AggreGuard™: VERO cells case study

Summary of case study

The reported findings highlight the potential to improve VERO upstream bioprocessing efficiency for existing batch/fed-batch modes using AggreGuard™.

Experimental design considerations:

- Cell type and microcarrier (MC) selection screens (*if data is unknown*).
- AggreGuard™ time-dose response screening in multi-well plates to evaluate cell type (*i.e., extracellular matrix composition, attachment behaviour, doubling time, etc.*), microcarrier selection and media formulation.

AggreGuard™ prevents MC aggregation.

Initial VERO cell-MC compatibility and AggreGuard™ time-dose response screening was completed using 6-well ultra-low attachment (ULA) plates. Consequently, VERO cells were grown on 1g of Corning® Untreated MCs in spinner flasks (SF) in 125mL media supplemented with 0.5% FBS, agitated at 60rpm post-seeding. After 3 days in culture, AggreGuard™ was added and maintained at a constant concentration in SF1 (**Figure 1A**), whilst no addition was made in SF2 (**Figure 1B**). Partial media exchanges (25%) were performed every 48h for both spinners, to maintain AggreGuard™ (SF1). MC adherent cells in SF1 showed no aggregation, eventually growing in layers (**Figure 2A**). MC aggregation was observed for SF2 from day 4/5, with increase in aggregate size until end of the culture (**Figure 2A)**.

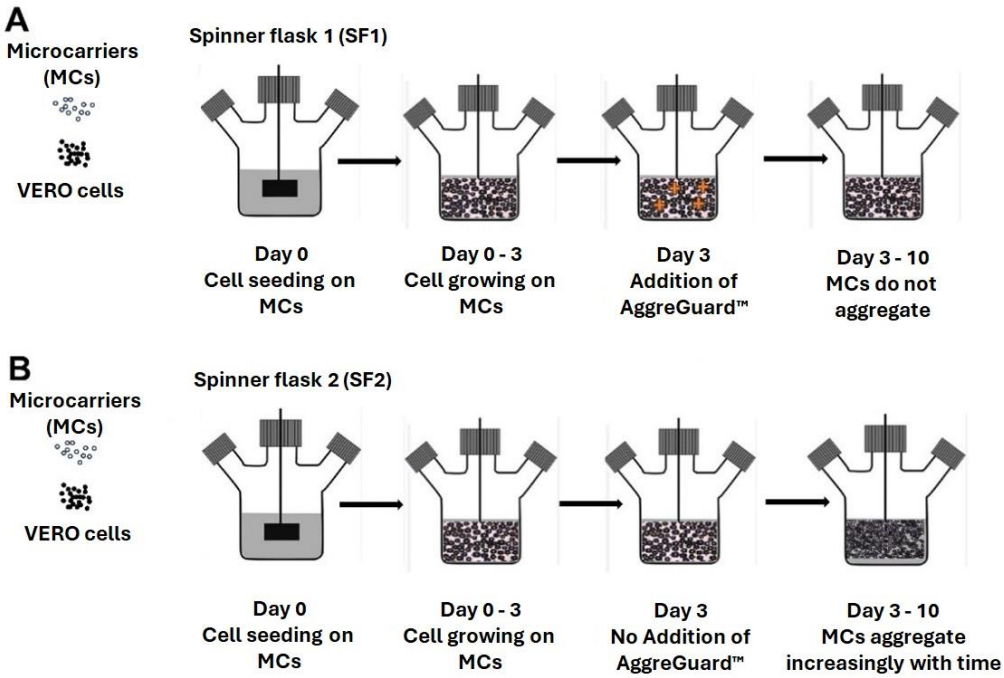

Figure 1. Experimental plan schematic. VERO cells were grown on MCs in spinner flasks with (A) or without (B) AggreGuard™ for the duration of the batch/fed-batch run. The presence of such supplement prevented the aggregation of MCs during the time in culture.

The presence of MCs aggregates is a common challenge in the field. The addition of AggreGuard™ prevented microcarrier aggregation for the duration of the bioprocess (**Figure 2A**). 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 (**Figure 2B, No AggreGuard**). Propidium iodide (**PI**) is typically used to determine the extent of cellular death in cell cultures, where a more intense signal indicates more cell death. The addition of AggreGuard™ not only prevented microcarrier aggregation, but also had a low PI signal intensity (**Figure 2B, With AggreGuard™**) at the end of the 10-day process.

