DNA Coated Gold Nanoshells for Laser Induced Antisense Drug Release in Cells

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The Big Picture

Research Goals

- Attach antisense DNA to gold nanoshells
- Introduce nanoshells to cells for intracellular delivery
- Activation via pulsed laser to release DNA in a time and position-specific manner

Real World

- Would enable time-resolved and spatial gene function studies (antisense, silencing RNA, and micro RNA)

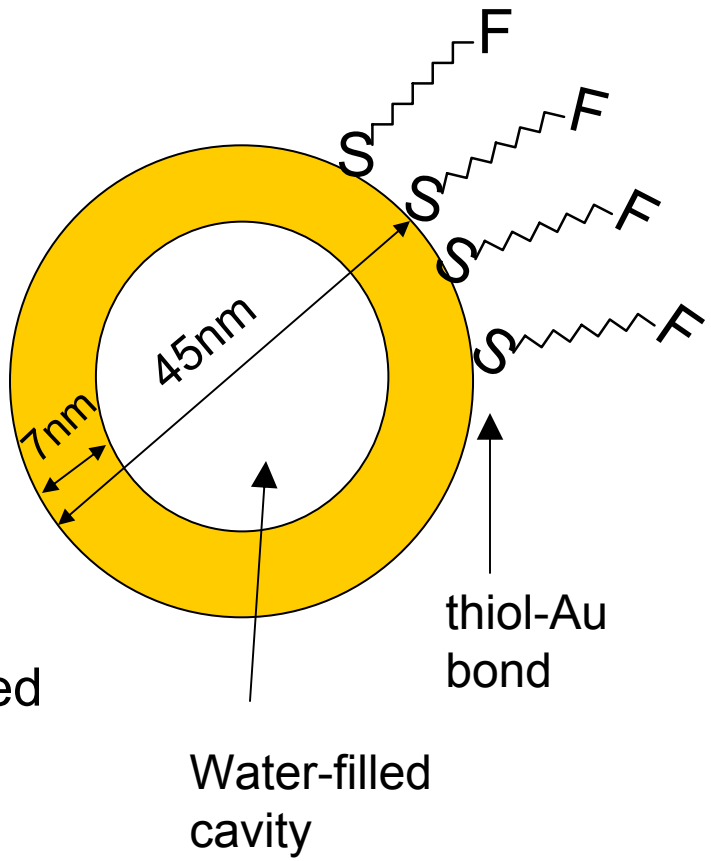
Overview

Research Objectives

- Coat nanoshells with thiol-DNA-dye
- Demonstrate delivery into cells
- Release DNA-dye via pulsed laser without killing the cells

