cells matched the growth rate of the cells on the MCs. Consistent with the first experiment, CellRev operated two spinner flasks in parallel, one with and one without CellRev's supplement. The experiment was conducted over three weeks with a media change every 48h (+0.5% FBS). Without the supplement, cell growth led to the expected MCs aggregation (Figure 3, right panel). With the supplement, VERO cells began to detach at steady state for the duration of the culture (Figure 3, left panel).

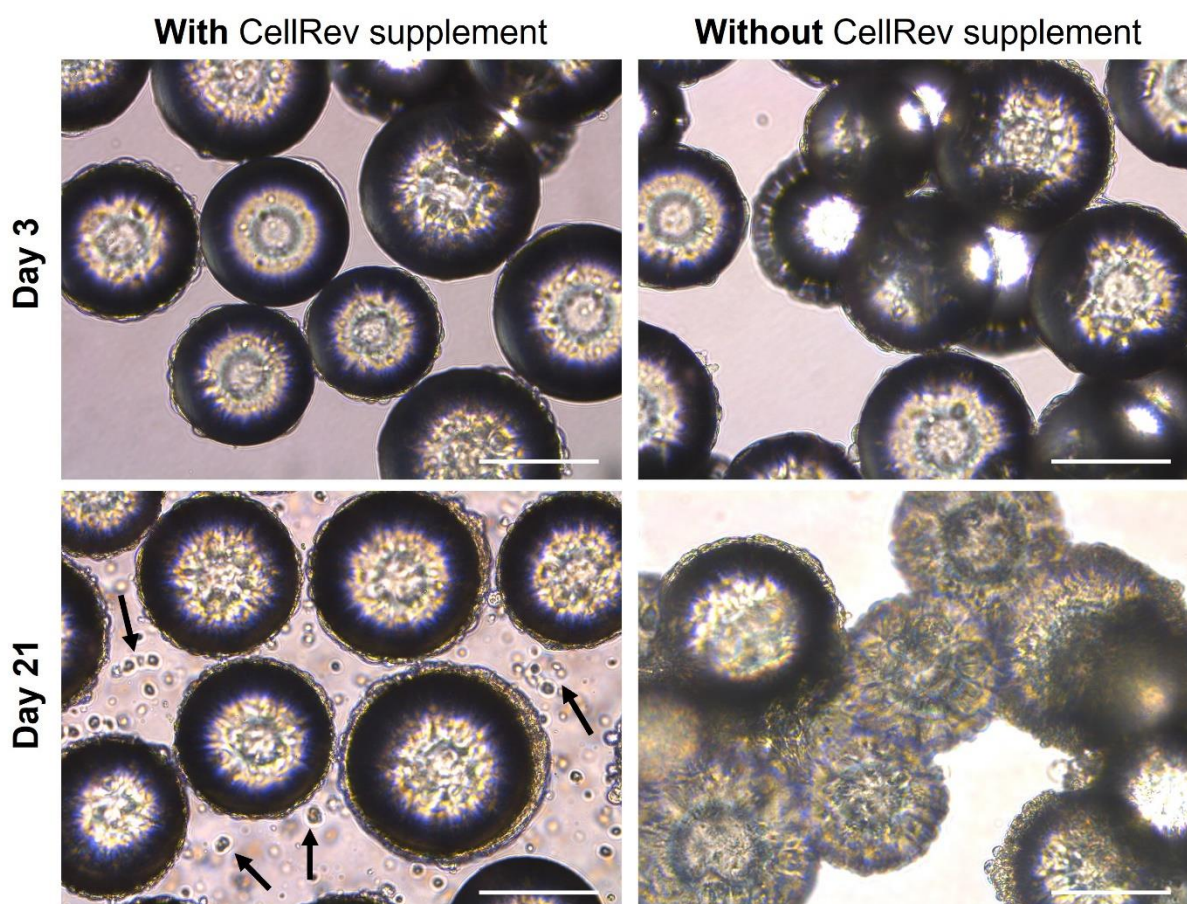


Figure 3. CellRev's supplement allows for continuous manufacture of VERO cells. Images show achievement of steady state of VERO cells over 21 days in culture (left panel; arrows highlight the detached cells present in the media). As expected, without supplement addition the MCs increasingly aggregated over the time in culture (right panel). Scale bars: 200µm.

Conclusion

CellRev have demonstrated the potential to improve existing VERO cell processes and the opportunity to transition to more efficient continuous processing over time. Through this study, CellRev has highlighted the opportunity to improve overall cell yield & viability by preventing MCs aggregation (section 1). In this case, avoiding the presence of aggregates allows for uniform distribution thus improved supply of nutrients and oxygen. Furthermore, demonstrated that VERO cells can be continuously detached at a steady state, thus are suitable for our continuous manufacturing platform (section 2).

CellRev can work with batch VERO processes to address production challenges. The company's media supplement can be integrated into existing biopharmaceutical manufacturing to facilitate intensification of the process. CellRev's continuous platform is also compatible and available in research format.

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.