Raw Material Variability in Biomanufacturing: A Case Study Using Poloxamer 188

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Introduction & Aim

Media for cell culture has evolved from recipes containing plant or animal-derived products, to fully chemically defined (CD) formulations [1] as an effort to reduce variability in bioprocess performance due to raw materials. A critical ingredient, poloxamer 188, has a history of variability [2] due to hydrophobic species (Fig. 1).

Intra-lot, lot-to-lot and vendor-to-vendor variability in raw materials can bring forth an imbalance of some key media ingredients which can directly impact the bioprocess by adding variations, losses in yield and adding concerns for compliance and changes in final product (Fig. 2). The present work looks to showcase the consistency in cell performance of different lots of critical raw material, Kolliphor® P188 Bio, a GMP version of poloxamer 188 that is a fit-for-function shear protectant used in biomanufacturing. In our study, we compared cell performance within, Intra-Lot, and between, Inter-Lot, two lots of Kolliphor® P188 Bio.

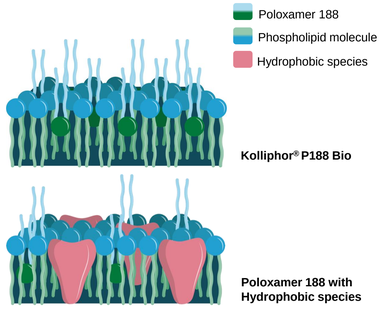


Fig. 1: Hydrophobic species are more surface active and can disrupt shear protection of poloxamer 188 even at low concentrations

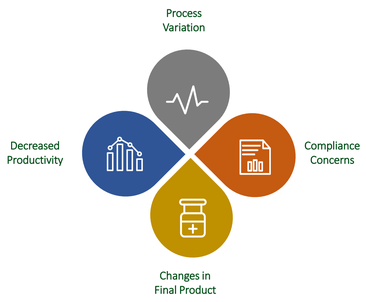


Fig. 2: How raw material variability can impact a bioprocess and final product

References:

[1] Yao et al., 2017, Reprod Med Biol. 16
[2] Biophorum Raw Material Risk Assessments, 2019

Materials & Methods

Drums of two lots of Kolliphor® P188 Bio were allocated into one of these categories: Early, Middle or Late depending on the time they were filled during the manufacturing cycle (Fig. 3). From these categories, we sampled n>>1 drums and used HEK293 cells to test cell viability over a 4-day period (Table 1). Samples were tested in triplicate.

Cell performance was analyzed using analysis of variance (ANOVA), where we compared cell performance in the presence of different drums from two lots of Kolliphor® P188 Bio. When comparing lots from within a lot, Intra-Lot, we found that both Kolliphor® P188 Bio lots had good performance indicated by high cell viability and there was no significant statistical difference in cell performance between the different groups of drums within a lot. When comparing drum groups of two lots of Kolliphor® P188 Bio, Inter-Lot, we found no statistically significant difference in cell performance between drum groups between the two lots. We compared these results to the cell performance of a Non-Bio Lot of poloxamer 188 which had much lower cell performance than the Kolliphor® P188 Bio lots tested.

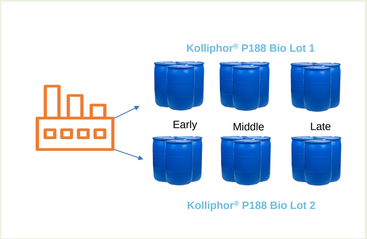
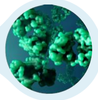


Fig. 3: How raw material variability can impact a bioprocess and final product

Table 1: Cell Culture Test Method Summary
Working volume of 50mL in a 500mL baffled shake flask
Shaking intensity of 300 RPM
P188 concentration @ 0.25g/L
Test duration is 4 days, with sampling every day
Cell line is non-clonal HEK293 (GMP-banked), cultured in HEK293 Medium
Seeding density is 3 x 10^5 cells/mL
Seeding set-up requires spin-down & resuspending cells 3x to remove all P188 carryover
Positive and negative controls are lots that have been identified as "suitable" and "underperforming", respectively



Results & Discussion

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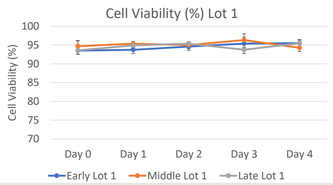
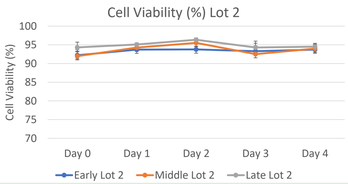
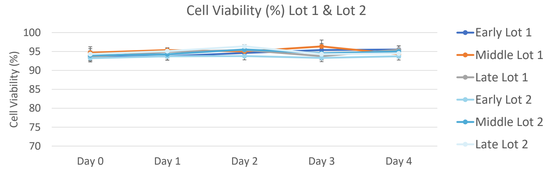
Intra-Lot Variability





Inter-Lot Variability



Conclusions

- Kolliphor® P188 Bio offers shear protection while reducing variability of the bioprocess.
- Kolliphor® P188 Bio provides consistent cell performance within and across lots.
- Using fit for function raw materials can help reduce the risk of variability.