# Investigation of Keratin's Impact on Protein Aggregation in Heat-Shocked Human Embryonic Kidney Cells

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## Background

- Heat shock leads to high levels of protein misfolding. These misfolded proteins can be properly refolded, marked for degradation, or directed to aggregate.[1]
- Protein aggregation (aggresome formation) occurs when the capacity of cellular folding or degradation pathways are exceeded (Figure 1, figure created by Connor Davis).[2]
- GFP-250 and GFP-cBSA are fluorescently tagged model misfolded proteins that may be used to image the general movement of misfolded proteins within a cell.
- Aggresomes provide cellular protection against cytotoxic misfolded protein configurations[3] We hypothesize that keratin's protective influence on shocked cells may stem from an interaction with the protein aggregation pathway.

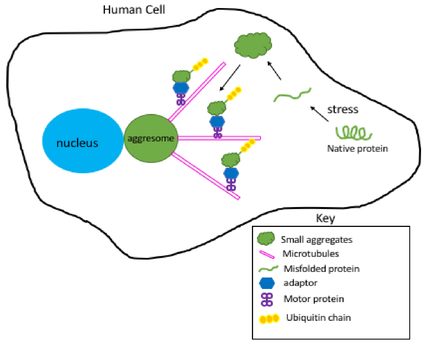



**Figure 1.** Proposed model of aggresome formation in which proteins that have been misfolded due to stress are directed toward a single juxtanuclear site via microtubules, adaptor proteins, and motor proteins.

## Methods

HEK 293 cells were grown at 37°C and transfected with a GFP-250 plasmid . Cells were treated with crude keratin solution (0.1 mg/mL) or gamma keratin solution (0.5mg/mL) 2 hours prior to heat shock or were left untreated. All cells were heat shocked at 43°C for 90 minutes, after which they were moved back to the 37°C incubator and fluorescently imaged in 30 minute increments.

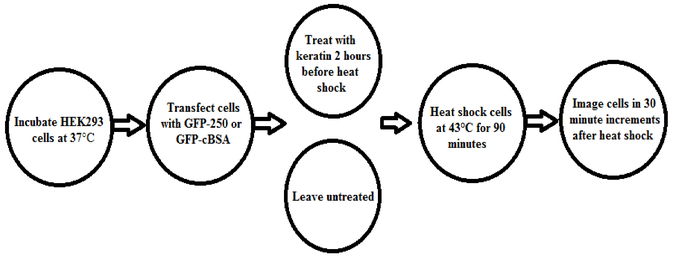



**Figure 2. Experimental procedure**

## Results

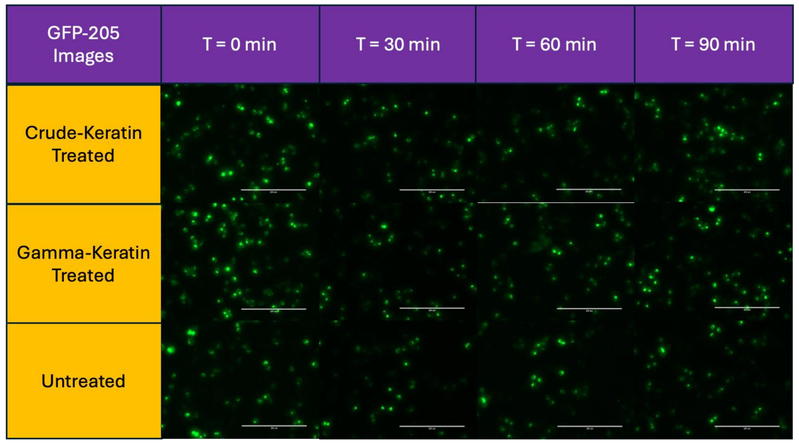



**Figure 3.** Representative fluorescent microscopy images taken of GFP-250 transfected HEK293 cells treated with keratin post heat-shock (T = time post shock). Crude keratin treated in the first row, gamma keratin treated in the second, and untreated in the last row

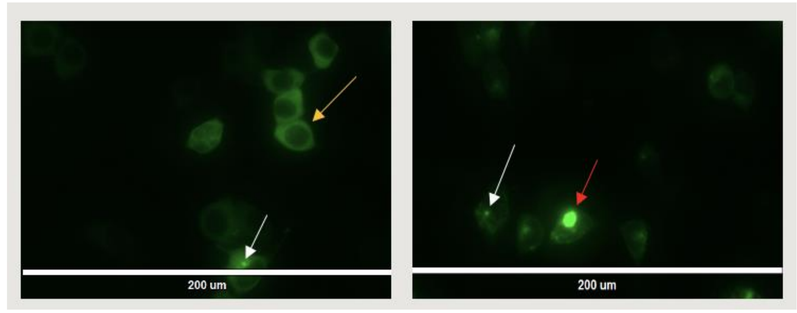
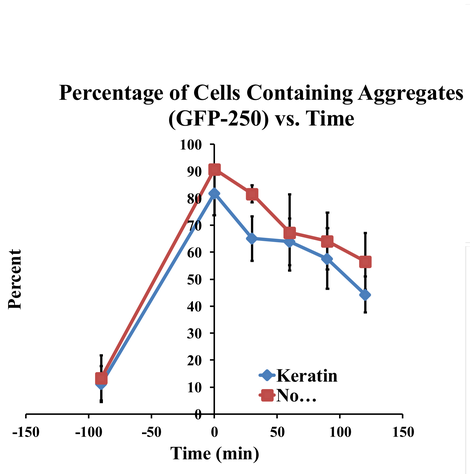
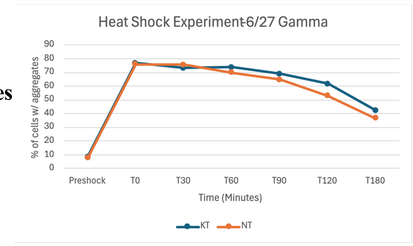



