Keep it Single: AggreGuard™ enables homogenous microcarrier cell culture environments

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Abstract

AggreGuard™, a media additive developed by CellRev, enables time-dose dependent control of cell-to-cell adhesion from cells growing on adjacent microcarriers. Cell-to-cell attachment in microcarrier-based cell culture induces aggregation and subsequent tissue formation in a bioprocess, resulting in decreased cell culture homogeneity and loss of bioreactor productivity. Vero cells are a classical workhorse cell line for vaccine and viral vector production, where cell-to-cell adhesion severely impacts bioprocesses with an associated loss in biomanufacturing efficiency, feeding through to cost of goods. In this study CellRev demonstrated how AggreGuard™ controls cell-to-cell mediated microcarrier aggregation for Vero cells grown on Kuraray microcarriers, a new polyvinyl alcohol (PVA)-based microcarrier. Evaluation of the interaction between AggreGuard™, microcarriers and Vero cells were done in disposable 125mL Corning® spinner flasks in a generic fed-batch approach. Results show that AggreGuard™: (i) prevented microcarrier aggregation; (ii) allowed cells to reach and maintain confluency on the microcarrier surfaces; (iii) improved culture homogeneity and (iv) allowed for higher cell viability. Data suggests that “keeping it single” prevents microcarrier aggregation with AggreGuard™ - a promising solution for bioprocess development while maintaining good cell viability.

Introduction

African green monkey kidney-derived epithelial cells (Vero) have been a crucial platform for vaccine production since being isolated by Yasamura and Kawakita in 1962[1], intrinsically more prone to viral infection. Vero cells have been approved to produce vaccines[1-3] such as Polio, Smallpox, Rabies, Japanese Encephalitis, Rotavirus, Vaccinia, and Influenza. The workhorse of legacy vaccines is also being used for the development of new vaccines[1,2] and viral vectors[1,3] for Polio, Ross River, West Nile, Chikungunya, Tick-Borne Encephalitis, Hepatitis A, and SARS-Coronavirus.

Microcarriers provide a surface to cell culture volume advantage for anchorage-dependent cells such as Vero cells. Adapting cells to microcarrier culture is not a trivial task for complex environments such as spinner flasks and stirred tank bioreactors. Besides general process parameter optimisation, cell-to-cell adhesion can induce microcarrier aggregation. Extracellular matrix (ECM) secretion and high cell confluency can cause the microcarriers to clump together, reducing the available surface area for cell attachment, and leading to uneven nutrient distribution which impaired cell growth, transduction efficiency and complicated harvesting processes. These challenges become amplified during scale-up to larger bioreactor systems where agitation, aeration and media feed strategies add an extra layer of complexity. Bioprocess optimisation can address some of these process challenges, but cell culture homogeneity in microcarrier processes remains an industry recognised challenge.

Kuraray microcarriers are made from polyvinyl alcohol (PVA), a synthetic polymer known for its biocompatibility, chemical stability, and water solubility, and coated by collagen[4]. These microcarriers provide 2,600cm^2/g of culture surface area and have an average diameter of 180 µm. Previous publications[4] demonstrated that Vero cells readily attached to these microcarriers and showed good proliferation and surface coverage. This application note investigated how CellRev supplement AggreGuard™, would influence a fed-batch process in spinner flasks for the expansion of Vero cells on Kuraray microcarriers.

Materials and Methods

The summarised materials used in this study can be found in Table 1. A brief discussion on methods follows.

Table 1: Materials used for this study

Components	Description
Cell line	Vero cells (ECACC Cat No: 84113001)
Microcarrier type	Kuraray PVA Microcarriers (Kuraray Life Innovation Promotion Group M11018SAC1-01GB)
Composition of medium	DMEM/F12 (Gibco Cat#31330028) enriched with 5% FBS (BioSera Cat No: 1001 018135116) and Penicillin-Streptomycin (Gibco Cat#51540122).
Dissociation agent	Gibco™ TrypLE™ Express Enzyme
Cell Culture Flask	175 cm^2 culture flasks (Greiner Bio-One Cat. No. 660175).
Spinner Flask	125 mL; Corning Cat. No. CLS3152
AggreGuard™	Developed by CellRev

Cell Culture: Vero cells were grown in DMEM/F12 supplemented with 5% FBS and Penicillin-Streptomycin, maintained in 175 cm^2 culture flasks before being seeded onto microcarriers in spinner flasks (Table 1).

