Tracking p14-induced cellular fusion through the visualization of cellular membranes and nuclei

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Introduction

Our lab has developed an alternative drug delivery platform that delivers cargo to cells of interest by causing fusion between the target cell and the drug delivery platform. This fusion bypasses the requirement of the drug to pass through the cell membrane.

Our aim was to track cellular fusion by labeling the cell membrane and nuclei, inducing fusion, and then tracking the fusion over time. We also set out to create a movie showing said fusion.

p14 FAST Protein Directed Cell Fusion
(*Fusion Associated Small Transmembrane*)

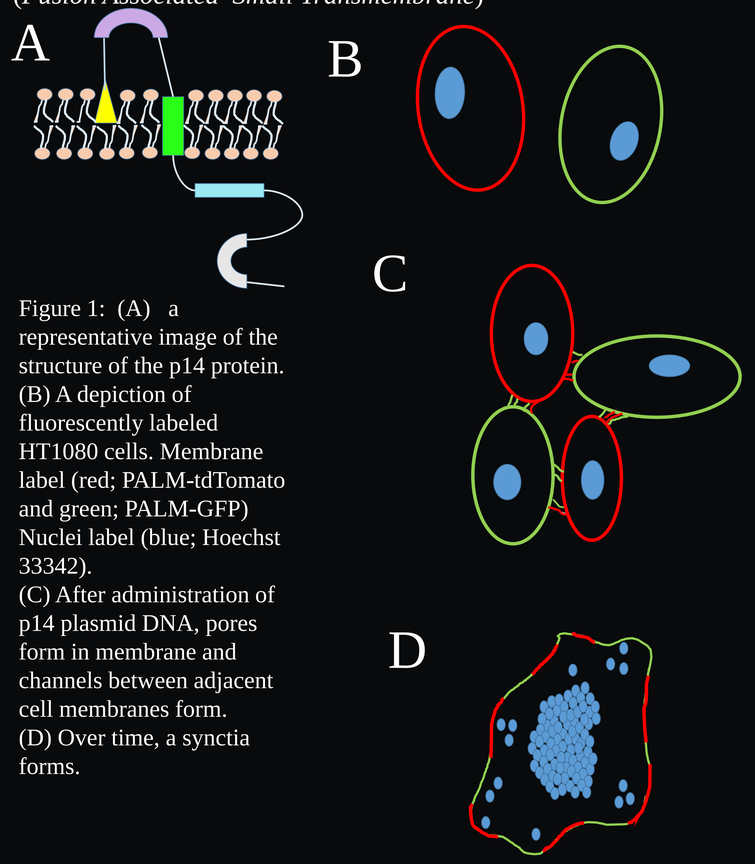


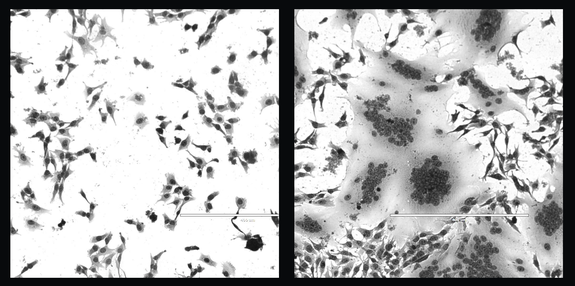
Figure 1: (A) a representative image of the structure of the p14 protein. (B) A depiction of fluorescently labeled HT1080 cells. Membrane label (red; PALM-tdTomato and green; PALM-GFP) Nuclei label (blue; Hoechst 33342). (C) After administration of p14 plasmid DNA, pores form in membrane and channels between adjacent cell membranes form. (D) Over time, a synctia forms.

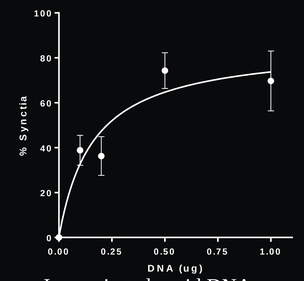
Methods

QM5 cells were used in the initial fusion functionality assay to track the effectiveness of p14 plasmid DNA to cause fusion. This provided the information for which DNA concentrations to use when tracking fusion in fluorescent HT1080 cells.
Three fluorescent labels were used: PALM-GFP and PALM-tdTomato (membrane labels), and Hoechst 33342, a blue DNA stain. The membrane stains remained on the cells throughout the experiment. Plasmids were transfected onto cells and ~2hs later images were taken for 15 minute intervals.