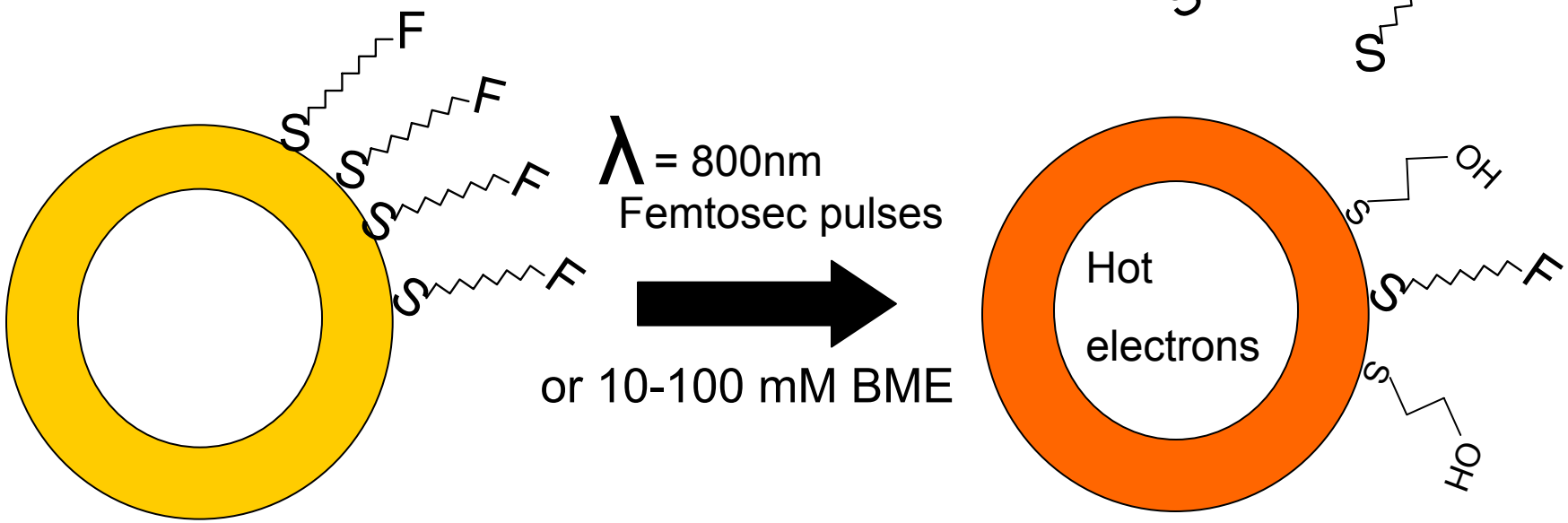
Approach

- Characterization of DNA-nanoshells
- Cell viability on nanoshell films
- Effect of variable conditions on pulsed laser release
  - laser power, laser exposure time
