Beginning Experiment: Nanoparticle Uptake in ARPE-19 Cells

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Purpose

- How does nanoparticle uptake work? Which surface proteins or receptors are used for nanoparticle uptake? How is cellular uptake measured?

- Hypothesis: If ARPE-19 cells are tested for cellular uptake with 0.2 micrometer carboxylate fluosphere dilutions, then there will be an increase in absorbance with a greater concentration of the fluorospheres than a smaller concentration.

- Independent Variable: Different dilutions of the 0.2 micrometer fluorospheres (The average size measurement was 276.8 nm)

- Dependent Variable: Nanoparticle uptake in ARPE-19 cells

- Controlled Variables: same bacterial medium was used (DMEM-F12), same machine to measure absorbance (Spectramax M5), cells were kept in incubation for the same amount of time at 37 degrees Celsius and 5% CO2, and same micropipettes were used to keep the measurements consistent and precise.
Background Research

- Researchers can now influence a receptor on the surface of a nanoparticle to resemble those on a specific cell type (Herd et. al).
  - This is useful in that it allows only certain cells that need the specific drug to be targeted.

- Nanoparticles have a very large surface to volume ratio which makes them easily identifiable by surface proteins on cells (Jiban et. al).
  - Proteins on the surface of cells actually compete for the nanoparticle surface so even small amounts of nanoparticles present extremely large surface areas available for protein binding (Jiban et. al)

- Absorbance can be measured through light given off by the nanoparticles and then through calculations, the percent uptake can be found.
**Protocol**

- ARPE-19 cells were taken from liquid nitrogen storage and heated in 37 degrees Celsius water bath for 2-3 minutes.
- Cell mixture was put into two small flasks and centrifuged for 5 minutes at 4000 rpm.
- The liquid was removed from both flasks and 1 milliliter of DMEM-F12 bacterial medium was added to each flask. The cells were suspended with the medium using a micropipette tip.
- Both were put into a larger flask with an air-filtered cap and more bacterial medium (5 milliliters) was added to the flask.
- Flask was put into incubator for 24 hours at 37 degrees Celsius and 5% CO2.
- After 24 hours, the bacterial medium was removed and a thin layer of 0.25% trypsin (2-3 milliliters) was added and put back into the incubator for about 5 minutes or until the solution appeared opaque.
- Solution in the flask was equally separated into two smaller flasks to be centrifuged once again at 4000 rpm for 5 minutes.
- The liquid part was removed and new bacterial media was added to the small flasks and re-suspended.
- Both of these mixtures were added to a larger flask with about 6 milliliters of new DMEM-F12 bacterial medium in it and incubated once again at 37 degrees Celsius and 5% CO2 in order to allow the cells to grow and have enough cells for each well in the 96 well plate.
Protocol Continued

- After 24 hours, cells were be transferred into the 96 well plate. The first two rows of the 96 well plate were filled with about 150 microliters of cell/ medium mixture in each well.
- The third row was filled with 150 microliters of cell culture medium. The well plate was once again incubated in 37 degrees Celsius and 5% CO2 for 24 hours.
- The nanoparticle was diluted down in decreasing concentrations using deionized water in a dilution series of 1-10, 1-25, 1-50, 1-100, 1-250, 1-500, 1-1000, 1-2500, 1-5000, and 1-10,000 dilutions of nanoparticle to water. 200 microliters of each of these solutions was put in a new 96-well plate and Spectramax M5 was used to measure the absorbance at 540 nm for the standard curve.
- The nanoparticle was diluted down with bacterial media DMEM-F12 having 1-10, 1-25, 1-50, and 1-100 dilutions only. 50 microliters of each of the 4 dilutions was put into 6 wells each so 1-10 dilution was put into the first 6 wells and then 50 microliters of the 1-25 dilution was put into the next 6 wells and etc. The plate was left alone for 5 minutes at room temperature.
- Solutions in the well were moved down (B to D and then A to C) and 200 microliters of PBS 1x solution was added to the first two rows of the 96 well plate. This process was repeated of moving down the cells and then previous PBS solutions into where the old cells were. New PBS solution was added to the top 2 rows until the bottom row was reached (should be 4 times).
- Absorbance was taken at 540 nm of all the wells. This entire protocol was repeated 3 times to better understand the data and be able to average the values.
Results

The individual data and calculations collected from the three trials of finding absorbance are all on labarchives.com.

The total percent RFU for the 1-10 ARPE-19 uptake was 2.679%, the 1-25 ARPE-19 uptake was 1.293%, the 1-50 ARPE-19 uptake was 0.476%, and the 1-100 ARPE-19 uptake was 0.054%.

Standard curve: absorbance of the dilution series of the polystyrene fluospheres. X-axis: concentration of the nanoparticles in the dilution series. Y-axis: RFU or Relative Fluorescence Unit is the measurement for absorbance. This value allows us to understand nanoparticle uptake in the ARPE-19 cells with the cells that have a higher absorbance having taken up more nanoparticles.
Discussion

- There are more nanoparticles that can be taken up by the ARPE-19 cells if there is a larger concentration of them in the cell culture medium than if there was a smaller concentration of them.

- There is more surface area on the ARPE-19 cells for the nanoparticles to attach onto and therefore, more nanoparticles can be taken up.

- With a lower concentration, there are more cells in the wells than nanoparticles so it is almost as though the cells are competing with one another to uptake the nanoparticles.

- This experiment was a good starting point in understanding nanoparticles and nanotechnology as more research was conducted in order to understand the importance of nanoparticles and nanoparticle uptake in drug delivery.
Conclusion

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- Cellular uptake and nanoparticle delivery was explored in this experiment and led to a higher understanding of the importance of these mechanisms in drug delivery.
Final Experiment: Silica and Gold Nanoparticle Toxicology Study

- Contacted Dr. Kompella’s collaborators to obtain silica nanoparticles (different sizes- 17, 24, and 54 nm) and gold nanoparticles (different shapes- spheres, rods, and octahedrals).

- Conducted silica adhering experiment to adhere a silica coat to the 17 nm gold spherical nanoparticles by adding sodium silicate and APS (advanced polymer solution used in chemical grafting) under vigorous magnetic stirring but it did not work as the gold nanoparticles started clumping together.

- Conducted size measurements for the gold nanoparticles using a Zetasizer.

- Created the Experimental Design for the gold and silica nanoparticle toxicology studies with the help of my mentor Ryan Kelley and Dr. Kompella. Started the gold nanoparticle toxicity studies, conducted 2 trials but all information and protocol must be kept at the University of Colorado Denver Anschutz Medical Campus.
Start of Experimental Design

My proudest moment...

Gold Nanoparticles (17, 24, and 54 nm)

Silica Nanoparticles for the tox studies
Works Cited