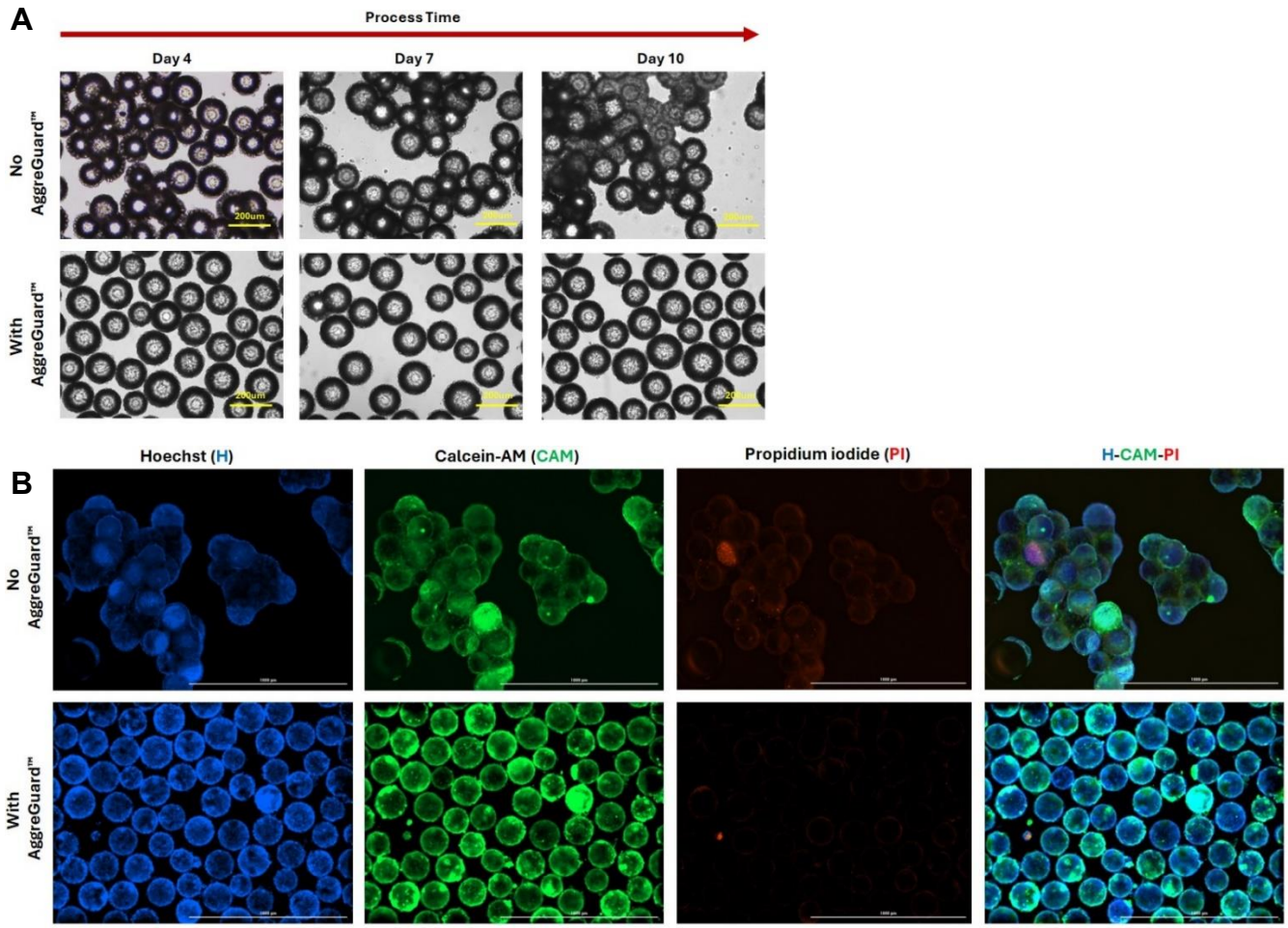

Figure 2. AggreGuard™ controls microcarrier aggregation. A) Brightfield images taken intermittently during the process shows that without AggreGuard™ there is significant aggregation as typically found with batch/fed-batch microcarrier bioprocesses. B) Qualitative cell health was much better (lower propidium iodide-PI signal) with the use of AggreGuard™ during the process. Scale bars in panel A correspond to 200µm and 1000µm for fluorescent images.

Qualitative analysis of the effect of long-term exposure of VERO cells to AggreGuard™ was done over a 60-day culture period, successfully preventing microcarrier aggregation (**Figure 3A**). Partial media exchange (25-50%) was done every 48-96h, with AggreGuard™ allowing a high cell viability (**Figure 3C, 95% vs. 75%)** at process end, with an overall improved cell yield (**Figure 3B, 80-fold vs. 63-fold**). Harvested cells were cultured on standard T-flasks (**Figure 3D**) and showed retained morphology and a preserved doubling time (~21h).