Microcarrier Protocol Development: The collagen coated PVA microcarriers were prepared as per the manufacturer's guidelines using DMEM/F12 and overnight hydration, followed washing, culture media immersion and transfer of the microcarriers to 125 mL Corning® disposable spinner flasks. A control (without AggreGuard™) and a AggreGuard™ supplemented process were tested in parallel. Each spinner flask was aliquoted with the equivalent of 310mg of microcarriers (18cm^2/mL) in 40 mL of culture medium (DMEM/F12 + 0.1% FBS), followed by seeding of Vero cells (17,000 cells/cm^2). The initial attachment phase lasted 3 hours and followed an intermittent stirring protocol (20rpm, 28 minutes off/2 minutes on) using a 2mag bioMIXdrive agitation system in a humidified incubator at 37°C, 5% CO_2. After cell attachment, full medium exchange was done with 5% FBS-DMEM/F12, to a total cell culture volume of 50 mL. Agitation speed was adjusted to compensate for increasing cell density on the microcarriers, reaching a maximum agitation of 50rpm by day 6. AggreGuard™ was added on day 3, when doublets and triplets of aggregates were observed in both spinner flasks. Total culture duration was 17 days, based on average duration fed batch runs used in |Vero cell bioprocess protocols. Partial media exchange (25%) was done daily, with the AggreGuard™ supplementation. The control flask only underwent partial media exchanges.

Culture Monitoring: Daily sampling was performed to qualitatively (brightfield microscopy) assess cell attachment on microcarriers, cell-surface coverage on the microcarriers and microcarriers aggregation status. This was complemented with Live-Dead staining (Hoechst/Calcein-AM/Propidium iodide) using a Cytation 5 (BioTek) and daily monitoring of glucose and lactate levels using handheld biosensors.

Results and Discussion

Cell attachment and growth: Results showed that by Day 1 most cells attached to the microcarriers, with adherence already visible 3h post-seeding (Fig. 1) for both spinner flasks. Twenty-four hours post-inoculation, the Vero cells adhered to and expanded across the Kuraray microcarriers, with significant surface coverage of the microcarriers observed on Day 3 (*Fig. 1*).

