From chemical to mechanical: A comparative analysis of cell lysis strategies for mammalian cells

A Youssef, D Bitnel, J Wagner, M Boscher, J Babic, B Larena Carnio, T Fischer, B Hager, C Zach, M Gora, T Kloetzler, A Schoberth, K Heller

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Abstract

Adeno-associated virus (AAV) gene therapy offers a revolutionary approach to treating a wide spectrum of genetic diseases. However, realizing its full potential hinges on the development of robust and scalable manufacturing processes providing high quality and high potency vectors at commercially viable costs. A critical bottleneck within this process is cell lysis, the controlled disruption of production cells to release the encapsulated AAV vectors. Most AAV serotypes mainly reside within or adhere to the production cells and are not significantly released to the supernatant, and the chosen lysis strategy to harvest rAAV significantly impacts both the yield of AAV vectors and the efficiency of downstream purification steps. In this study, we evaluated and compared various cell lysis strategies specifically tailored for AAV gene therapy manufacturing. We investigated a range of methods, including:

- Chemical Lysis: This method employs mild detergents or salts to selectively permeabilize the cell membrane, allowing the release of intracellular components without compromising the integrity of the AAV vectors. However, selecting the appropriate chemical and optimizing its concentration are crucial to ensure efficient lysis while minimizing unwanted interactions with the AAV particles. We compared in this study various commercial and in-house cell lysis reagents.

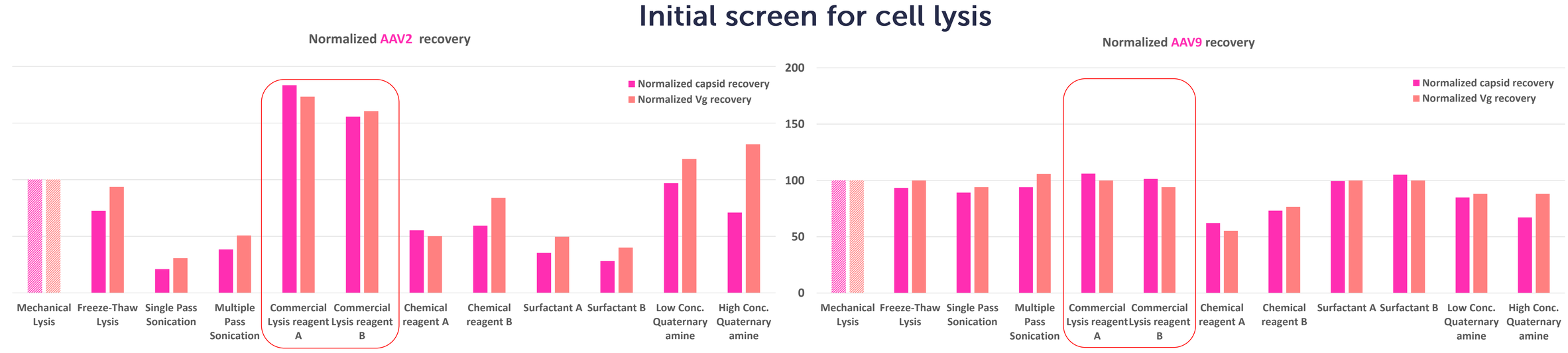
- Sonication: Sound waves are used to disrupt the cell membranes in this method. While offering scalability and relative ease of implementation, sonication can generate significant heat, potentially affecting the stability and functionality of the AAV vectors.

- Mechanical Lysis: This approach utilizes high pressure homogenization to physically disrupt the cell membranes, releasing the intracellular contents. While efficient, it can also damage the delicate AAV particles, necessitating careful optimization of the selected pressure.

We assessed each method's impact on several key parameters. Cell lysis efficiency was evaluated through recovery calculations of the AAV vector yield, determined using digital droplet PCR (ddPCR) and enzyme-linked immunosorbent assay (ELISA). As a follow-up to this study, it is planned to investigate the compatibility of the lysate generated by chemical lysis with that generated by our current gold standard (mechanical lysis) for subsequent downstream processing steps, particularly focusing on factors like lysate viscosity, turbidity, pH and conductivity.