- Initial studies using a chemical release (excess thiol)



Nanoshell Structure and Surface Chemistry

DNA: HSC_6H_{12}-5'-CGC ATT CAG GAT(F)- 3'



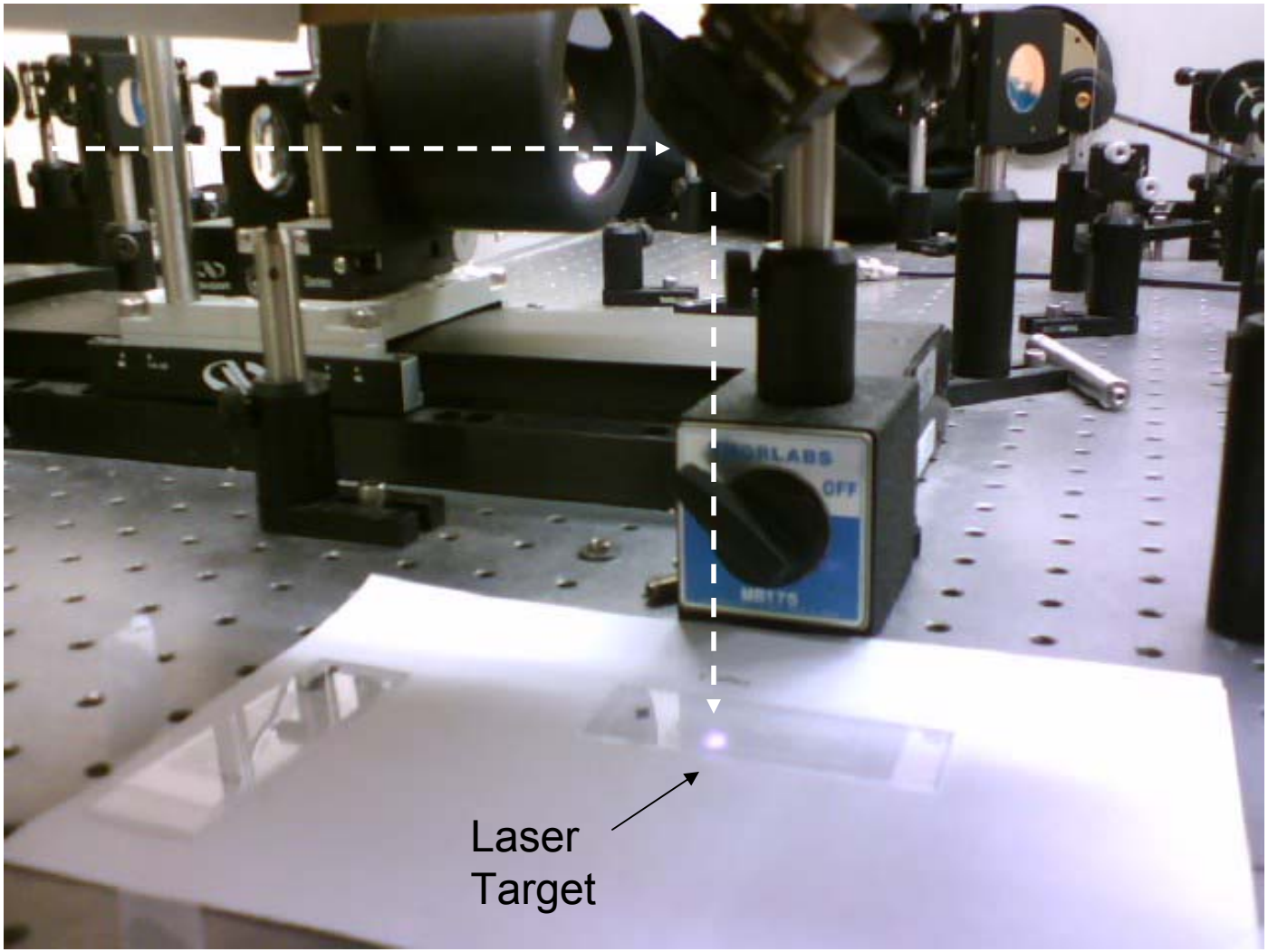
~95% quenched fluorescence

(near the NS surface)