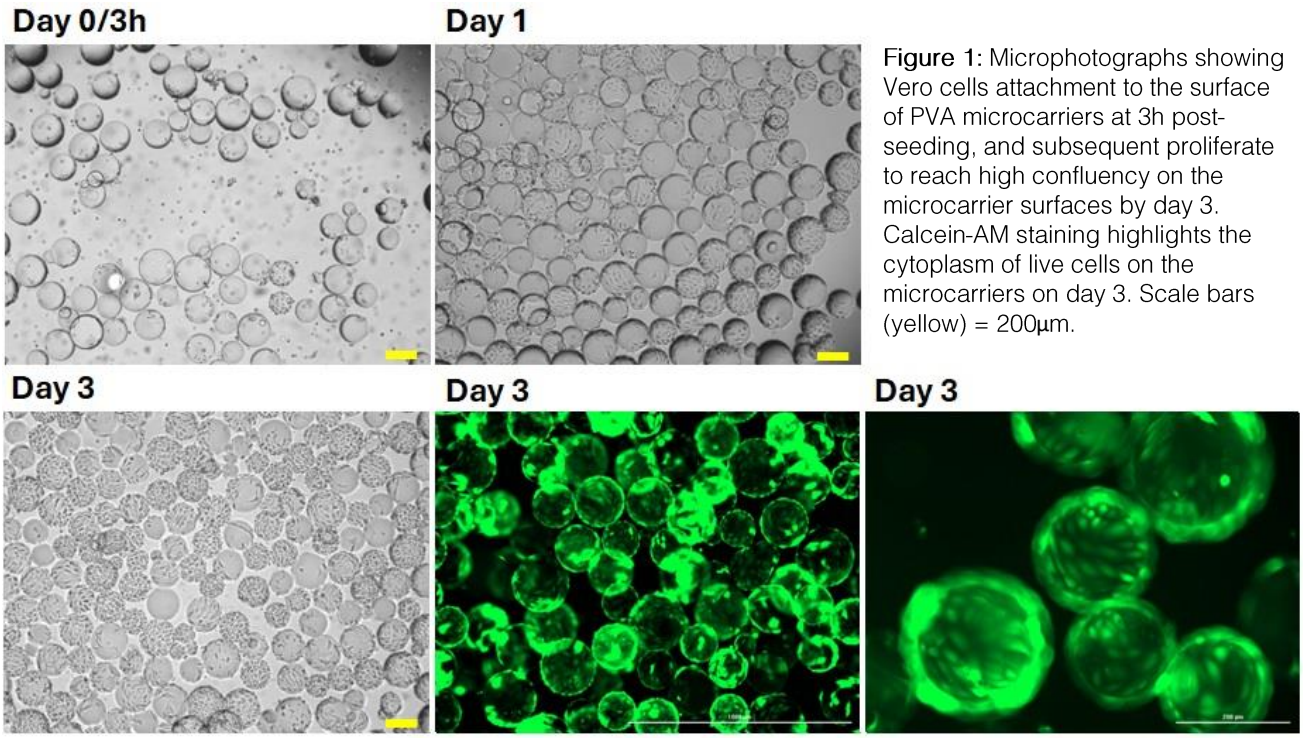

Figure 1: Microphotographs showing Vero cells attachment to the surface of PVA microcarriers at 3h post-seeding, and subsequent proliferate to reach high confluency on the microcarrier surfaces by day 3. Calcein-AM staining highlights the cytoplasm of live cells on the microcarriers on day 3. Scale bars (yellow) = 200µm.

AggreGuard™ prevents microcarriers aggregation: When the cells reached ~70% confluency on Day 3, comparable for both spinner flasks, AggreGuard™ was added to one spinner flask and not to the control flask. Microcarrier aggregation was clearly observed in the control spinner from Day 5 (*Fig. 2*), which, as expected, worsened over time. In contrast, the spinner treated with AggreGuard™ exhibited no aggregation throughout the entire experiment (*Fig. 2*), suggesting that AggreGuard™ effectively inhibits this process.

When samples from both spinners were stained with Calcein-AM and propidium iodide, they revealed that while both groups had microcarriers covered with live cells (*Fig. 3*), the control group had significantly more dead cells, as indicated by the propidium iodide staining (*Fig. 3*). This highlights AggreGuard™'s role in not only prevents aggregation but improves microcarrier culture homogeneity and supports healthier cell growth.

