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Characterizing Mesoporous Carbon-Based Dopamine as an Efficient Carrier for Treatment in HeLa Cells

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ABSTRACT

Cancer is at the forefront of one of the deadliest diseases to plague our nation. In attempts to find definite cures, nothing has been completely successful in lowering the mortality rate of this disease. Materials research, however, has been presented to be a viable alternative by targeting nanoparticles; these molecules can be customized to hold chemicals within their hollow structure that only affect mutated cells. This experiment utilizes mesoporous carbon-based dopamine nanoparticles to treat HeLa cells, which are baseline to most strains of cervical cancer. The hypothesis stated that if mesoporous carbon-based dopamine is introduced to cancer cells in increasing concentrations, the higher end of the variable spectrum will have a high mortality rate and lesser targeted effect. After being transferred to a 96-well plate, the cells were introduced to the nanoparticles and incubated for 7 days until the testing. The stability and retinance capabilities of the MCBD were trialed using assays such as an IC-50 for viability, photothermal for heat capacity, and photostability for cell divisibility upon exposure to nanoparticles. After analysis, the data presented that the 100 ug/mL was the most efficient concentration to infiltrate the cell and not be deteriorated by the activation heat exposure; all three curves created from the assay data determined that too high of a concentration tended to kill the cell and higher heat levels on the other concentrations destroyed both. Hence, these results can be further applied to alternative cancer treatment research fields and the further investigation of nanoparticles.

Background

Modern medicine has evolved to microscopic treatment and digital sensing using brand-new technology that can work to save patients more efficiently than before. One of the most successful but still new methods is the use of nanoparticles or materials with a nanoparticle diameter in dimension. These innovative materials can enter the body and target specific organs or cells more accurately with a higher dissolution rate, making them an optimal treatment for targeted treatment for a specific illness.

One of the broadest and common types of cancer is cervical cancer, which affects the reproductive system, specifically the cervix, of women. In 1951, a line of cervical cancer cells was harvested illegally from a woman named Henrietta Lacks and immortalized, later being known and publicly distributed as the HeLa cell line. These cells replicate at an exponential rate, reaching 80% confluency in a few short days of starting a new culture; this made them viable for this experiment. Their rapid growth rate along with adaptability makes them flexible enough for cancer research, and introducing a doreign particle with cellular survival demonstrated that the cell line was adequate for testing.

Nanoparticles were first introduced in the scientific community in recent years, presenting them as the new medical technology of the future. They were designed to be able to enter the body in a different way than normal medications, either nasally and sublingually. Although scientists have run into environmental disputes,

the research and data currently circulating have allowed for the advancements of nanoparticle discovery and experimental success. Alterations in the chemical compositions have made it possible for a broad spectrum of applications, including medical imaging -- optic and MRI -- and gene/drug therapy -- applicable to cancer, neurodegenerative diseases, etc.

This experiment focuses on the gene/drug therapy target aspect. Nanoparticles can be divided based on what chemical they use to concentrate or target specific cells in the body, such as dopamine and epinephrine. These chemicals are imbued into mesoporous particles like carbon and silica; while silica is the most commonly researched and successful alternative, this study uses mesoporous carbon dopamine. Carbon is more advantageous in that it can be used as a chemical carrier and has been characterized as being one of the most tolerant elements to high temperatures. The last trait is what makes it particularly applicable in biochemical engineering and medical studies, since more environments that these nanoparticles are exposed to reach contrasting extremes. The mesoporous carbon being infused with dopamine is one of the new areas of studies, seeing how it mimics favorable drug carriers used for drug treatments.

One of the most important factors in nanoparticle research is determining the best way to ease the particle into the cell. This is part of a particular process called engulfment, where the cell releases vesicles or extends the cell membrane to surround and absorb the molecule attempting to enter the cell. If the cell detects it as a harmful foreign substance, it'll expel it quickly. Certain covering or structures, like mesoporous carbon have been proven to enter the cell with high sensitivity to be expelled as a "security measure." This process works hand-in-hand with nanoparticle delivery and the photothermal effect since the latter depends on there to be sufficient concentration of the cell to be released when exposed to directed heat.

