Assessing Branched Amphiphilic Peptide Capsules (BAPC) Delivery of Short Interfering RNA (siRNA) to Mammalian Cells

Laman Mamedova and Barry Bradford

Department of Animal Sciences and Industry, Kansas State University

Objectives

Primary: To evaluate knockdown of target protein following antisense siRNA delivery to GFP-CHO cells with BAPC versus current commercial transfection reagents. Secondary: To assess cellular viability through cryopreservation following BAPC treatment.

Experimental design

Transfection efficiency. CHO-GFP cells were transfected with the following double-stranded RNA complexes: 1) siRNA237 (5' gcagcacgacuucuucaag 3' and 5' cuugaagaagucgugcugc 3') and 2) mission siRNA universal negative control (Millipore). GFP-CHO and CHO (negative control) cells were resuspended in maintenance media and plated at 1×10^5 cells/well in 24-well plates. Treatments were initiated after cells reached ≥ 75% confluence. For comparison of transfection reagents, we used the following reagents combined with 25 nM siRNA:

- Lipofectamine 3000 (ThermoFisher) per manufacturer's instructions
- TransIt-X2 (Mirus Bio) per manufacturer's instructions
- BAPC with fluorescent tracker (633 nm) in 3 variants: 25 nm mean particle size at concentrations of 0.01, 0.1, or 1 µM, pre-incubated with siRNA for 30 min

The transfected cells were incubated at 37°C with 5% CO_2 in a humidified incubator for 48 h. The suppression of each target transcript were evaluated using fluorescence microscopy and the Guava® easyCyte Flow Cytometer System (Millipore) to detect green (GFP) fluorescence.

Negative control siRNA treatments were used to assess off-target effects of reagents on cell function, whereas siRNA237 were used to assess target knockdown. Cell viability were assessed using rezasurin method (Riss et al., 2004).

***Viability after cryopreservation*.** The 3 BAPC variants from experiment 1 were assessed for potential carryover effects on cryopreserved cells. Positive and negative control treatments and BAPC treatments, complexed with negative control siRNA and siRNA237, were applied to GFP-CHO cells in triplicate in 6-well culture plates. After 48 h, cells were cryopreserved in FBS with 10% DMSO and maintained in liquid nitrogen for one month. After storage, each vial was thawed and cells were plated back on 6-well plates. 48 h later, metabolic activity (accounting for both expanded cell populations and metabolic activity per cell) were assessed by rezasurin metabolism.

Results:

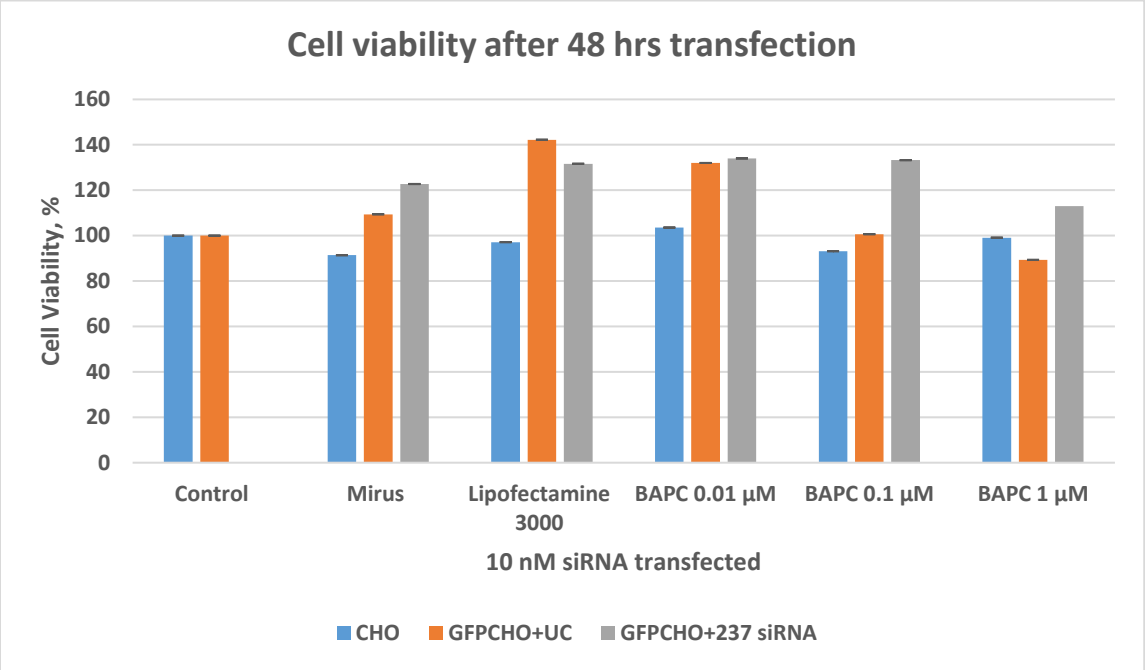

Figure 2A.

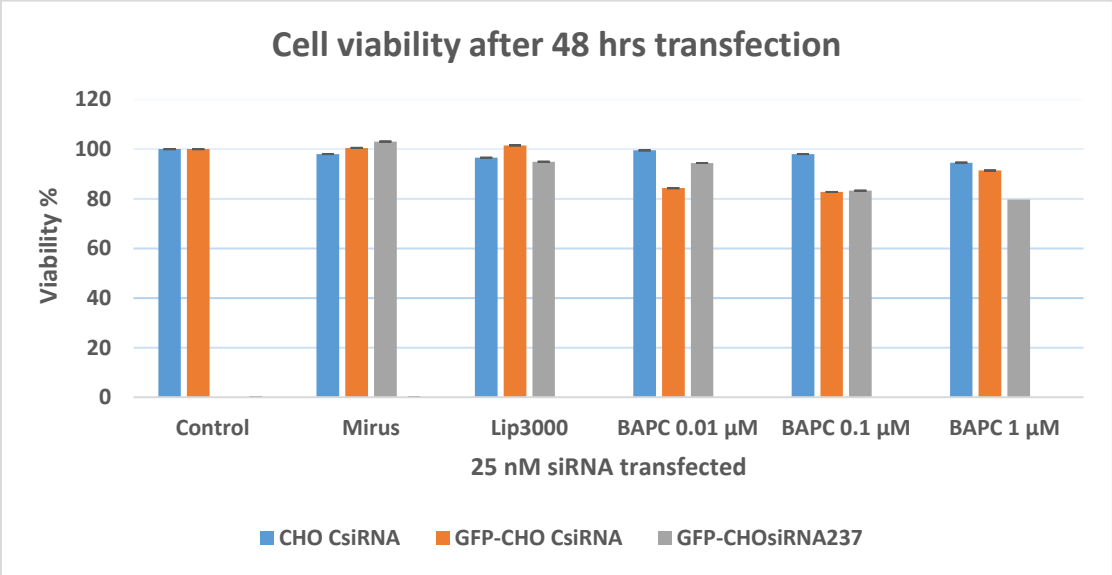

Figure 2B.

Figure 2 A and B. ***Cell viability after transfection.*** BAPC were dissolved per BAPtofect instructions using pure water and $CaCl_2$. 10 nM siRNA and BAPC, were incubated for 30 min, and then added to cells. Medium was changed after 4 h of transfection and cells were incubated for an additional 44 h. Cells were then treated with 10% resorufin and cell viability measured after 4 h incubation. 2B. Cells were transfected with 25 nM siRNA as described for Figure 2A and no medium were changed after 4 hours of

transfection. Cell viability were assessed with 10% resorufin and cell viability measured after 4 h incubation.