10x to 20x increase in fluorescence

(diffusion away from NS)

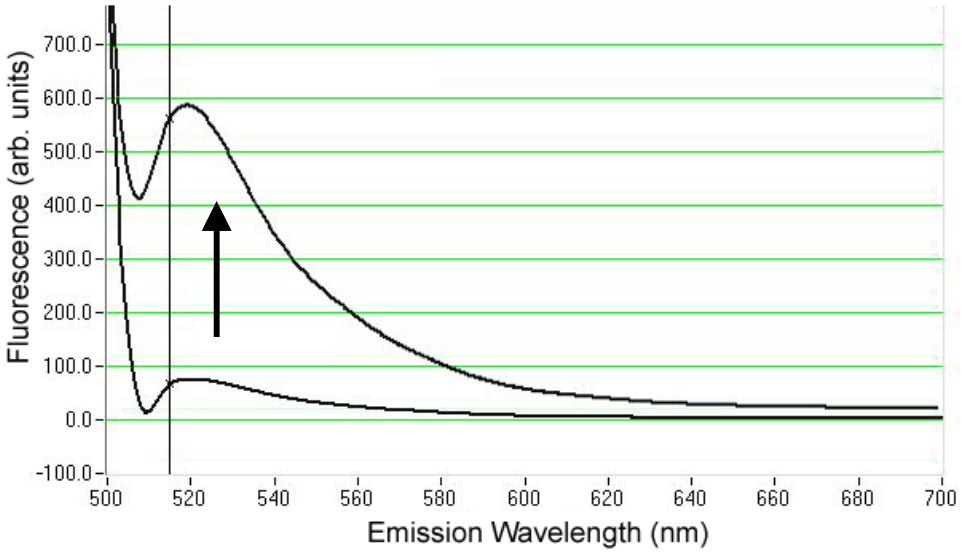
Pulsed Laser Releasing DNA



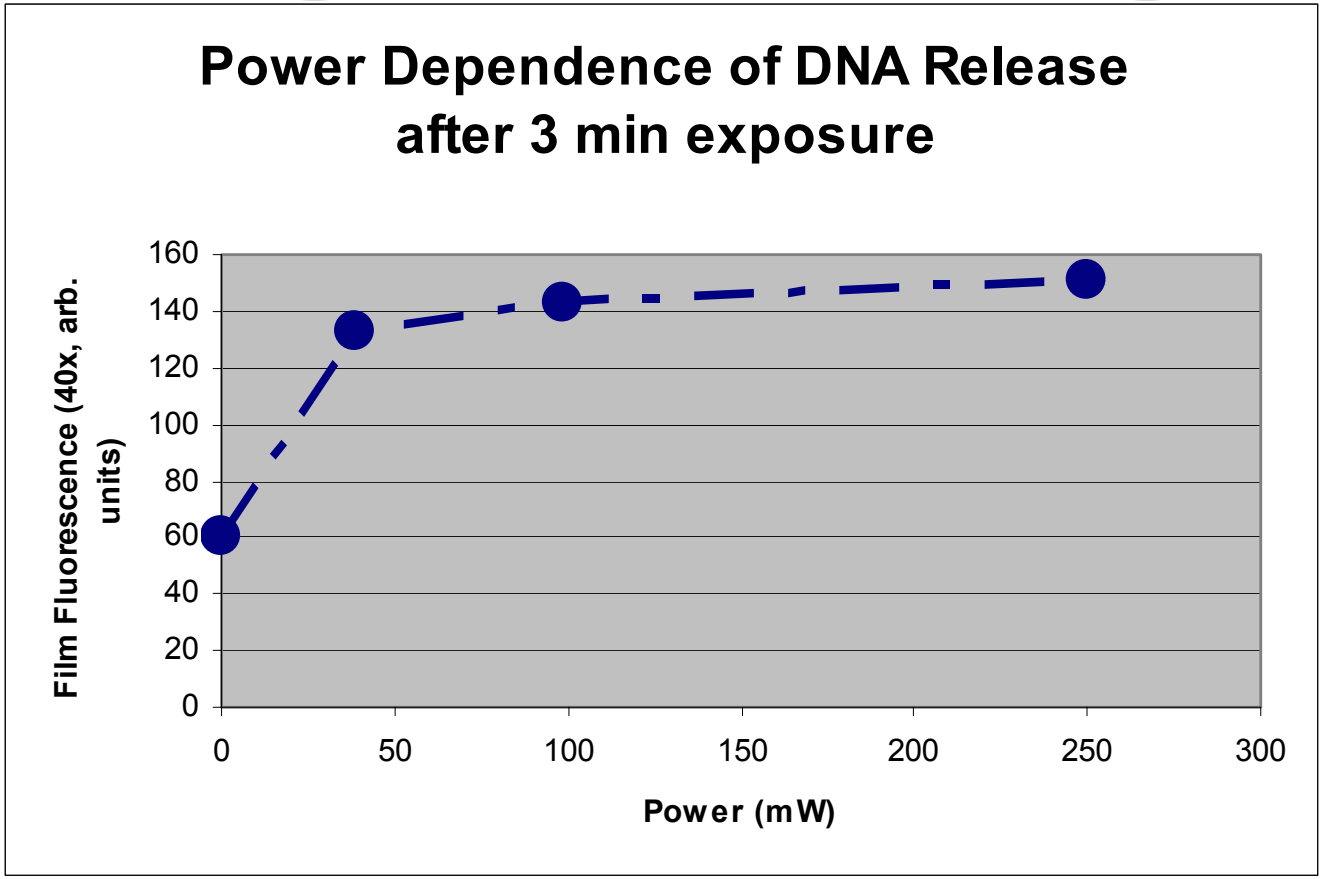
Verifying DNA Release using Fluorescence