Extreme temperature resistance is necessary in medication carriers, since upon entering the body, there is a drastic rise in temperature and acidity depending on target location. And the most efficient way to test this tolerance is using the photothermal effect -- a phenomenon associated with electromagnetic radiation using thermal energy (lasers). This exposure can outline the limits to which the nanoparticles can continue to function as a carrier and not dissolve prematurely along with testing their rate of heat absorption upon direct exposure.

Introducing the idea of using mesoporous carbon-based dopamine as an alternative treatment to cancer is viable since it would present another treatment besides chemotherapy. More specialized, it would target specific cells or infected areas without harming the healthy cells while being efficient and effective. Chemo-photothermal therapy would be completely restructured and redesigned, so that less toxic chemicals have to interact with the unaffected organs.

Purpose: The purpose of this experiment is to utilize the photothermal effect of mesoporous carbon-based dopamine for the biological application to efficiently target cancer cells.

Hypothesis: If mesoporous carbon-based dopamine is introduced to cancer cells in increasing concentrations, the higher end of the variable spectrum will have a higher mortality rate and targeted effect.

Materials: 96 well plates, luminometer, incubator, HeLa cells, confocal microscope, trypsin, PBS, T-75 flasks, 50 mL test tubes, sterile hood, pipette, light microscope, cell counter (hemocytometer), trypan blue dye, Cell Counting Kit 8 (CCK-8) assay dye, F12K media

Safety: In order to assure safety when coming into contact with hazardous materials or cultures (and for the protections of the cells), the researcher used a properly certified lab coat and goggles with nylon gloves under the sterile hood to assure proper protection. 70% ethanol was used to sterilize all work spaces and instruments to assure no bacteria or airborne pathogens could come into contact with cultures or research in addition to being transported in and out of the lab. All lab safety protocols were diligently followed.

Procedure

1. Maintaining cells
    1.1. If not being split nor seeded, conduct the following procedures:

1.1.1. Wash the T-75 flask with 7 mL of PBS and aspirate to remove dead cells and allow space for new ones to grow
1.1.2. NOTE: Do not add trypsin nor wash flask to not kill or detach dead cells

2. Splitting HeLa cells
   2.1. Dispose or vacuum cell media
      2.1.1. NOTE: place both media and trypsin in warm bath to bring to 37ºC prior to use
   2.2. Wash the T-75 flask with 7 mL of PBS and aspirate
   2.3. Add 5 mL of trypsin to the flask and incubate for 10 minutes at 37ºC w/ 5% CO2
      2.3.1. NOTE: use tapping motion to force cells to aggregate instead of clump
   2.4. Add 10 mL of media (the goal is 15 mL of suspension for new flask) and use pipette to fix the last group of adhered cells
   2.5. Transfer to 13 mL conical tube and count cells
   2.6. Add counted suspension into flask and incubate at 37ºC w/ 5% CO2
      2.6.1. NOTE: rock the flask back and forth to spread the cells -do NOT- use a swishing motion
3. Seeding HeLa cells into well plates
   3.1. Dispose or vacuum cell media
      3.1.1. NOTE: place both media and trypsin in warm bath to bring to 37ºC prior to use
   3.2. Wash the T-75 flask with 7 mL of PBS and aspirate
   3.3. Add 5 mL of trypsin to the flask and incubate for 10 minutes at 37ºC w/ 5% CO2
      3.3.1. NOTE: use tapping motion to force cells to aggregate instead of clump
   3.4. Add 10 mL of media (the goal is 15 mL of suspension for new flask) and use pipette to fix the last group of adhered cells
   3.5. Transfer to 13 mL conical tube and count cells
   3.6. Transfer 250 uL of suspension into each well plate according to the variable format
   3.7. Add 250 uL of PBS to the perimeter wells surrounding experimental groups and incubate with cover at 37ºC w/ 5% CO2
   3.8. Add remaining suspension into flask and incubate at 37ºC w/ 5% CO2
      3.8.1. NOTE: rock the flask back and forth to spread the cells -do NOT- use a swishing motion
4. Counting cells
   4.1. Take 10 uL of suspension and add 2 uL of trypan blue
   4.2. Mix the new solution using sterile pipette tip
   4.3. Use the pipette to add the suspension to the hemocytometer, using a slight amount of pressure to create a suction between the cover and liquid
   4.4. Formula
      4.4.1. $total\ cells/mL = total\ cells\ counted \times \frac{dilution\ factor}{\#\ of\ squares} \times 10^4$
5. Concentration chart
   5.1.