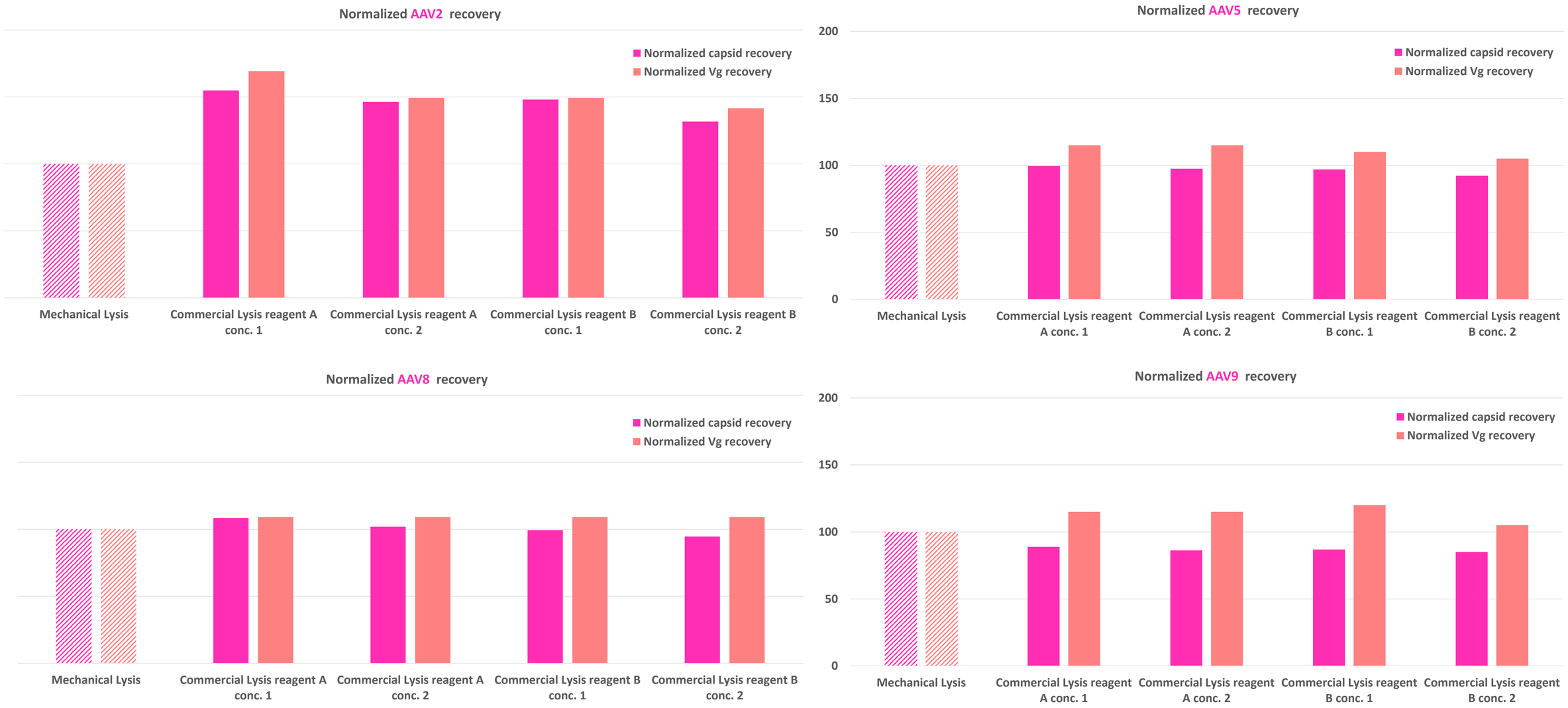
By presenting a comparative analysis of these diverse lysis strategies, we aim to highlight their relative strengths and weaknesses in terms of cell lysis efficiency, product quality impact and potential for large-scale manufacturing. Our current manufacturing platform is strong, reliable, and delivers excellent results. We're also actively developing next-generation processes to push efficiency, quality, and cost-effectiveness even further. Paving the way for wider clinical application and improved patient outcomes.

Initial screen for cell lysis



The data shows capsid recoveries using Gyrolab immunoassay as well as vector genome recoveries using ddPCR against our proprietary mechanical lysis method. Our mechanical lysis method is still our best option for AAV9 with the advantage that no further chemicals or reagents are added, that require DSP removal. However, for AAV2, we observed that commercial lysis reagents were superior to mechanical lysis, hence these two candidates were further tested against a panel of other serotypes.

Confirmatory evaluation across multiple serotypes



The two commercial candidates from the first trial were tested using different AAV serotypes at two different concentrations each and evaluated using capsid and genome titer recoveries. We observed that the cell lysis effect was still maintained when decreasing the concentration of the lysis reagent. We also observed that cell lysis efficiency of our proprietary mechanical lysis was still comparable to the chemical lysis except for only the AAV2 serotype that showed an advantage in recovery when cells were lysed chemically. There was no impact of Full:Empty ratios with the different lysis methods. This demonstrates the importance of developing modular manufacturing platforms, that allow adaptation to specific needs of given vectors and AAV serotypes.

Summary

Our modular platform is designed to evolve with the rapid advancements in the AAV field. By continuously innovating and optimizing key areas, such as cell lysis unit operation, we ensure our clients can effortlessly integrate cutting-edge technologies. Our recent trials have yielded promising data with chemical lysis reagents, particularly for AAV2, which we'll further evaluate for their impact on downstream processing. Alongside key vector quality parameters including encapsidated impurities and vector potency. This commitment to optimization aligns with our broader goal of supporting clients throughout their product lifecycle, from early-stage development to commercial manufacturing. We prioritize yield, quality, risk management, and route of administration-specific needs, tailoring our platform to meet the unique requirements of each product.

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ahmed.youssef@ascend-adv.com