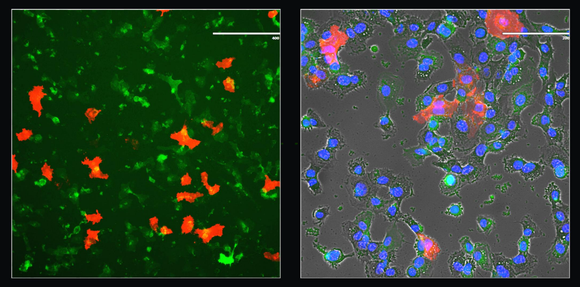
Results

QM5 cells without Fusion DNA alongside fused QM5 cells.



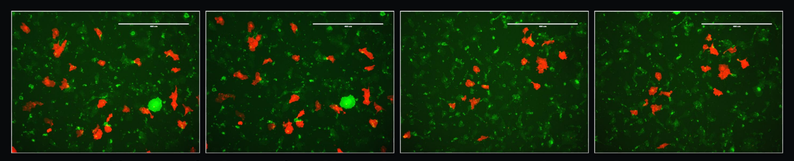


Increasing plasmid DNA transfected into QM5 cells increases fusion.

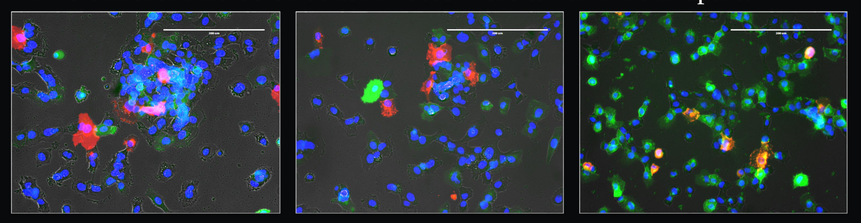


HT1080 cells with p14 plasmid DNA and membrane labels; HT1080 cells with nuclei and membrane labels.

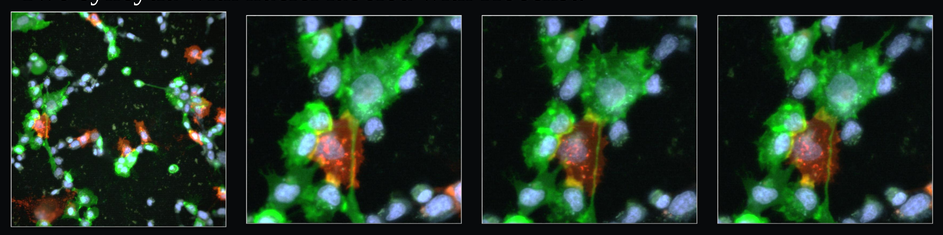
We discovered that the Hoechst needed to be applied later, as it binds the DNA and stops expression of the P14 fusion protein. We found that the addition of the DNA stain after 4 hours circumvented this problem.



Two different fields of cells. Photos were taken 1 hour apart.

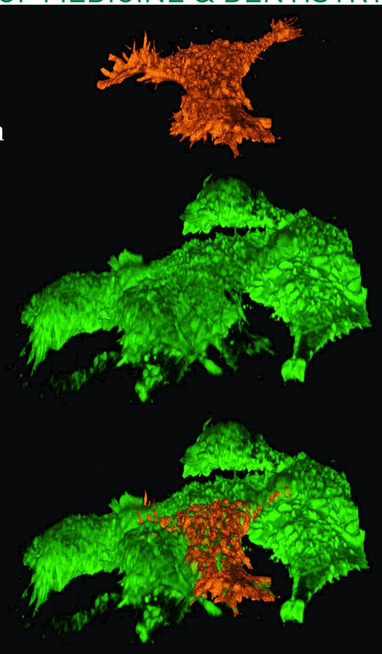


Two syncytia with nuclei labeled with Hoechst.



A full field after three hours; a close up of cells fusing taken 30 minutes apart.

3D image of HT1080 cells in the process of fusion.



Conclusion

We were able to easily see fusion with the QM5 cells, but experiencd some difficulty using the Ht1080 cells. Several trials have varying results and imaging of cell fusion is ongoing.

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