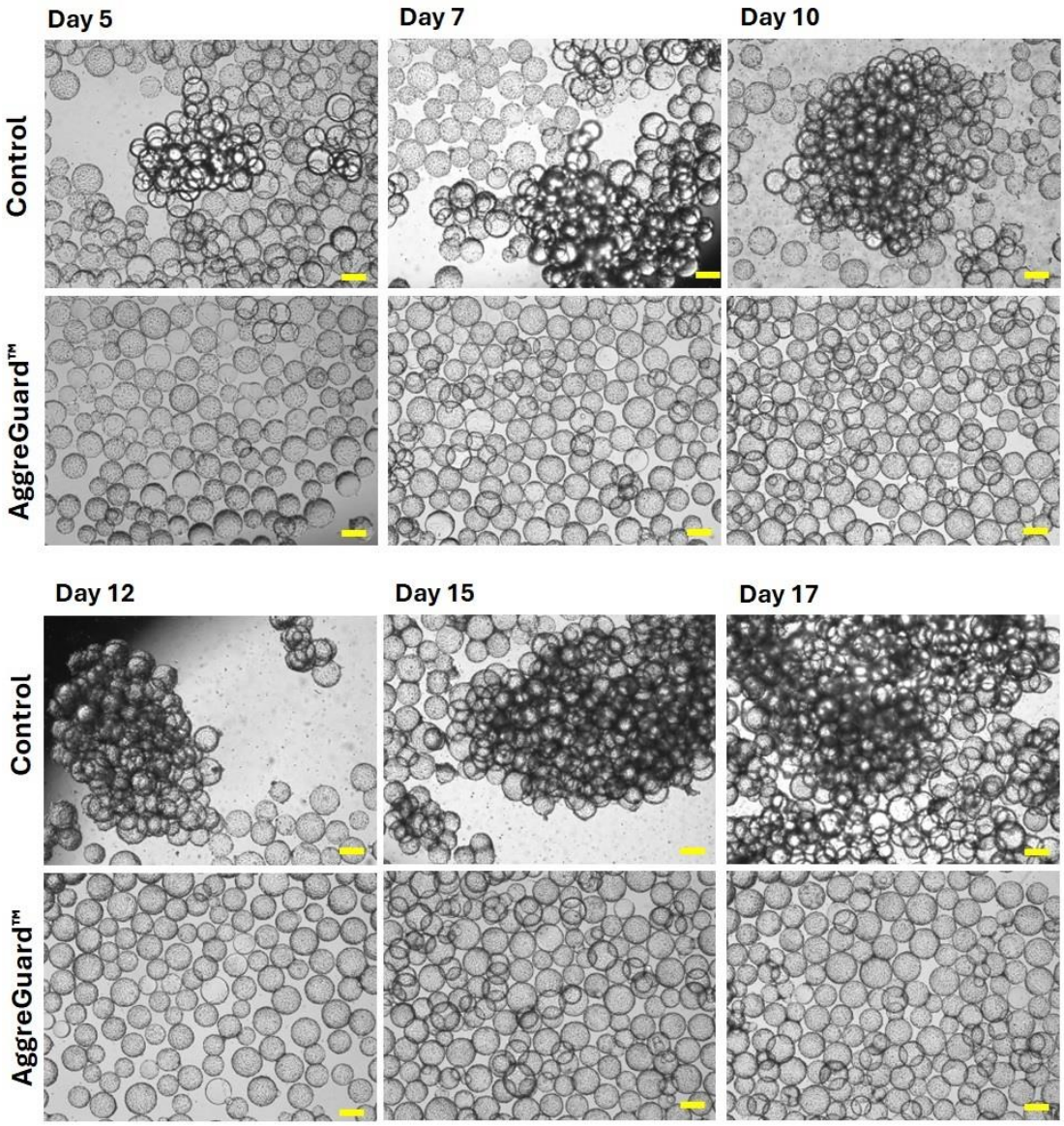

Figure 2: AggreGuard™ prevents cell-to-cell mediated microcarrier aggregation over 17 days of fed-batch culture. The control spinner flask shows increasing microcarrier aggregation over the process until the end of the experiment. Scale bars: 200µm

Glucose consumption and lactate secretion: Glucose levels drop rapidly in both experimental conditions from day 1 to day 3, which is expected with increased cell proliferation. On day 3, glucose levels stabilize around *5 – 7 mmol/L* (*Fig. 4*) for both groups with a similar trend observed for lactate (*Fig. 4; 5 – 8 mmol/L*). Interestingly, the AggreGuard™-treated culture exhibits a minor increase in glucose consumption compared to the control. Live-Dead staining indicates this glucose consumption could be attributed to more viable cells in the AggreGuard™ treated spinner flask (*Fig. 3*). Daily media exchanges help to maintain steady lactate levels (< 10 mmol/L) and was comparable between both spinner flasks.