Tube #	concentration	drug	media
1	2 mg/mL	10 mg	5 mL (H_2O)
2	200 ug/mL	400 uL (1)	3600 uL
3	150 ug/mL	300 uL (1)	3700 uL

4	100 ug/mL	2000 uL (2)	2000 uL
5	50 ug/mL	2000 uL (4)	2000 uL
6	25 ug/mL	2000 uL (5)	2000 uL
7	10 ug/mL	1600 uL (6)	2400 uL
8	5 ug/mL	2000 uL (7)	2000 uL
9	1 ug/mL	400 uL (7)	3600 uL
10	0.1 ug/mL	400 uL (8)	3600 uL

6. CCK-8 assay
   - 6.1. Make CCK-8 ratio with dye and media
     - 6.1.1. Use 10:100 ratio (dye to media)
     - 6.1.2. Vortex to make solution consistent
   - 6.2. Aspirate media from inner well plate and then add 250 uL of PBS to the variable wells
     - 6.2.1. NOTE: rock back and forth or side to side but -do not- swirl
   - 6.3. Aspirate off PBS and add 110 uL of CCK-8 solution made in step 1 to each variable well
   - 6.4. Incubate at 37ºC with 5% CO2 for 1-4 hours
   - 6.5. Wrap in aluminum foil and transport to the biomedical engineering lab at the end of the hall
   - 6.6. Use plate reader (settings at 450 and normal) and read plate without lid
   - 6.7. Save and email results for analysis
7. Photothermal/photostability assay
   - 7.1. Photothermal effect was studied in mesoporous carbon-based dopamine in a 96-well plate.
   - 7.2. Prepare solutions of treatment variables in the following concentrations: 0,5, 10, 25, 50, 100, 200, 400, 800
   - 7.3. Load 250 uL of each solution into the plate according to the designated concentration gradient.
   - 7.4. Expose each well to 808 nm NIR laser (1 W output power) for 10 min (measure temperature with a thermocouple every 10 seconds).
   - 7.5. The photostability of the particles was also measured using identical concentration solutions: 0,5, 10, 25, 50, 100, 200, 400, 800.
   - 7.6. The solutions were each exposed to the 808 nm NIR laser (1 W output power) for 10 minutes, measuring the temperature and drug quantity every 10 seconds.

Discussion

The development of a successful nanoparticle treatment could have drastic implications for the medical community. By testing photostability and photothermal capability, this experiment works to prove that mesoporous carbon-based dopamine would be a viable therapy with benefits unavailable elsewhere. Characterizing its properties allows for the official inquiry into the extent of its applications as a target-specific medication. The goal of this research was to further delve into exploring new mechanisms in which to deliver therapeutics for diseases like cancer, and being able to determine its properties, both individually and inner-cell, is crucial to this process. Cancer treatment needs to be improved by drastic measures, since current chemotherapy and radiation tends to have a side effect killing healthy cells, making the patient worse.

Targeted treatment, like that of mesoporous carbon-based dopamine, allows for three independent factors to be manipulated and improved. First, it allows scientists or doctors to be able to ease the MCBD into complete and "proper" engulfment to target specific types of cell, like that which make up tumors to destroy them. Secondly, the photothermal and photostability assays help in the determination of the application to which the nanoparticle could be heated up enough to release the filling chemical without killing the cell. MCBD is a heat-dependent release particle, so without an established successful NIR range, there is a higher probability of cell death and failure of particle activation. Lastly, the chemical within the mesoporous carbon can be altered depending on the type of disease it is treating, like gene therapy, or the imaging function it is carrying out, such as contrast for PET scans or MRIs.