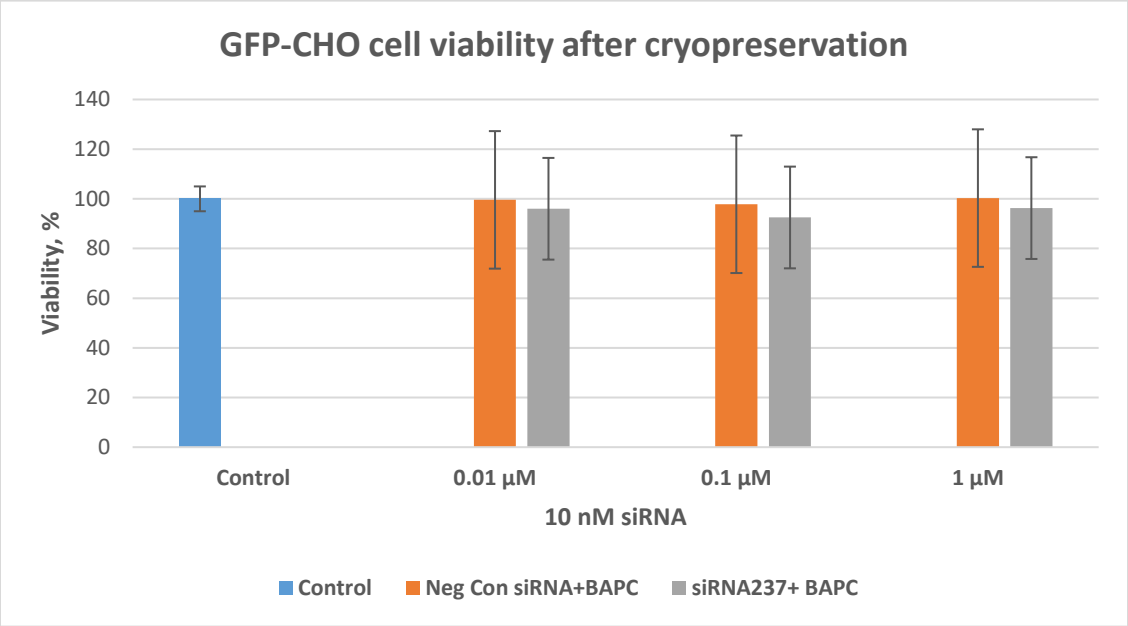

Figure 3.

Figure 3. ***Viability after cryopreservation***. GFP-CHO cells transfected using different concentration of BAPC (0.01, 0.1 and 1 µM) with 10 nM negative control siRNA and siRNA 237 for 48 hours. After 48 h, cells were cryopreserved in FBS with 10% DMSO and maintained in liquid nitrogen for one month. After storage, each vial was thawed and cells plated back on 6-well plates. 48 h later, metabolic activity (accounting for both expanded cell populations and metabolic activity per cell) were assessed by rezasurin metabolism. Total of 6 replicates per treatment.