- **Gold nanoshells quench DNA-dye fluorescence**
  - Laser causes DNA separation from NS and increases the fluorescence of the solution
  - Quantitated using a fluorimeter and through fluorescence imaging with a microscope

Pulsed Laser release (10x increase)



Microscope Imaging Release from NS-Films



Note: No immediate cell death at these powers!

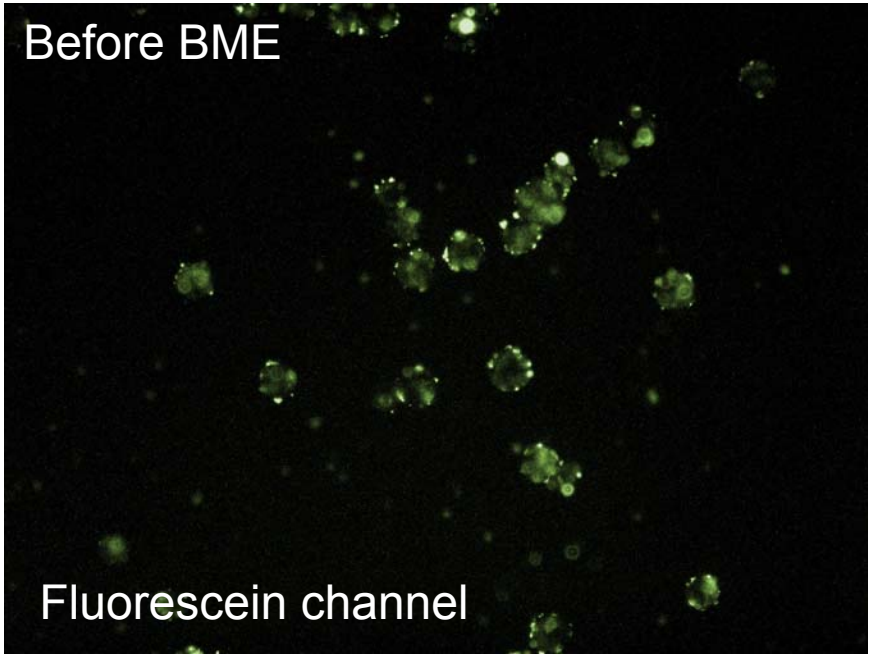
DNA Transfection Strategies

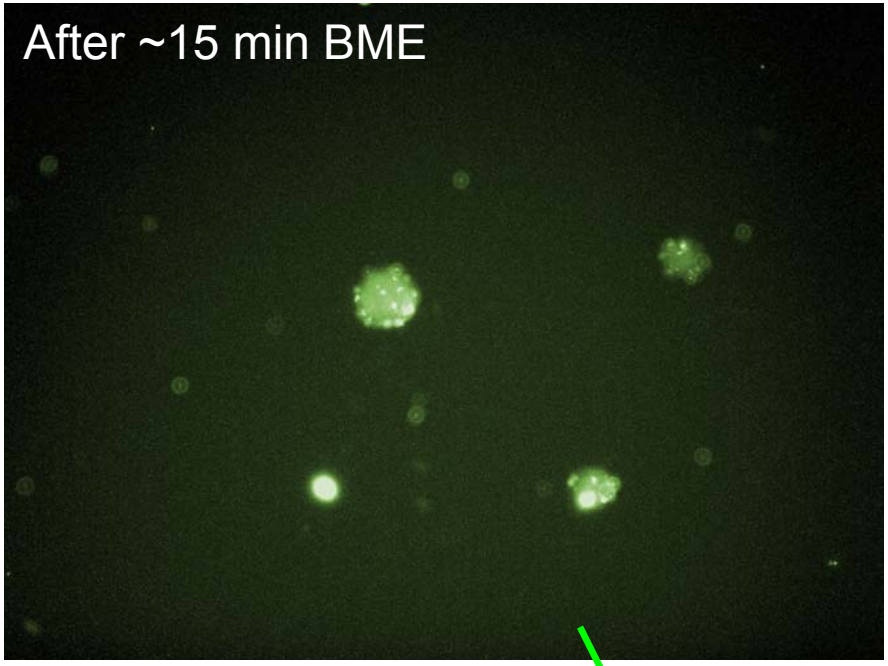
- Simple Incubation
- Cationic Polymers
  - Electrostatically-induced endocytosis
- Targeting using Peptides or Antibodies
- Receptor-Mediated Endocytosis
  - Transferrin
- Viral Capsids
  - Adenovirus

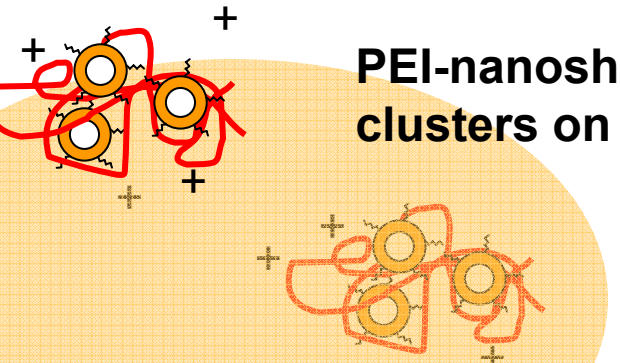
- **Our approach: Cationic Polymer-Nanoshell Composites in solution and films ("Surfection")**
  - Branched-Polyethylenimine (PEI)
  - HeLa (cervical cancer cells, cultured on plates)

Fu-Hsiung Chang et al. Surfection: a new platform for transfected cell arrays. *Nucleic Acids Research*, 2004, Vol. 32, No. 3