Data Analysis

The data from the assays create a structured baseline for general use of cell viability with median levels of MCBD in the cells. Higher concentrations tend to kill off the cells quickly while also engulfing less nanoparticle amounts into the cell, so the IC-50 helps determine to which extent 50% of the cell population is still alive with enough particle concentration within the cell to reach desired treatment release. In both IC-50's, the data showed that the 100 ug/mL concentration was the most preferred option in regards to maintaining cell viability while also achieving maximum potential nanoparticle concentration.

The photothermal assay was useful in determining the maximum heat release point, showing that cells have a limit to the amount of exposure before degradation that allows for sufficient release. It supports the theory of successful heat release under NIR laser concentration within that wavelength and the sensitivity of the carbon lining to breakdown and open its pores to release the dopamine into the system. Similar to the IC-50, the concentration provided stability in all trials at around 100 ug/mL, providing another reason to determine this as the baseline for most efficient release.

Photostability assays test for the stability of the cell to release the dopamine without breaking down or expelling the nanoparticle. It was successful in showing that at high concentrations (or anything exceeding) 200 ug/mL, there was rather immediate cell death or no acceptance of the nanoparticle into the membrane.

Application

This project has vast applications among the scientific community; they are the future of medical research, and having multiple areas of effect allows them to impact almost every strain of complex disease for patients. First and foremost, it presents a new alternative to chemotherapy that is a lot safer for the patient and also diminishes treatment time. Cancer patients would no longer have to subject their bodies to toxic chemicals that kill their healthy cells to make them worse before they can improve. Another possibility is the expansion of data on targeted drug therapies. By altering the carrier structure element or the filling within the nanoparticles center, it will allow for additional diseases to be tested and treated that either do not have a cure, or whose treatment is harmful to the subject. Lastly, this project has great implications in the advancement of nanoparticle research; since it is such a new field, there is much to be researched and different aspects to be studied. By introducing this field of cancer studies using nanoparticles, it provides a pathway into the greater study of them across all scientific fields.

Limitations

Though this experiment was as regulated as possible, there are still some potential limitations that could have minorly impacted the project. First, the CCK-8 viability test is slightly sensitive to light, so any exposure outside

of the incubator during transfer to plate reader (minimized by aluminum foil as much as possible) could have slightly modified the results. Another factor is the testing done using the laser. Due to human error, it is possible that the data collected went over a second during the 10-sec collection period, along the change would not have made a difference in the data since over the period of time, it would have resulted the same.

Future Research

Because of the broad span of applications this research has, it is best to say that any future research can be applied towards mostly the drug or gene therapy subject. Primarily, a significant advancement that could be made is in the gene therapy field, where nanoparticles like mesoporous carbon can be manipulated into convenient RNAi vectors; these alterations can repair errors causing down syndrome or predispositions to cancer. Furthermore, nanoparticles can have different outer constructs and filling particular to each medication necessary, and changing the outer covering allows for different heat ranges for different release or cell targets. To conclude, another possible variation would be modifying the wavelength or strength of the laser in order to maximize the release without delivering too much radiation into the body of the patient.

