Characterization of Engineered AAV Capsids from Different HEK293 Cell Culture Fractions, Crude Lysate Versus Cell Pellet Material (Abstract 529)

CAPSIDA BIOTHERAPEUTICS

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Introduction and Objectives

Recombinant adeno-associated virus (rAAV), using wildtype serotypes, has emerged as a promising delivery vehicle for gene therapies. To address the limitations of existing serotypes, Capsida is engineering novel capsids using a proprietary process of library generation and in vivo screening. These engineered capsids are selected for improved tissue tropism, enhanced transduction efficiency, and reduced toxicity profiles when compared to wildtype serotypes providing the potential to treat both rare and common diseases across all ages. As part of Capsida's end-to-end gene therapy solutions, we characterized different production methods for engineered capsids focusing on product distribution, capture method, and removal of process-related impurities. This work using engineered capsids:

1. Assesses product distribution (vg/ml) in distinct fractions (CPL = Cell Pellet Lysate, SN = Supernatant, CL = Crude Lysate) of cell culture.
2. Identifies differences in residual profiles (i.e, hcDNA) in the different cell culture fractions.
3. Evaluates different purification methods (affinity chromatography and iodixanol gradient purification) assessing process-related impurities and other practical considerations given the stage of a program.

Methods and Materials

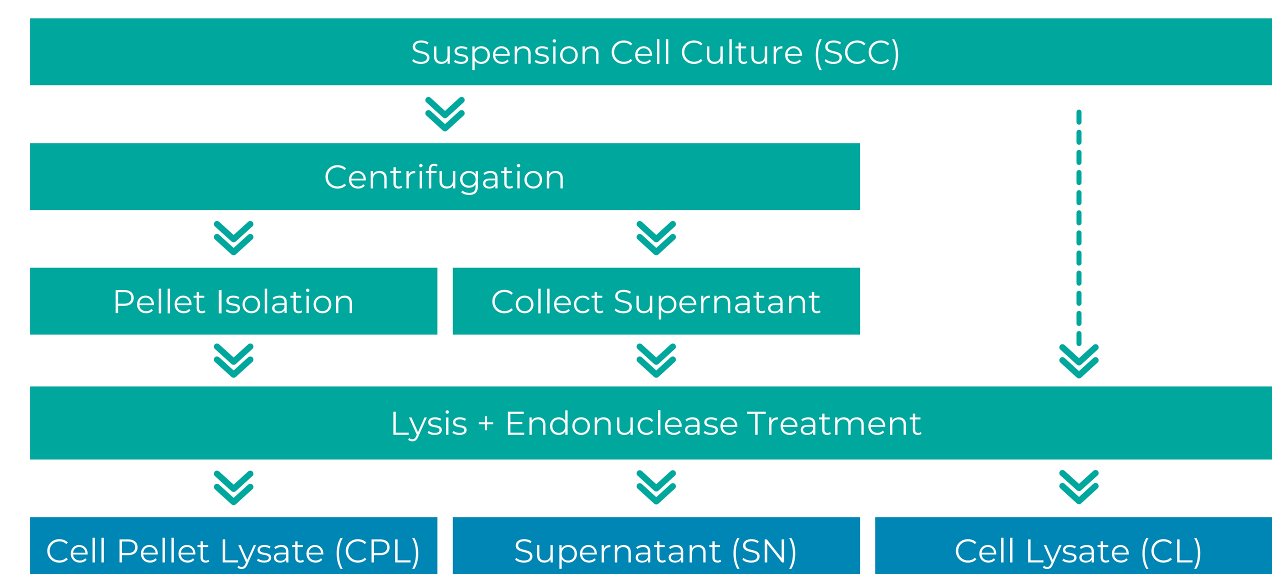


Figure 1. Fraction Collection

Material is generated in a stirred bioreactor which controls temperature, agitation, pH, and dissolved oxygen (DO). Bioreactor material is lysed 72 hours following transfection.