Chemical BME-Release: Nanoshell-PEI Solution-based Transfection with HeLa



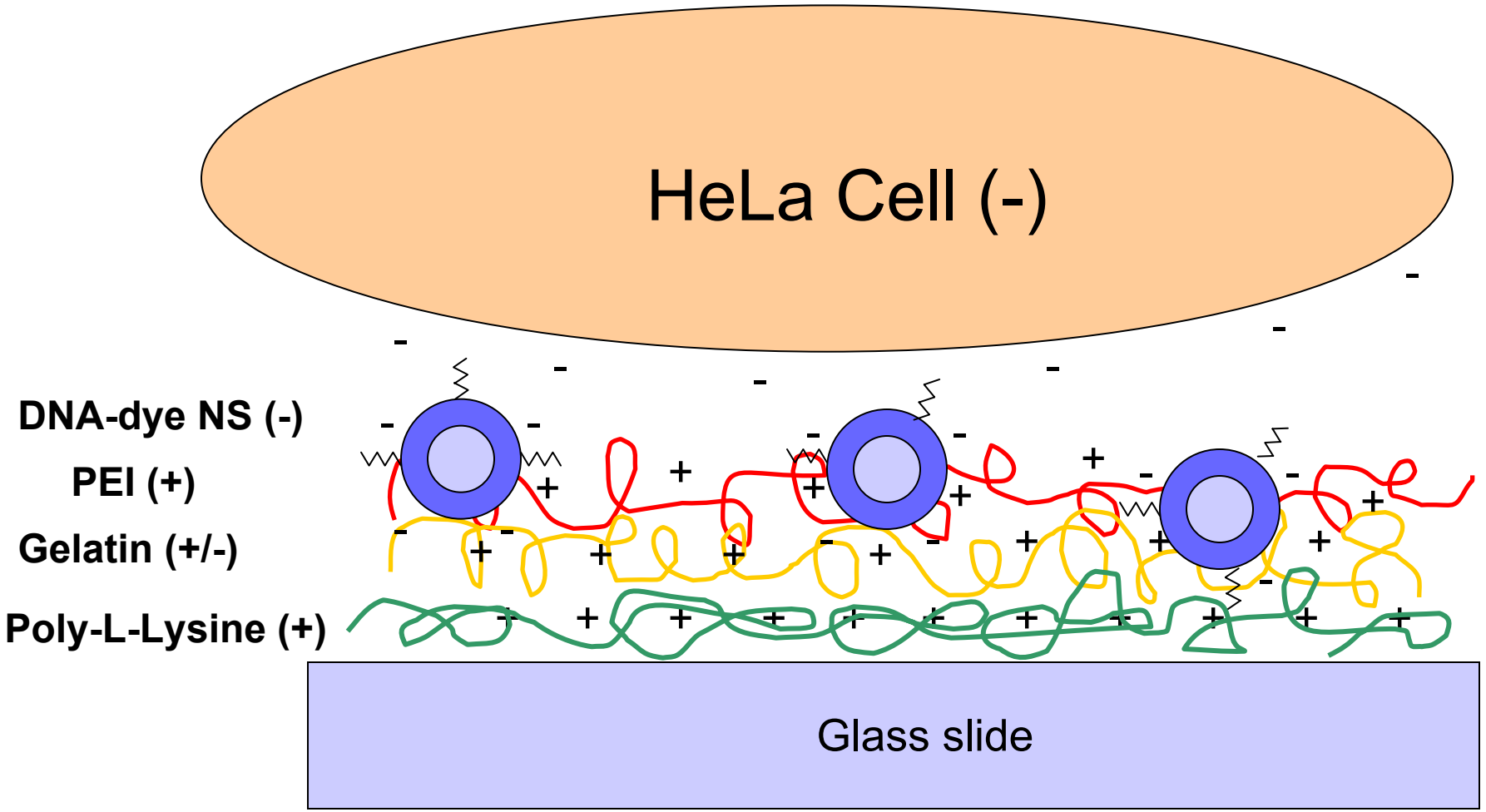




PEI-nanoshell clusters on cells

Fluorescence increases after BME release of DNA-dye

Surfection with HeLa Cells



Cell Viability on Surfection Films

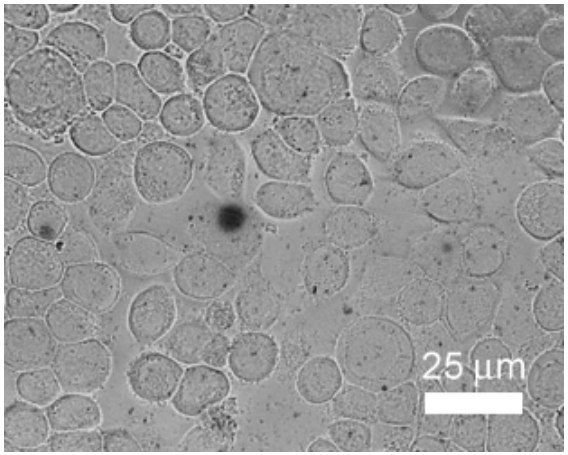
Optimal Surfection composition:

0.01% PLL (100kD) for 15 min

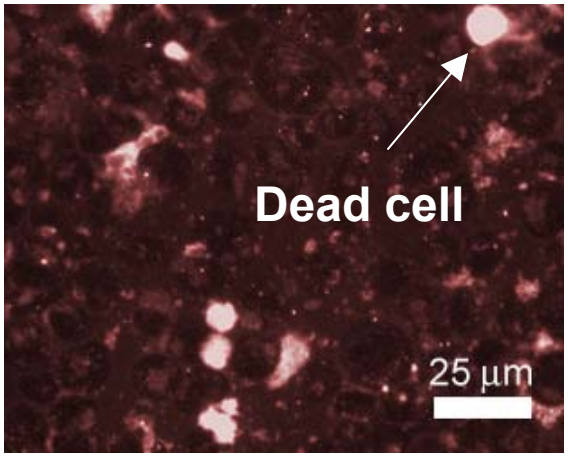
1% branched-PEI / 1% Gelatin mixture for 15 min