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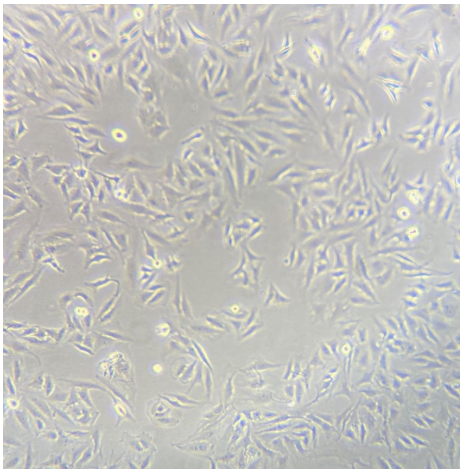
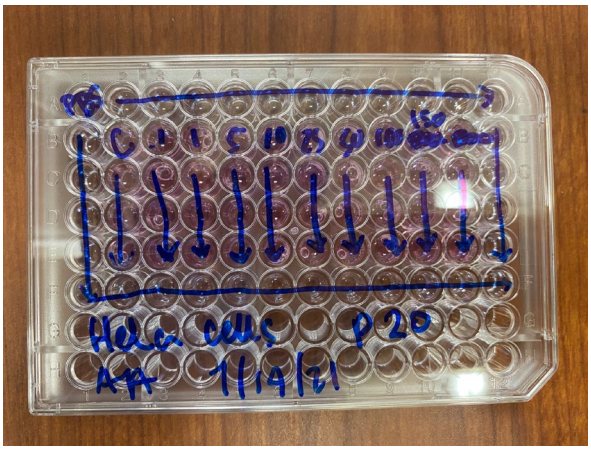
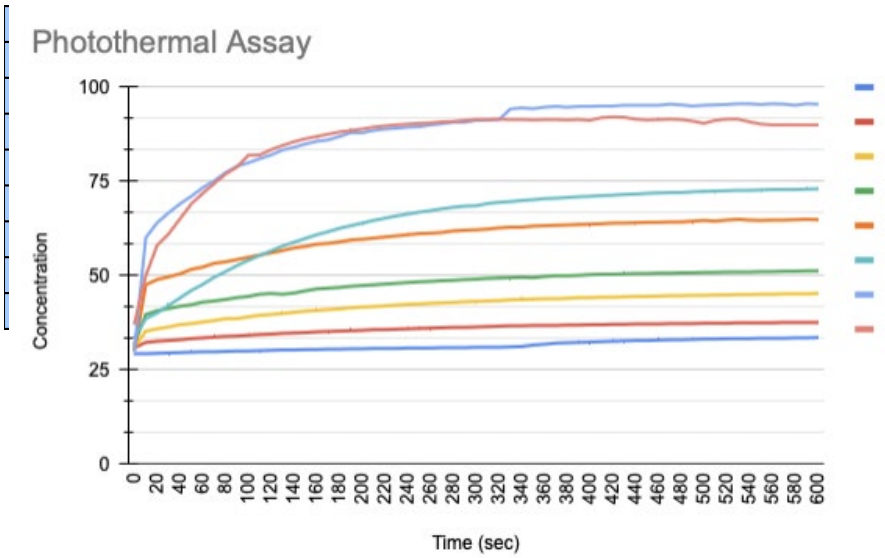
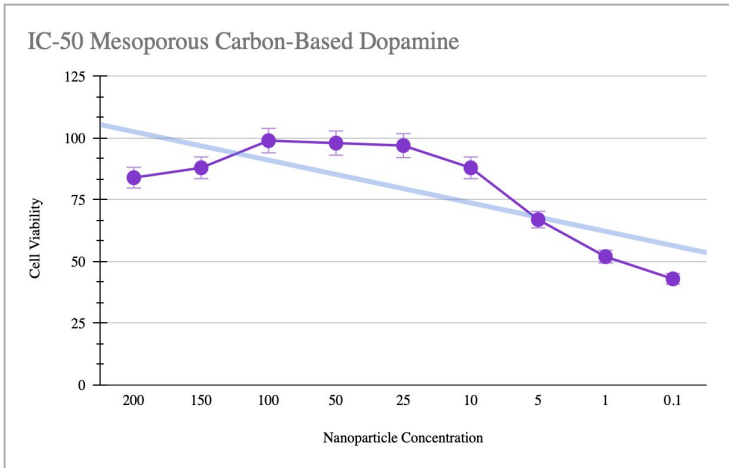
Visuals






Photothermal Assay
Concentration
100
75
50
25
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600
Time (sec)


IC-50 Mesoporous Carbon-Based Dopamine
Cell Viability
125
100
75
50
25
0
200 150 100 50 25 10 5 1 0.1
Nanoparticle Concentration

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