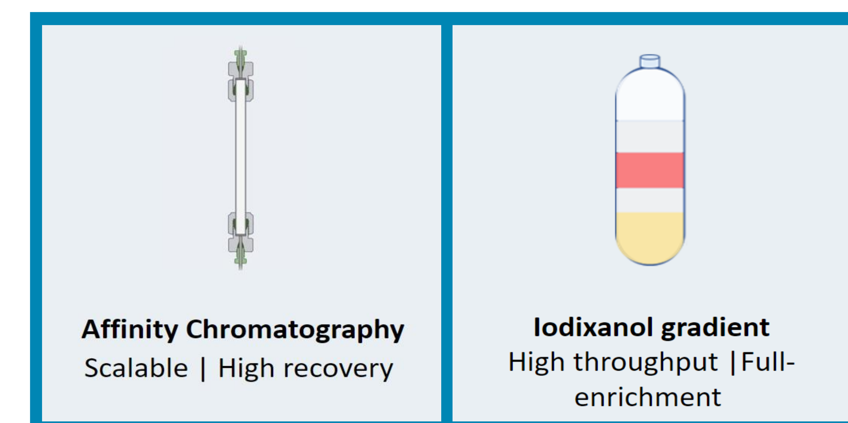


Figure 2. Methods of Purification

Material was purified using affinity chromatography or iodixanol ultracentrifugation gradients (IDX). In early product development, IDX offers a higher throughput method for purifying the smaller volumes of many different cargo-capsid combinations. For larger scale productions, affinity chromatography has advantages.

Results

Product Distribution by Fraction

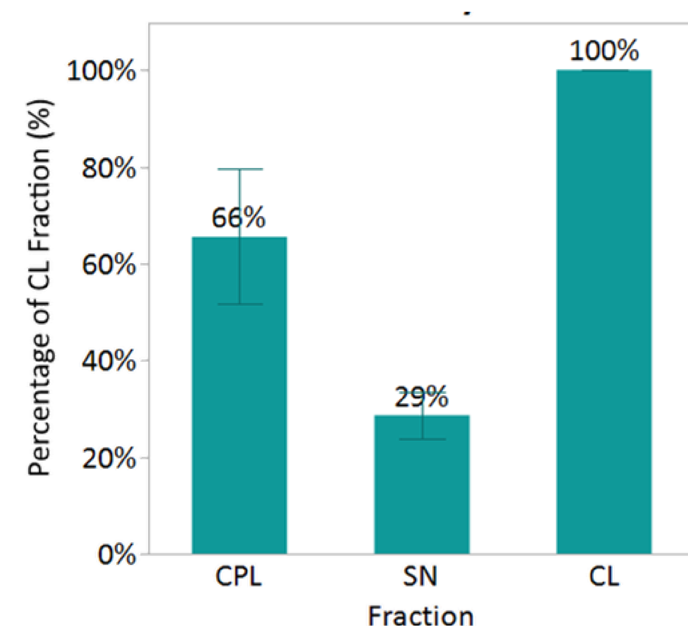


Figure 3. Product Distribution by Fraction, Three Different Engineered Capsids Screened:

Titer analysis (vg/ml) indicates that 2/3 of the total material can be found in the CPL. Material was produced for three distinct engineered capsid types, with no significant difference observed in fraction yields between capsid types. The following were used to calculate % distribution:

$$SN_{vg/ml} * V_{SN} / V_{SCC} * CL_{vg/ml}$$

$$CPL_{vg/ml} * V_{CPL} / V_{SCC} * CL_{vg/ml}$$

Where V_{SCC} is the total cell culture volume used to generate the fraction.

Host Cell Protein (HCP) by Purification Method

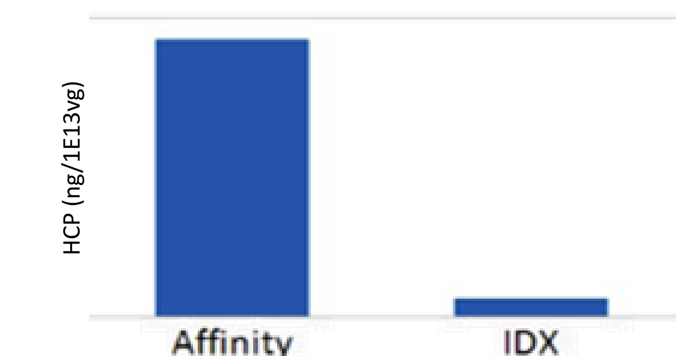


Figure 5. IDX material has lower HCP levels

CL material taken through IDX purification has lower HCP levels compared to affinity. An affinity purification train typically has subsequent polishing steps.