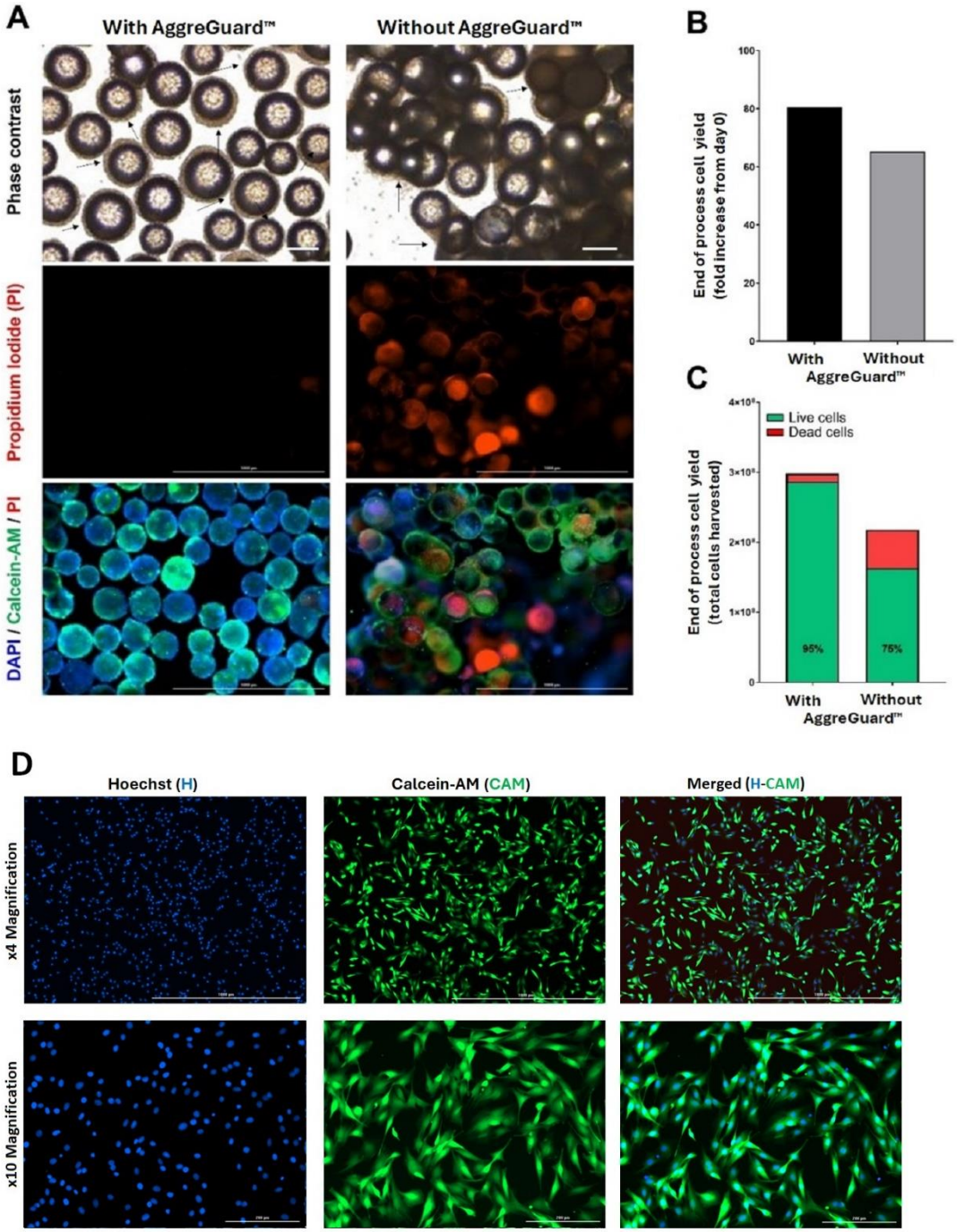

Figure 3. AggreGuard™ exposure over long term increases final cell yield. A) Images taken at end of the process showing the presence of dead cells in the MC aggregates (without AggreGuard™), whilst no MC aggregates or dead cells are visible with the addition of AggreGuard™. Cells were harvested from the MCs at the end of the process from both spinners with both yield (B) and viability (C) higher when AggreGuard™ was used. D) Qualitative phenotype assessment of the VERO cells was done to determine morphology and doubling time, where both characteristics were normal. Scale bars in panel A correspond to 200µm and 1000µm for phase contrast and fluorescent images, respectively.

Conclusion

AggreGuard™ improves microcarrier-based cell culture for VERO cells, without affecting the basic phenotype of the cells. This demonstrates high potential to improve existing VERO cell processes, where workflow integration is with minimal disturbance to existing media addition strategies.