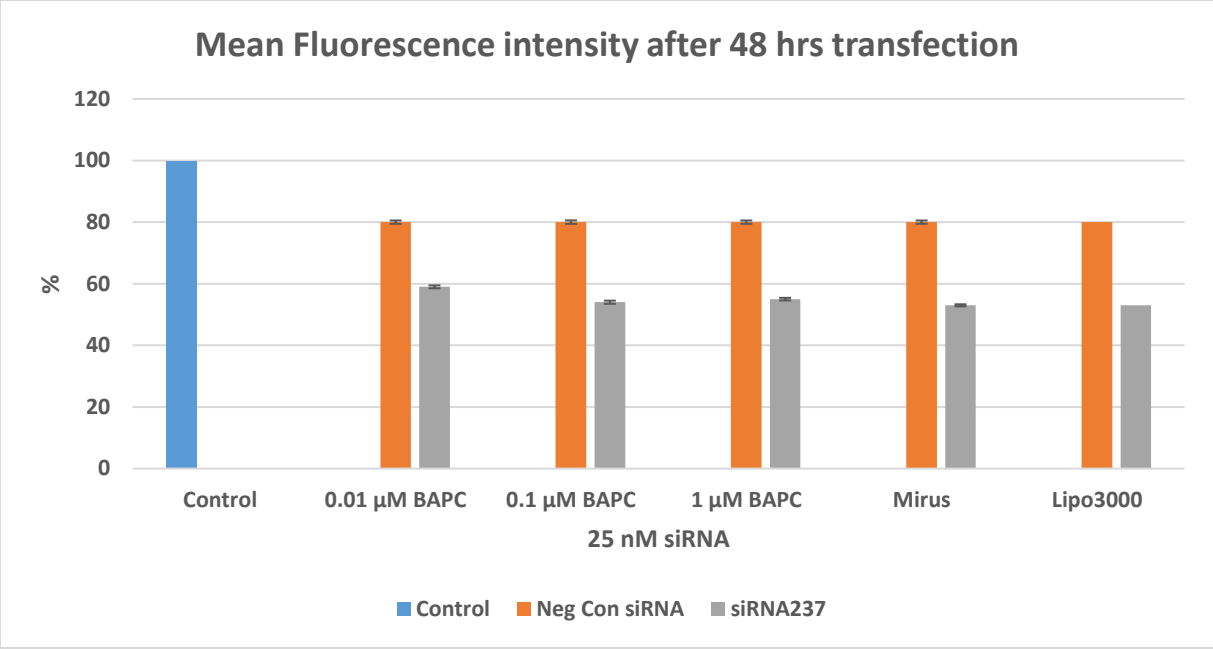

Figure 4A.

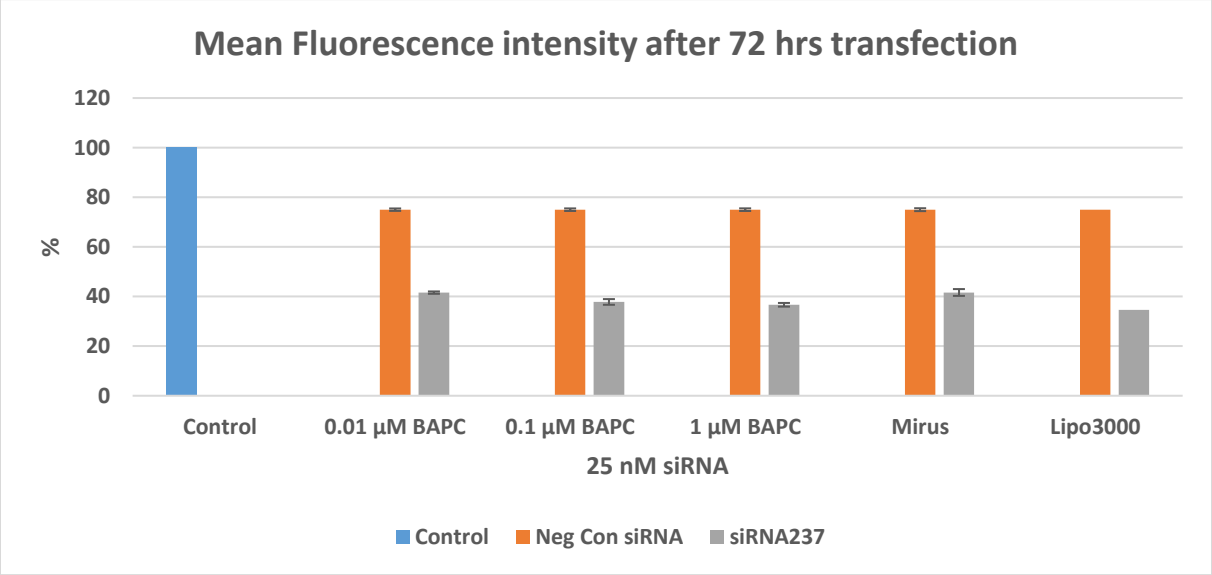

Figure 4B.

Figure 4 A and B. ***Transfection efficiency.*** GFP-CHO cell fluorescence 48-72 hrs. after transfection with negative control siRNA and siRNA237 using three different transfection reagents. GFP-CHO cells were transfected with 25 nM negative control and target siRNA 237. Cell medium has not been replaced after 4 hrs. transfection. There are 8 replicates per treatment.

Conclusion: BAPC at lower concentrations (0.01, 0.1 and 1 µM) does not reduce cell viability. BAPC at three different concentrations assessed knockdown siRNA 237 about 50 % after 48-72 hours. There is no

difference between BAPC and other transfection agents such as Mirus or Lipofectamine 3000. There is no dose effect between 0.01 – 1 μM BAPC. BAPC did not affect cell viability after one-month cryopreservation.