Product Distribution in Wildtype Capsid Serotypes

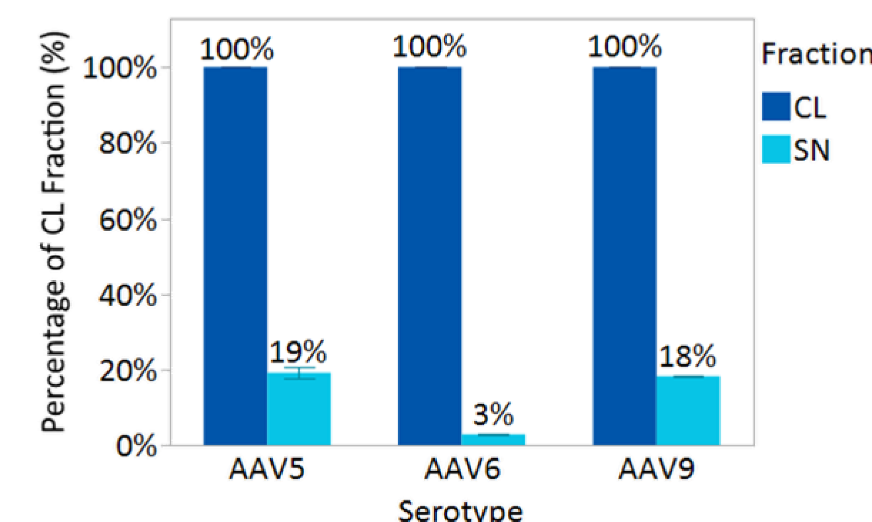


Figure 4. Titer Distribution by Serotype

A number of wt capsids were tested for product distribution profiles. Results indicate product distribution profile can differ based on serotype.

Normalized Encapsidated hcDNA (ng/1E13vg) by Fraction

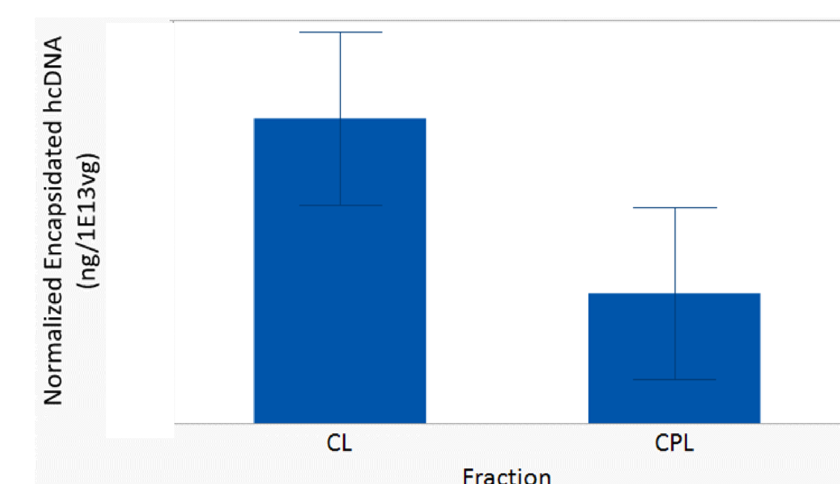


Figure 6. hcDNA Profiles Stirred Bioreactor Runs (n=11)

4L bioreactor batches of CL material were purified via affinity chromatography. A parallel CPL sample was collected for each run and purified by IDX purification. Crude lysate fraction (CL), which includes the supernatant, has higher encapsidated hcDNA.

