Characterization of factors that influence the yield and quality of rAAV produced using HSV co-infection

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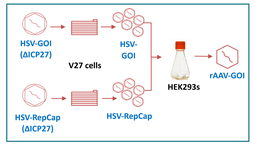
Abstract

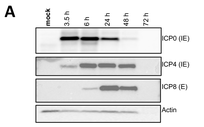
- Increased yield and quality is a key goal in rAAV manufacturing, particularly for diseases that require high systemic doses
- For rAAV production in shake flasks, rHSVs encoding gene of interest (rHSV-GOI) and Rep-Cap (rHSV-RC) were co-infected in HEK293 cells
- Several factors influenced rAAV yield including HEK293 cell density, shaker speed, and the sequence of infection of the two viruses
- Multiplicity of infection (MOI) and cell culture medium were identified to have the most significant impact on rAAV yield and quality
- Characterization of HSV proteins was performed to gain understanding of the rHSV-mediated rAAV production process

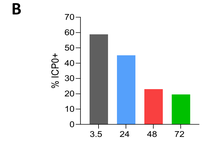
rAAV production using HSV

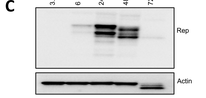


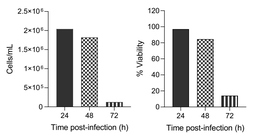
Results

Characterization of HSV Infection









- HEK293s co-infected with rHSV-GOI and rHSV-RC at MOI 2:2
- Sampled at various timepoints for analysis

Figure 1.

(A) Temporal expression of HSV proteins in HEK293 cells co-infected with rHSV-RC and rHSV-GOI. Western blot analysis of ICP0, ICP4 and ICP8.

(B) Percent ICP0-positive cells by FACS.

(C) Induction of AAV Rep protein .

(D) HEK293 cell viability & density at 24, 48, & 72 h post-infection.

Impact of Cell Density, Shaker Speed, and Order of rHSV Infection on rAAV Production

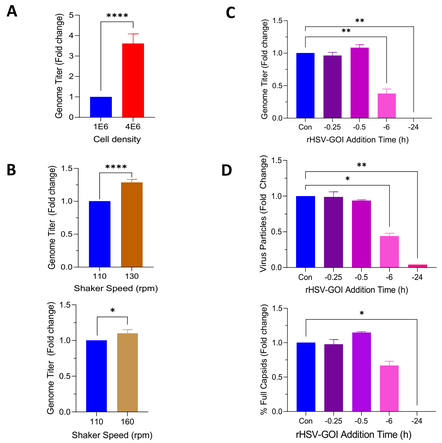


Figure 2.

(A) rAAV yield 3-4 fold increased in cultures infected at 4E6 cells/mL.

(B) Modestly increased rAAV yield with shaking speed increase from 110 to 130 or 160 rpm.

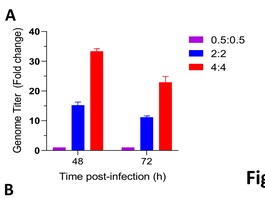
(C) Impact on rAAV yield at 72 h, when two rHSVs sequentially infected; rHSV-GOI first at various timepoints prior to infection with rHSV-RC. Similar yield at early rHSV-GOI timepoints, much lower yield when rHSV-GOI added 6 or 24 h prior to rHSV-RC.

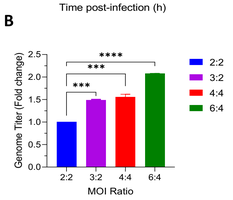
(D) Total rAAV particles measured by capsid ELISA. %Full capsids: GOI titer divided by total capsids. Significantly lower %full capsids when rHSV-GOI added 6 or 24 h prior to rHSV-RC infection.

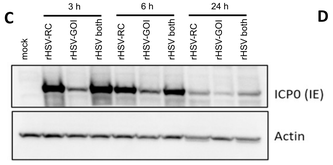
- Statistical Analysis in GraphPad Prism using unpaired t-test or Ordinary One Way ANOVA

MOI is an Important Determinant of rAAV Yield

rHSV-RC Infects HEK293 cells more efficiently than rHSV-GOI







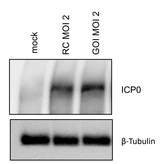


Figure 3.

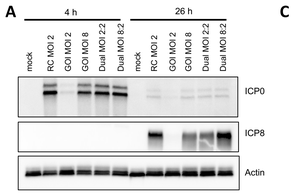
(A) MOI (rHSV-GOI:rHSV-RC) significantly impacted rAAV yield. GOI titers at 48 h and at harvest (72 h).

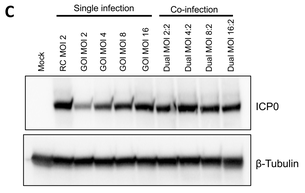
(B) Increased rHSV-GOI to rHSV-RC MOI ratio resulted in increased rAAV yield

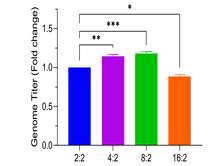
(C) ICP0 level in HEK293s infected with individual rHSVs (MOI of 2) or co-infected at MOI 2:2. Higher ICP0 expression (~4-fold) from rHSV-RC vs rHSV-GOI.