**Figure 4.** Fluorescent microscopy images show cells with and without aggregates. The yellow arrow shows a cell that has taken up the plasmid but is not undergoing protein aggregation. White arrows show examples of protein aggregates. Red arrow demonstrates an intensely fluorescing region that obscures the ability to determine whether aggregates are present. Cells with such regions were not included in counts.



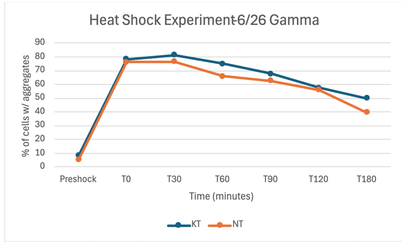




**Figure 5A,B, & C. A)** Prior studies in our lab showed that GFP-250 transfected cells that were heat shocked recovered more rapidly when treated with crude keratin. Measured as a percentage of cells with aggregates. The graphs show percentage of GFP-250 transfected cells containing aggregates over time pre- and post-heat shock **B)** Shocked cells are compared to crude keratin-treated and untreated shocked cells. **C)** Figure B comparison repeated.

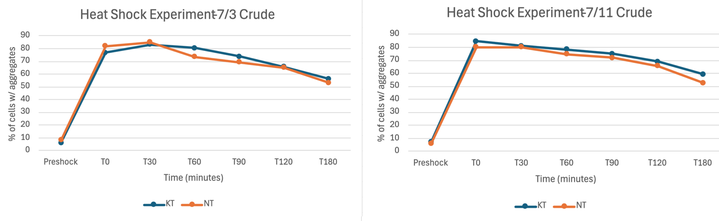



**Figure 6A & B.** The graphs show percentage of GFP-250 transfected cells containing aggregates over time pre- and post-heat shock. **A)** Shocked cells are compared to crude keratin-treated and untreated shocked cells. **B)** Figure A comparison repeated.

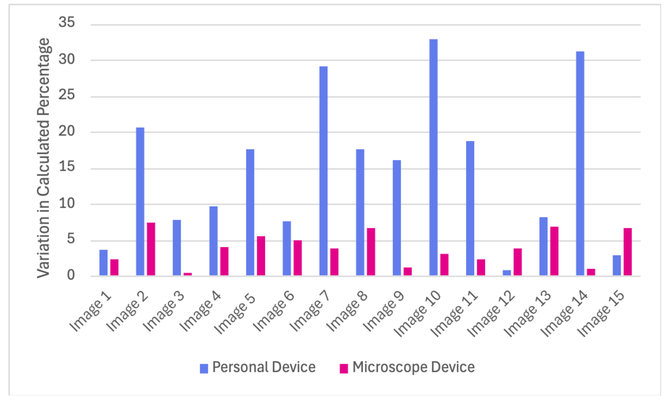



**Figure 7**. The graph shows comparisons of variation of percentage of cells with aggregates between images taken on a personal device and microscope device.

## GFP-250 Results

Our experiments suggest that the heat shock model system is not working appropriately (Figure 5B,&C/Figure 6A &B). Prior studies performed in our lab (Figure 5A) show cell removal of aggregates over time as an indication of recovery *and* a statistically significant effect on recovery in keratin supplemented cells. Our recent studies showed no statistically significant effect on the two types of keratin treat cells. However, did show evidence of recovery over time. The device used for image analysis may impact the results. Comparing the type of device used showed high variation based on device.(Figure 7)

## Conclusion and Future Work

- Prior results in GFP-250 transfected cells suggest that keratin may affect formation and/or clearance of aggregates. However, it's unclear to what extent.
- Our current work did replicate the heat-shock model of recovery shown previously but did not indicate being impacted by keratin. Optimizing work did show impact of type of device on data collection. Further studies will be done to further optimize our system before continuation of keratin studies.
- After optimization, future work will monitor the effects of varying concentrations of gamma and crude keratin in the heat shock model .
- Cell fixation and staining of the endogenous protein ubiquitin will be performed in future trials to ensure that the GFP-250 model misfolded proteins do not interfere with results.

### Acknowledgments

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