30 min Nanoshell deposition in PBS

50% coverage with HeLa, 10% FBS

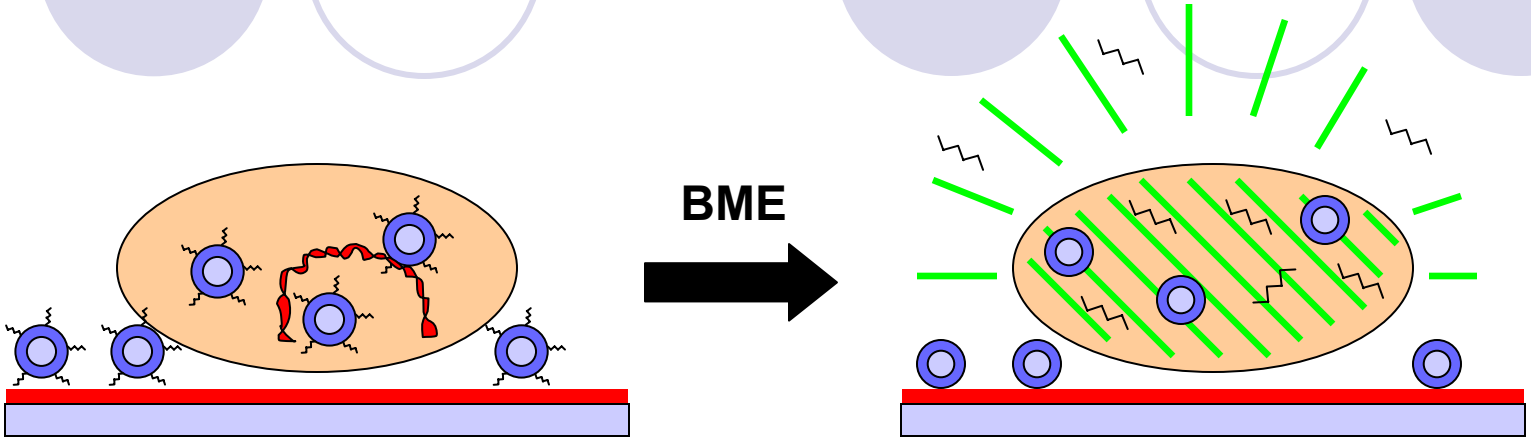


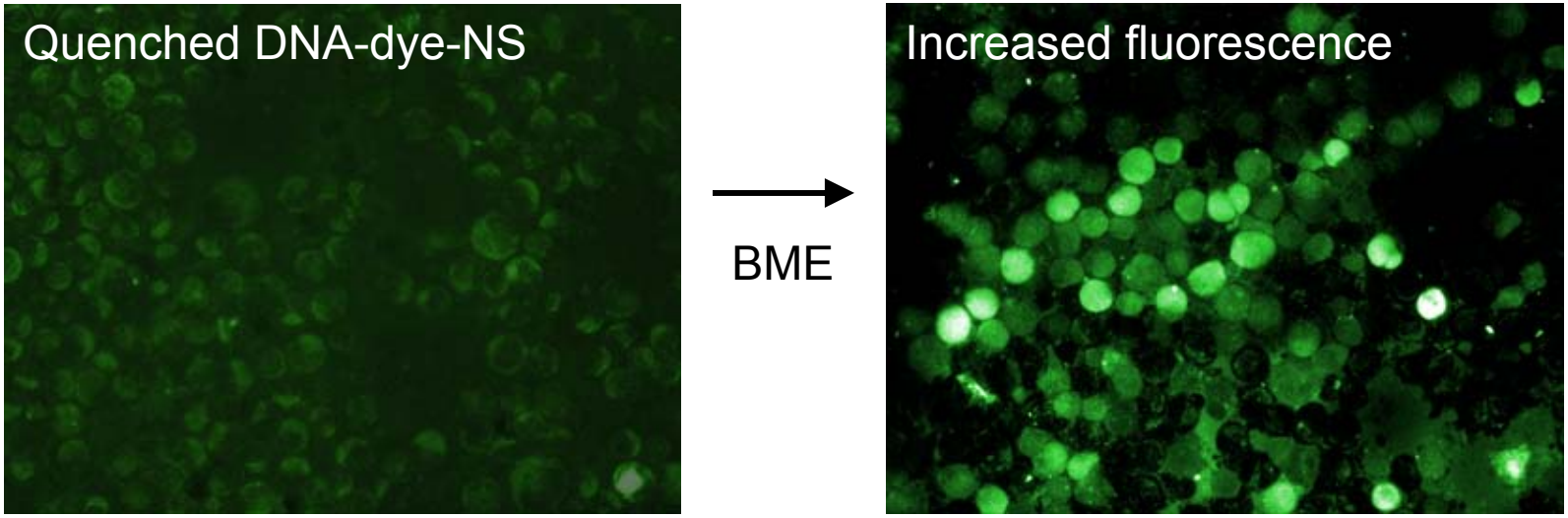
White light



Trypan Blue fluorescence Filter

BME Release of DNA-Dye inside HeLa Cells

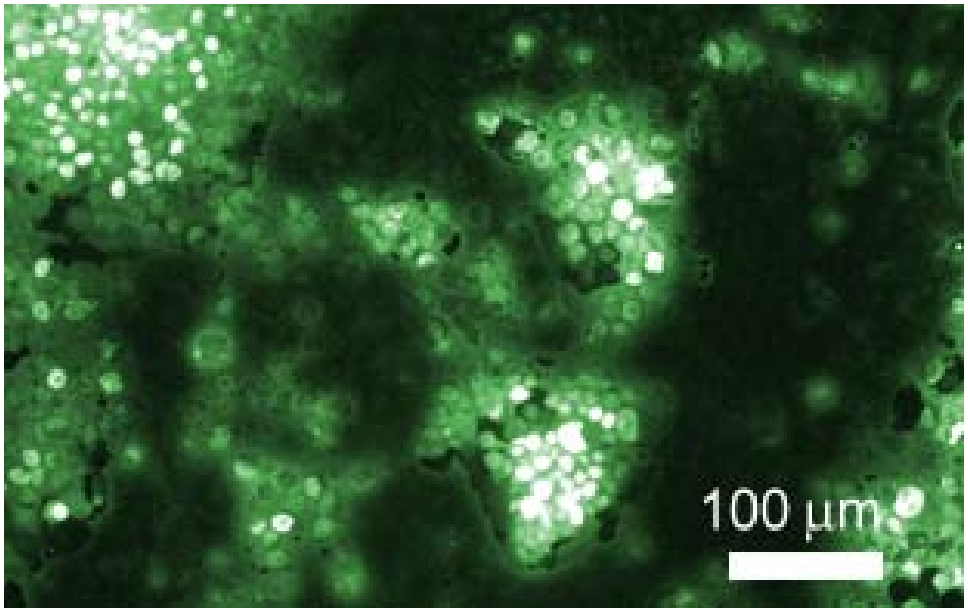


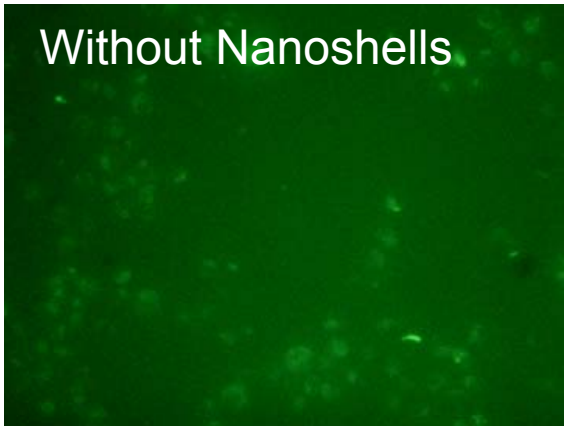


BME Release of DNA-Dye inside HeLa Cells

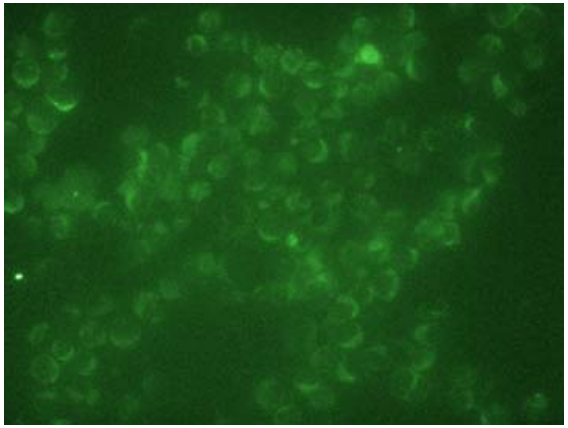
Top: A wide field view of the BME-released DNA-dye observed in ~ 40% of cells.

Bottom: A control experiment shows no increase in fluorescence when nanoshells are absent





BME



Analysis Summary

- DNA release from NS was observed at moderately low laser powers after 3 min exposure, or by addition of BME
- Cell death was not observed at powers much higher than needed for DNA release (~50 uJ/pulse per cm^2)
- Verified that PEI-Gelatin films allow for good cell viability
- Uptake of DNA-nanoshells was demonstrated using chemical release with fluorescence increase

Future Plans

- Show laser release of DNA inside cells
- Improve surfection technique
- Use more stable fluorescent dyes
- Deliver known, chemically-resistant, antisense DNA sequences
- Verify gene knockdown
- Use other cell lines to test generality

Acknowledgements

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References

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Thank You