Comparison Of Purification Methods

Normalized Encapsidated hcDNA (ng/1E13vg) by Fraction and Purification Method

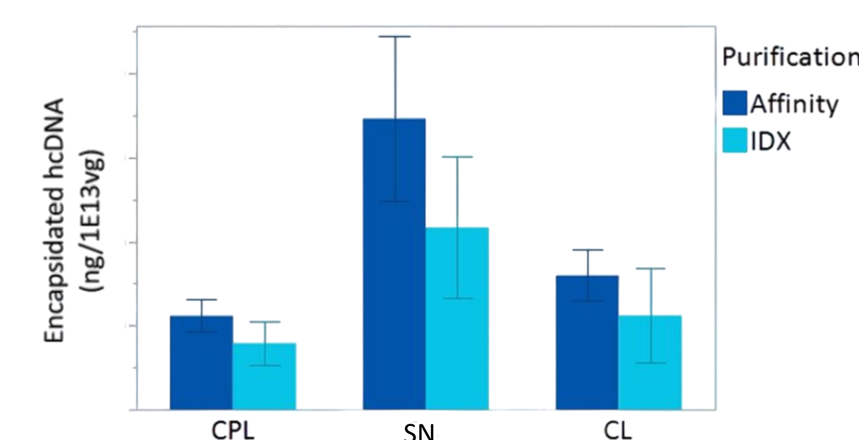


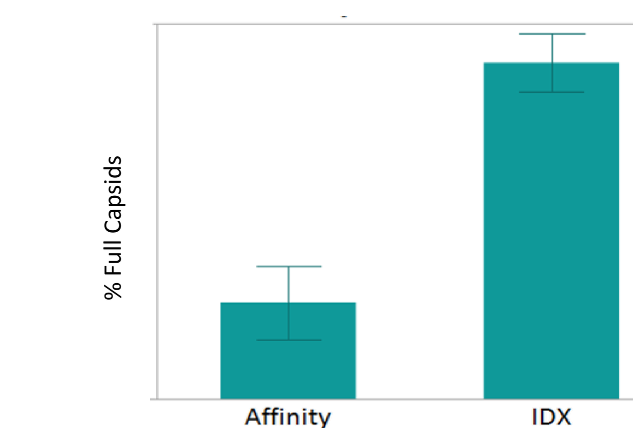
Figure 7. Comparison of Purification Methods

Identical starting material and fractions were purified by both affinity and IDX. Results were normalized for comparison. Data suggests differences in product profile depending on purification method. IDX shows lower levels of hcDNA, likely due to removal of mispackaged capsids. Both methods of purification suggest similar trends in impurity profiles.

Note: results presented are from analysis of independent fractions. When weighting the CPL and SN fractions by the partition coefficient, the calculated hcDNA value is within 8% of the measured CL number, refer to equation:

$$hcDNA_{CL} = CPL_W * hcDNA_{CPL} + SN_W * hcDNA_{SN}$$

A Full Enrichment by Purification Method



B Recovery by Purification Method

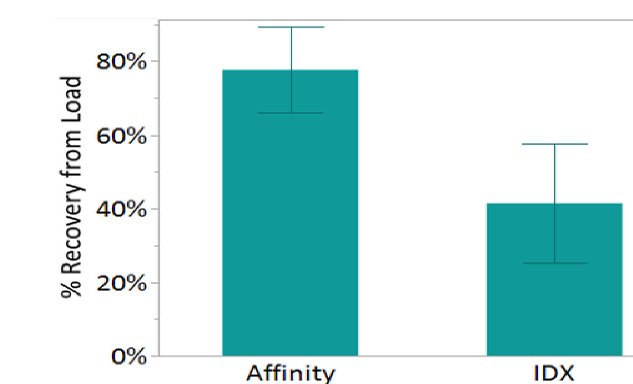


Figure 8. Purification Methods have Distinct Advantages

Purification by IDX yields 3x full capsid enrichment while affinity yields minimal enrichment (Fig 7.A). Affinity offers better product recovery over IDX (Fig 7.B).

Note: affinity-based processes undergo further enrichment steps to remove partials and mispackaged capsids.

Conclusions

The development and characterization of engineered capsids by Capsida marks a significant advancement in the field of gene therapy delivery systems. Our data from 11 different lots of HEK293 suspension material spanning three different capsids show a consistent partitioning profile. Most of the vector resides in the cell pellet 72 hours post-transfection. Notably, the CPL fraction showed lower hcDNA impurities when compared to the SN fraction which is an important consideration when developing harvest approaches and selecting process parameters.

Both affinity and IDX purification trains provide material with consistent impurity profiles. Each train has advantages and disadvantages. Selection depends on the user's objectives e.g., yield, throughput, impurity levels. The work presented here provides insights that can be applied to the manufacturing of engineered capsids, an exciting modality that has enormous potential to address unmet medical needs in both rare and common diseases across all ages.

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2. Vandenberghe, LH., Xiao, R., Lock, M., Lin, J., Korn, M., & Wilson, JM. (2010). Efficient serotype-dependent release of functional vector into the culture medium during adeno-associated virus manufacturing. Human Gene Therapy, 21(10), 1251-1257. https://doi.org/10.1089/hum.2010.107.

Acknowledgements

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