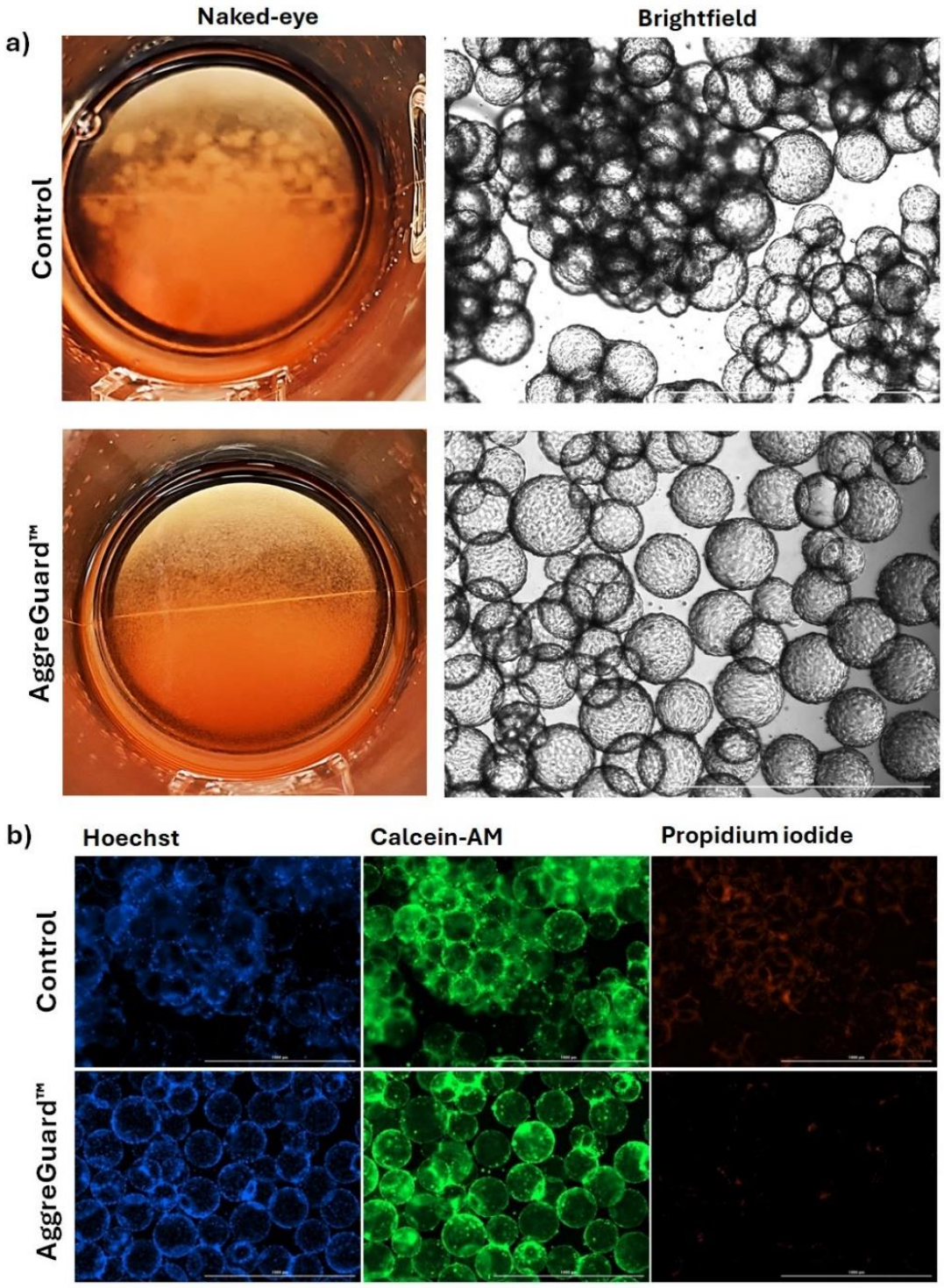

Figure 3: a) Naked-eye observation (captured with a mobile phone camera) correlates with qualitative brightfield microscopy - AggreGuard™ prevents microcarrier aggregation when compared to control. Fluorescent staining (b) shows no aggregation when AggreGuard™ is present in culture, with cells spread homogenously over microcarrier surfaces and no bead-to-bead adhesion. The propidium iodide signal is significantly more pronounced in the control spinner flask where dead cells are prominently present on the cell-to-cell adhesion

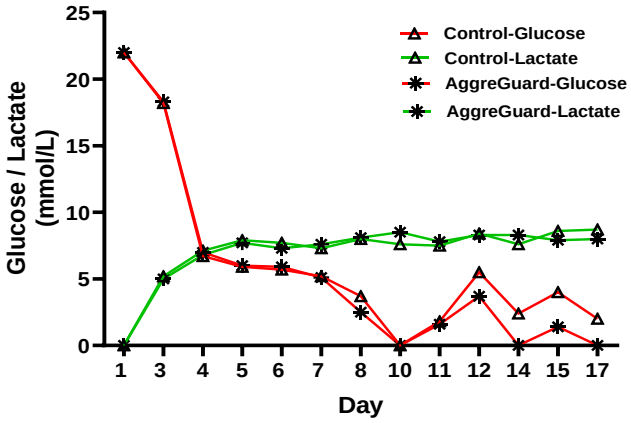

Figure 4: Glucose consumption and lactate production in Vero cell cultures grown on PVA microcarriers.

Conclusion(s)

The aim of this study was to determine if AggreGuard™ was compatible with a novel PVA microcarrier develop by Kuraray. The results indicate the following:

- Vero cells readily attached to the Kuraray microcarriers, with proliferation observed until full microcarrier surfaces was covered by the cells.
- AggreGuard™ maintained the cell density on the microcarriers for the whole experimental duration of 17 days.
- AggreGuard™ effectively prevented Kuraray microcarrier aggregation and improved cell culture homogeneity.
- The overall cell viability was improved when AggreGuard™ was present in cell culture, compared to control.

References

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