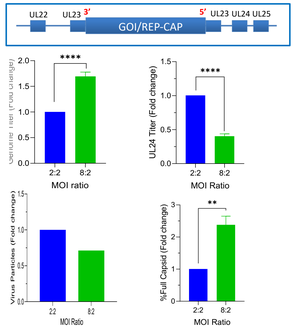
(D) Similar ICP0 expression V27 cells infected with individual viruses (MOI 2) at 4 h post-infection.

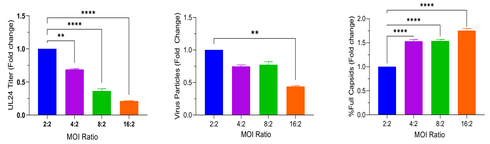
MOI Ratio Studies











- UL24 by ddPCR as surrogate for HSV mispackaging
- Total capsids measured by AAV9 ELISA; % Full capsids calculated by dividing GOI titer by total particles.

Figure 4.

(A) Low levels of ICP0 and ICP8 from rHSV-GOI infection at MOI 2 were significantly increased to rHSV-RC levels when rHSV-GOI MOI was increased 4-fold to MOI 8.

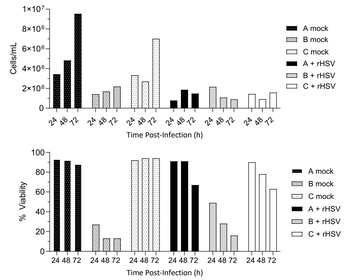
(B) rAAV yield increased 1.5-fold in cells dual infected at MOI 8:2 versus 2:2. HSV mispackaging (measured by UL24 ddPCR) significantly decreased at 8:2 and % Full capsids was doubled in 8:2 vs 2:2. Balancing rHSV MOI to achieve equivalent infection results in increased rAAV yield with improved quality attributes.

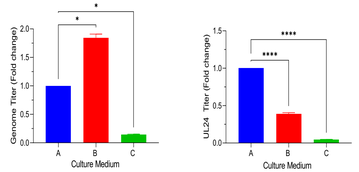
(C) Dose responsive increase in ICP0 expression with increasing MOI of rHSV-GOI.

(D) Modestly improved rAAV yield with increasing rHSV-GOI MOI ratio.

(E) Significant rAAV quality improvements with increasing rHSV-GOI in MOI ratio..

Strong Impact of Culture Media on rAAV Yield & Quality





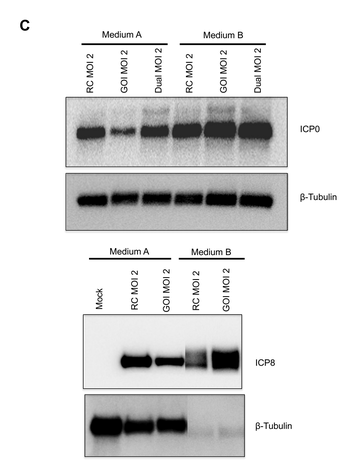


Figure 5.

(A) Cell density and viability in medium A, B or C at various timepoints in culture in the presence or absence (mock) of rHSV co-infection. Lower cell density and viability in medium B.

(B) Higher rAAV yield and reduced mispackaged HSV DNA (UL24 titer) from HEK293 cells infected and cultured in medium B versus A or C.

(C) Increased ICP0 expression (HSV infectivity) in medium B versus A. Equivalent levels of ICP0 in cells infected with rHSV-GOI or rHSV-RC, i.e., medium B achieved re-balancing of rHSV infectivity to achieve increased yield and quality.

Conclusions

- ICP0 is a useful surrogate marker for HSV infection. Quantification of ICP0 to measure and balance rHSV infectivity is a useful tool to improve rAAV production in HEK293 cells
- Increased HEK293 cell density significantly, and faster shaking speed modestly, increased rAAV yield.
- Sequencing of viral addition revealed that simultaneous addition of the two rHSVs for dual infection was optimal for rAAV production.
- Changing MOI of rHSV to HEK293 cells had an MOI dose-responsive impact on rAAV production.
- Less efficient rHSV-GOI infection of HEK293 cells but not V27 cells was unexpected.
- Increased rHSV-GOI (~4-fold) MOI ratio balanced infectivity and gave higher rAAV yield and quality.
- Culture medium had a significant impact on rAAV production; rAAV yield was significantly higher and UL24 much lower in medium B versus A. Higher rHSV infectivity in medium B may have contributed. Strikingly, infectivity of rHSV-GOI was significantly higher, and equivalent to rHSV-RC in medium B.
- Selection of culture medium was a critical factor in rAAV production using HSV.
- The relationship between culture medium and HSV infectivity with its consequent impact on MOI for rAAV production was unexpected and illustrates the value of studies that characterize the biology of HSV infection and rAAV production.